Author Index
&
Presentation Abstracts

102nd Conference of Research Workers in Animal Diseases

December 4-7, 2021

Chicago Marriott Downtown Magnificent Mile

Chicago, IL

Conference of Research Workers in Animal Diseases
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CRWAD 2021 President's Message

December 2021

Welcome to the 102nd Conference of Research Workers in Animal Diseases. I have the honor and privilege of serving as president of CRWAD this year and I am pleased to share with you the outstanding Conference we have this year.

I am pleased we are able to hold an in-person event this year for those who are able to travel to Chicago. As we work through the challenges (and opportunities) the global pandemic continues to present, I am also pleased CRWAD is offering a virtual participation option for those who are not able to travel at this time. The full content of our onsite program, in addition to a number of virtual abstract presentations, will be available for all registered attendees to access in an easy to use format from now until June of 2022.

Many thanks to the CRWAD council members and program committee for their continued commitment to putting together the best program for you. A special thank you to Dr. Paul Morley, CRWAD Executive Director, and program committee co-chairs Dr. Brandy Burgess and Dr. Heather Wilson for their work to bring you a program that has a record number of invited abstracts and a panel of eminent featured speakers.

I feel strongly that CRWAD is an important link in the research we all do. I joined Michigan State University in 1974. My advisor, Dr. Gordon Carter, told me that if I want to succeed in my professional career as a researcher, I must try to attend CRWAD a regular basis. I have been attending this CRWAD since 1975, and only missed one year. CRWAD has been useful, not only for my academic growth and success, but also for my personal growth and development as a member of this great scientific community.

I also strongly believe this conference provides an opportunity for graduate and post-doctoral students and junior scientists to demonstrate their research skills and talents by presenting their data to other scientists. We have a very diverse group of researchers, academicians, industry folks, and members of state and federal agencies from all over the world. CRWAD's annual Conference provides an ideal environment and opportunity to cross-pollinate your research. It is also a great place for students and junior scientists to network and develop connections for collaborative research ideas and long-term growth and success.

I am happy to be a part of CRWAD. I am glad you are part of this year's Conference. It is a great organization and Conference because of you and how you participate every year to enrich yourself with knowledge and research ideas, while seeking contacts for future research opportunities and collaboration

Dr. M. M. Chengappa, DVM, PhD, AVCM Diplomate
CRWAD President

CRWAD 2021 Featured Speakers

"Development of microbiota-directed complementary foods for treating childhood undernutrition."
Jeffery I. Gordon, MD
CRWAD Keynote Speaker
Washington University School of Medicine in St. Louis
Department of Pathology & Immunology
Sunday, 12/5/2021 2:00 PM

"Pathogens from the Host Point of View"
L. Garry Adams, DVM, PhD
ACVM Distinguished Microbiologist
Texas A&M University
Veterinary Medicine & Biomedical Sciences
Veterinary Pathobiology
Monday, 12/6/2021 8:30 AM

"Applying knowledge of immunological correlates to vaccine design for prevention of chlamydial abortion"
Gary Entrican, PhD, BSc
AAVI Distinguished Veterinary Immunologist
The University of Edinburgh
Monday, 12/6/2021, 2:00 PM

"Risk management in an ever more complex world"
Dirk Pfeiffer, DVM, PhD
AVEPM Calvin Schwabe Award
City University
Centre for Applied One Health Research and Policy Advice
Tuesday, 12/7/2021, 8:30 AM

CRWAD 2021 Featured Speakers

"Precision livestock farming: From where we came, where to go?"
Daniel Berckmans, PhD, MSc
Precision Agriculture and Animal Health Special Symposium
Katholieke Universiteit Leuven
Leuven, Belgium
Sunday, 12/5/2021, 5:00 PM

"USDA-NIFA Programs in Precision Livestock Farming and Future Outlook"
Kathe Bjork, DVM, MSPH, PhD
Precision Agriculture and Animal Health Special Symposium
Institute of Food and Agriculture-Division of Animal Systems
USDA-NIFA
Sunday, 12/5/2021 3:00 PM

"Application of precision technologies on livestock farms"
Dipti Pitta, PhD
Precision Agriculture and Animal Health Special Symposium
University of Pennsylvania
Sunday, 12/5/2021 4:15 PM

"USDA-NIFA Programs in Precision Livestock Farming and Future Outlook"
Steven Thomson, PhD
Precision Agriculture and Animal Health Special Symposium
Agricultural and Biosystems Engineering
Institute of Food Production and Sustainability
USDA-NIFA
Sunday, 12/5/2021 3:00 PM

CRWAD 2021 Featured Speakers

"Brucellosis and Reproductive Disease: A Current Update"
Angela Arenas-Gamboa, DVM, PhD, DACVP
AAVI Featured Speaker
Texas A& M University
College of Medicine
Monday, 12/6/2021, 5:15 PM

"Industry Perspective on the Value of Animal Health Research"
Lisa Becton, DVM
Vaccine Network Featured Speaker
Swine Health
National Pork Board
Sunday, 12/5/2021, 10:30 AM

"Aquaculture production and vaccinology: Current and past hurdles"
Mark David Fast, BSc, MSc, PhD
Vaccine Network Featured Speaker
University of Prince Edward Island
Pathology and Microbiology
Monday, 12/5/2021, 8:30 AM

"A molecular perspective on the development of fetal immunotolerance to BVDV"
Hanah Georges, PhD
AAVI Featured Speaker
Colorado State University
Monday, 12/6/2021, 3:00 PM

CRWAD 2021 Featured Speakers

"Host responses following third trimester maternal PRRSV2 infection"
John Harding, DVM, MSc
AAVI Featured Speaker
University of Saskatchewan
Western College of Veterinary Medicine
Monday, 12/6/2021, 4:15 PM

"Better Bridging Science and Animal Health Policy"
Wantanee Kalpravidh, PhD
AVEPM Featured Speaker
Scientific Advisory Committee for the School of Global Health
Faculty of Medicine
Chulalongkorn University
Tuesday, 12/7/2021, 11:15 AM

"*Campylobacter jejuni* sets the stage for disease by manipulating the signaling of intestinal epithelial cells"
Michael Konkel
ACVM Featured Speaker
Washington State University
Veterinary Microbiology and Pathology
Monday, 12/6/2021, 10:30 AM

"A Paradigm Shift in the Use of Vaccines and Vaccination in Global Control of Avian Influenza"
David Swayne, DVM, PhD, DACVP, DACPV
Vaccine Network Featured Speaker
United States National Poultry Research Center
USDA- ARS
Sunday, 12/5/2021, 11:15 AM

CRWAD 2021 Featured Speakers

"Maximizing USDA surveillance data for improved influenza vaccines for swine"
Amy Vincent, PhD
Vaccine Network Featured Speaker
USDA Research Veterinary Medical Officer
Sunday, 12/5/2021, 9:15 AM

"Creative Destruction and One Health: Policy, Science & Practice in a Post Normal World"
David Waltner-Toews, DVM
AVEPM Featured Speaker
University of Guelph
Tuesday, 12/7/2021, 10:30 AM

"Coronaviruses and cats and humans: where are feline coronaviruses and SARS-CoV-2 alike"
Gary Whittaker, PhD
ACVM Featured Speaker
Cornell University, College of Veterinary Medicine
Department of Microbiology and Immunology
Professor of Virology
Monday, 12/6/2021, 9:15 AM

"Intrauterine immunization during breeding"
Heather Wilson, BSc, PhD
AAVI Featured Speaker
University of Saskatchewan
Western College of Veterinary Medicine
Department of Veterinary Microbiology
Monday, 12/6/2021, 4:45 PM

CRWAD 2021 Featured Speakers

"A novel epitope- and structure-based vaccinology platform empowers broad-spectrum and precision vaccine development"
Weiping Zhang, PhD
ACVM Featured Speaker
University of Illinois
College of Veterinary Medicine
Monday, 12/6/2021, 11:15 AM

"The cost and the lessons of the Covid pandemic"
David Zilberman, PhD
AVEPM Featured Speaker
University of California Berkeley
Department of Agricultural & Resource Economics
Tuesday, 12/7/2021, 9:15 AM

Fellows of the Conference of Research Workers in Animal Disease
-- 2021 Inductees --

Fellows of the Conference of Research Workers in Animal Diseases represent an eminent cadre of scientists from all types of research careers, including academia, industry, and government. Election as a CRWAD Fellow is a lifetime honor and all Fellows meet the highest standards of professional ethics and scientific integrity.

Scientists recognized as CRWAD Fellows have distinguished research careers evidenced by the outstanding impact and importance of their work, and their ability to communicate and interpret science to stakeholders and the public. Fellows have made significant contributions to scientific literature reflecting fundamental discoveries and/or innovative applied research in animal health and disease, population health, and translational medicine. Reflecting the tradition and spirit of CRWAD, mentoring of young research scientists in furthering their careers is an important contribution of CRWAD Fellows.

CRWAD Fellows are scientists who have made sustained and notable contributions to CRWAD through service or participation in the CRWAD organization and annual meetings.

CRWAD is pleased to announce the inaugural Fellow Inductees in conjunction with the 2021 Conference.

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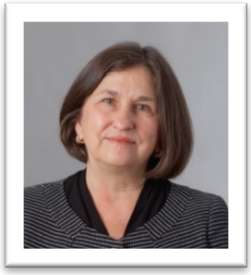
Lorraine M. Sordillo-Gandy, PhD

Fellows of the Conference of Research Workers in Animal Diseases
-- 2021 Inductees --



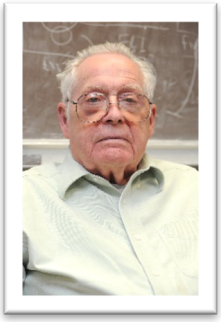
Cynthia L. Baldwin, PhD
University of Massachusetts Amherst

Yung-Fu Chang, DVM, MS, PhD
Cornell University

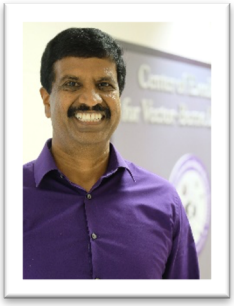
Norman F. Cheville, DVM, MS, PhD
Iowa State University

Lynette Bundy Corbeil, DVM, PhD
University of California – San Diego

Roy Curtiss, III, PhD
University of Florida



William C. Davis, PhD
Washington State University



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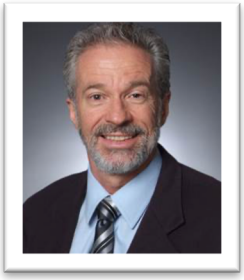

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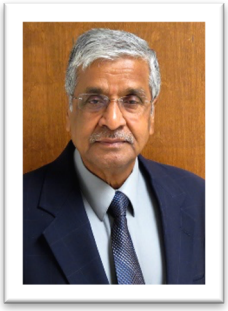
Laura L. Hungerford, DVM, MPH, PhD, CPH, FNAP
Virginia Tech

Jun Lin, PhD
University of Tennessee



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Kansas State University

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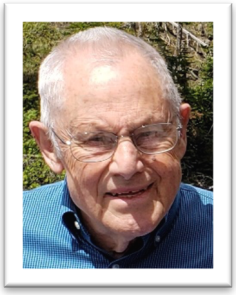

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Please visit www.crward.org/fellows_directory/ for biographical information about all Fellows of the Conference of Research Workers in Animal Diseases

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1 - Aquaculture production and vaccinology: Current and past hurdles

M. Fast Atlantic Veterinary College. mfast@upei.ca
Session: Vaccine Network Featured Speaker, Dec. 5, 8:30 - 9:15 AM

Cultivation of freshwater and marine shellfish and finfish species occurs worldwide and can range from land-based to open-ocean facilities. While capture fisheries have experienced a plateau in production since the 1980s, aquaculture production has experienced 3-10% growth annually; and since 2016-2017 has accounted for over 50% of the aquatic animals processed for human consumption. Freshwater carps, tilapia and catfish, account for the greatest tonnage, while salmonids are associated with the most technologically advanced culture practises. In order to supply the global markets with product year-round, increased intensification, staggered stocking and harvest, along with vertical integration of operations has evolved for several of these species. However, continued sustainable growth of aquaculture, is impeded by outbreaks of viral, bacterial and parasitic disease. Salmon aquaculture has frequently led the way in innovative solutions to these problems, likely afforded in part by the high price of the fillet in comparison to other finfish species with smaller margins for research. During the 1980s, salmon aquaculture was hampered by infectious bacterial disease (*Vibrio* and *Aeromonas* spp.) that required heavy reliance on antibiotic usage within the industry. However, the first vaccines were put in use in the late 80s and as oil emulsion and combination vaccines were introduced in the 1990s, antibiotic use in salmonid aquaculture precipitously declined, despite the exponential increase in production. Now vaccination is common practise across multiple species, each with their own unique issues in adapting comparative immunological knowledge to hundreds of diverse aquatic species (600 unique species, including invertebrates, chondrosteans and teleosts farmed globally as of 2017, FAO); and relating laboratory-based vaccine efficacy testing to 'real-world' field protection. Add to this the current threat of rising marine and freshwater temperatures, as a consequence of global climate change, in poikilothermic hosts in which immunological robustness is heavily linked to the temperatures they are reared at, presents a unique set of challenges for the industry to overcome in the near future. Some of the basic differences in innate immunity and immunological memory in fish species, in comparison to endothermic production animals, will be discussed in light of these challenges, as will some of the potential opportunities for success in the future.

Notes:

2 - Maximizing USDA surveillance data for improved influenza vaccines for swine

A. Vincent[1], T. Anderson[1], Z. Arendsee[1]. [1]National Animal Disease Center, USDA-ARS. amy.vincent@usda.gov
Session: Vaccine Network Featured Speaker, Dec. 5

Objective

Influenza A virus (IAV) is a common cause of respiratory disease in swine and other mammals, including humans. Zoonotic infection of humans with swine adapted IAV occurs sporadically, and IAV from swine caused the 2009 H1N1 pandemic (H1N1pdm09). Although swine IAV notably shaped human seasonal IAV with H1N1pdm09, human seasonal IAV genes, including H1N1pdm09, frequently spill into swine populations. Rather than a sweeping replacement of prior endemic IAV, these incursions add to the current diversity with subsequent maintenance of new lineages of IAV with distinct surface glycoproteins, hemagglutinin (HA) and neuraminidase (NA). IAV possess segmented RNA genomes with 8 gene segments that can reassort when more than one unique strain infects a host. Reassortment adds to the diversity of endemic swine IAV, as HA and NA and 6 other genes undergo frequent mutation and continued evolution during transmission in swine herds. Although endemic subtypes in swine are limited to H1N1, H1N2, and H3N2, phylogenetic analysis of HA and NA genes demonstrate many lineages and clades for each subtype.

Methods

Due to the urgent need to monitor swine IAV for swine and human health, USDA APHIS implemented a voluntary surveillance system in swine through veterinary diagnostic laboratories and the National Veterinary Services Laboratories.

Results

The system generated over 9,000 public HA and NA sequences and over 2,400 whole genome sequences to date. Virus isolates are available through the repository. Analyzed and aggregated sequence information from the surveillance system are reported on the USDA APHIS Swine Health site, Iowa State University FLUture site, and an ARS National Animal Disease Center octoFLUshow site.

Conclusions

Monitoring trends in genetic clades of HA and NA genes provides basic metrics to make vaccine strain decisions. Antigenic characterization of HA and NA further informs vaccine strain selection. The presentation highlights the current trends in the USDA swine surveillance data with interactive visualization tools and antigenic characterization to aid in vaccine strain selection.

Financial Support
U.S. Department of Agriculture, Agriculture and Research Services; U.S. Department of Agriculture, Animal and Plant Health Inspection Services

Notes:

3 - Vaccine development and use in swine production – What key concepts can be applied from COVID-19 pandemic?

L. Becton National Pork Board. lbecton@pork.org
Session: Vaccine Network Featured Speaker, Dec. 5, 10:30 - 11:15 AM

The U.S. Pork industry represents a very diverse production that spans different geographies, breeds, genetics, demographics, farm type and size. Despite these differences, a common goal for all producers is the desire to maintain excellent swine health. Healthy pigs provide the foundation for safe, sustainable, and efficient protein production. Vaccines are a critical component of herd health plans and as shown by the 2012 USDA NAHMS Swine Survey Part II report, producers have embraced strategic use of this technology to prevent critical diseases from harming their herds.

The current COVID-19 pandemic has challenged all aspects of public health, diagnostic and vaccine research, and response. In late 2020, the American Medical Association outlined the concept of "herd immunity" to help explain the need for vaccination against COVID-19. This terminology was used extensively by public health officials during vaccine discussions. This discussion was very pertinent to swine production and agriculture since developing and maintaining herd immunity through strategic use of vaccination is a key component of population medicine. Useful lessons from COVID-19 continue to be made for management of disease symptoms, outcomes and spread. This is especially true in areas regarding rapid development, assessment, distribution, and administration of vaccines to targeted populations. Many experiences and lessons learned from COVID-19 can, and where possible, should be directly applied to swine production to continuously improve herd health.

Notes:

4 - A paradigm shift in the use of vaccines and vaccination in global control of avian influenza

D. Swayne Agricultural Research Service. david.swayne@usda.gov
Session: Vaccine Network Featured Speaker, Dec. 5, 11:15 - 12:00 PM

High pathogenicity avian influenza (HPAI) is a devastating disease of poultry and wild birds that can cause high mortality and negatively impact livelihoods and trade. Historically, HPAI in poultry has been met with stamping-out programs with eradication of most outbreak. However, stamping out alone is unsustainable against entrenched or re-introduced HPAI viruses and control efforts could benefit from broader use of vaccines. In a few poultry outbreaks, the HPAI viruses have become entrenched, with a few countries utilizing vaccines as an additional management tool which has led to the control of the virus in some countries and elimination in others. Globally, vaccination has not been uniformly accepted as a control tool, but scientific data produced over the past decade has supported a greater use of vaccines in control and eradication programs. Influenza virus mutation, i.e. drift in the hemagglutinin, has generated antigenically divergent field viruses which need multiple seed strains for protection. Fortunately, newer vaccine platforms are more easily updated to achieve a closer match and improved protection. This has been augmented by the adoption of accelerated national regulatory processes for approval and deployment of revised/updated seed strains in non-replicating vaccines such as RNA-particle, DNA and inactivated whole influenza A virus vaccine platforms. Also, some technologies favor mass field vaccination that could eliminate the need for capture and vaccination of individual meat chickens, reducing labor, complex logistics and cost of vaccination. These include vectors based on Newcastle disease virus and other avian paramyxovirus, and Marek's disease herpesvirus (rHVT). The rHVT is especially favored as it can be automated in the hatchery for in ovo or post-hatch vaccination and persist to replicate and produce longer term immunity. Finally, deletion mutants of influenza A virus have a restricted growth, reduced reassortment potential and reduced transmissibility hold promise for immunization in the field by spray vaccine application.

Notes:

5 - Description of feedlot animals culled for slaughter, revenue received, and associations with reported U.S. beef market prices

L. Horton[1], B. Depenbusch[2], D. Pendell[3], D. Renter[1]. [1]Center for Outcomes Research and Epidemiology, College of Veterinary Medicine, Kansas State University, [2]Innovative Livestock Services, Inc, [3]Department of Agricultural Economics, College of Agriculture, Kansas State University. lhorton@vet.k-state.edu
Session: Epidemiology -1, Dec. 5, 8:30 - 8:45 AM

Objective

The primary objective of this study was to describe cattle characteristics and revenue received for feedlot animals culled for slaughter from Central Kansas feedlots, and associations with prices from U.S. beef cattle markets.

Methods

Animal- and lot-level data were collected from four participating Kansas feedlots between Dec. 2018 and Nov. 2020. Data also were sourced from USDA reported weekly market prices for U.S. fat cattle, feeder cattle, cull cow, and boneless beef trimmings. Associations between actual prices received for feedlot culls (from individual animals) and weekly beef market prices were tested using Spearman's correlation coefficients. The characteristics of feedlot culls (N = 2,922) were assessed using descriptive statistics, and general and generalized linear mixed models that accounted for clustering.

Results

Common reasons for culling were musculoskeletal/trauma (49.7%) and respiratory disease (40.9%). Revenue was returned more frequently from animals culled for musculoskeletal/trauma reasons (99.1%) compared to those culled for respiratory or "other" reasons (96.7 or 96.3% respectively; $P < 0.01$). On a carcass basis, the mean price received (± 95% CI) for culled animals was 87.40 (86.70 to 88.10) $/45.5 kg, which resulted in a mean return of 434.81 (427.22 to 442.40) $/animal (including animals that returned no revenue). Prices received for feedlot culls were significantly correlated with the majority of U.S. beef markets; and while overall correlations were relatively weak, they generally improved when culls were categorized by weight groups. The strongest correlations with feedlot cull prices were with national dressed Breaker cow prices (75% lean; over 500 lb [227 kg]), for which $r = 0.26$ overall, and ranged from $r = 0.23$ to 0.77 when categorized by weight.

Conclusions

In this population, the reason for culling was associated with revenue, and dressed Breaker cow prices resulted in the strongest relationship with prices received for feedlot culls, which may be used as an indicator for estimating revenue from culled feedlot cattle.

Notes:

6 - Veterinary involvement in cattle health and production record-keeping on U.S. cow-calf operations

W. Jumper[1], R. Wills[2], C. Huston[1], D. Smith[1]. [1]Department of Pathobiology and Population Medicine, College of Veterinary Medicine, Mississippi State University, [2]Department of Comparative Biomedical Sciences, College of Veterinary Medicine, Mississippi State University. isaac.jumper@msstate.edu
Session: Epidemiology -1, Dec. 5, 8:45 - 9:00 AM

Objective

The objective of this study was to identify factors associated with veterinary involvement in cattle health and production record (CHPR) collection and analysis on U.S. cow-calf operations.

Methods

We anonymously surveyed 14,294 cow-calf producers across the U.S. Multivariable logistic regression using manual forward selection was used to test demographic factors for association with veterinary involvement in the collection and analysis of CHPR.

Results

A total of 3,741 (26%) responses were received, with 3,641 (97%) respondents actively involved in cow-calf production. Of those, 2299 (63%) said a veterinarian was influential in cow-calf operation management decisions. A veterinarian was paid to collect/record CHPR by 156 of 3611 (4%) of respondents, and 495 of 3603 (14%) respondents paid a veterinarian to analyze CHPR and provide management advice from that information. If the service was available, 736 of 2934 (25%) respondents said they would pay a veterinarian for CHPR management services. Factors associated with willingness to pay a veterinarian for CHPR management services were region (midwest: OR=1.6, 95%C.I.=1.2,2.2; mountain: OR=1.4, 95%C.I.=0.9,2.0; northeast: OR=1.6, 95%C.I.=1.0,2.4; north plains: OR=1.9, 95%C.I.=1.2,2.8; southeast: OR=2.0, 95%C.I.=1.4,2.8; south plains: OR=1.7, 95%C.I.=1.2,2.4; compared to west), cow-calf operation is primary income source (OR=0.8, 95%C.I.=0.6,0.9; compared to alternative primary income source), and education level (Bachelor's degree or less: OR=1.1, 95%C.I.=0.9,1.4; post-graduate degree: OR=1.3, 95%C.I.=1.0,1.7; professional degree: OR= 2.4, 95%C.I.=1.6,3.4; compared to High-school diploma or less).

Conclusions

A meaningful number of cow-calf producers indicated willingness to pay a veterinarian for services that include CHPR collection and analysis depending on region, primary income source, and education.

Financial Support
Supported by the Mikell and Mary Cheek Hall Davis Endowment for Beef Cattle Health and Reproduction, Mississippi State University Foundation.

Notes:

7 - Effects of eye patches on corneal ulcer healing and weight gain in stocker steers on pasture

G. Maier[1], J. Davy[2], L. Forero[2], H. Bang[3], K. Clothier[4], J. Angelos[5]. [1]Department of Population Health & Reproduction/ University of California Davis., [2]Cooperative Extension, Division of Agriculture and Natural Resources, University of California, [3]University of California Davis, Division of Biostatistics, Department of Public Health Sciences, [4]California Animal Health and Food Safety Lab, University of California-Davis, [5]Department of Veterinary Medicine and Epidemiology, University of California -Davis. gumaier@ucdavis.edu
Session: Epidemiology -1, Dec. 5, 9:00 - 9:15 AM

Objective

Infectious bovine keratoconjunctivitis (IBK) is a painful ocular disease of cattle. The objective of this study was to evaluate the efficacy of eye patches in reducing corneal ulcer size due to IBK over time, and to compare time to healing for ulcers and weight gain in cattle when eye patches are used versus a control group.

Methods

A group of 216 clinically normal crossbred beef steers were followed between April and August 2019 and evaluated weekly for the development of IBK. Cattle with IBK received standard treatment and were randomly assigned to either an eye patch or no eye patch stratified by ulcer severity score. All treatment and control group animals were housed in a pasture separate from the rest of the cohort for a maximum of 28 days or until clinical cure. Corneal ulcer areas and body weights were measured twice weekly for steers in treatment and control groups. Weights of all steers in the cohort were recorded three times during the entire trial period.

Results

The primary outcome, rate of corneal ulcer healing, was faster (P = 0.001) for lesions in eyes receiving an eye patch as evaluated by a linear mixed model that controlled for ulcer severity score at enrollment and previous IBK in the opposite eye. In a Cox Proportional Hazards model adjusted for severity score at diagnosis, the hazard ratio for ulcer healing was 1.62 (95% CI 1.02 – 2.56, P=0.04) for eyes that received a patch compared to eyes that did not.

IBK tended to affect the secondary outcome, average daily gain (ADG) with 0.45 (± SE 0.01) kg in steers with IBK versus 0.42 (± SE 0.12) kg in those that remained free of IBK (P = 0.06). No difference in weight gain was observed between patched (0.47 (± SE 0.02) kg) and non-patched cattle (0.43 (± SE 0.02) kg; P = 0.22).

Conclusions

Though the placement of cattle with IBK into a separate pasture from unaffected cohorts may have confounded weight gain, the design provided an avenue to evaluate the rate of ulcer healing from IBK. It appears the more rapid rate of healing with the use of an eye patch warrants the use of this treatment in addition to antimicrobial therapy.

Notes:

8 - Production indicators for reporting suspicions of highly pathogenic avian influenza virus outbreaks in farmed ducks

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Session: Epidemiology -1, Dec. 5, 9:15 - 9:30 AM

Objective

Highly pathogenic avian influenza (HPAI) is a viral infection characterized by inducing severe disease and high levels of mortality in poultry. Increased mortality, drops in egg production or decreased feed or water intake are used as indicators for notification of suspicions of HPAI outbreaks. However, infections in commercial duck flocks may result in mild disease with low mortality levels, compromising, thereby, notifications. Hence, there is a need to identify quantitative indicators of infection in duck flocks which can be used as notification thresholds for effective early detection.

Methods

Data on daily mortality, egg production, feed intake and water intake from Dutch broiler and breeder duck flocks not-infected (n = 56 and n = 11 respectively) and infected with HPAI (n = 13, n = 4) were used for analyses. Data from negative flocks were used to assess the baseline (daily) levels of mortality and production parameters and to identify potential threshold values for triggering suspicions of HPAI infections and assess the specificity (Sp) of these thresholds. Data from infected flocks were used to assess the effect of infection on daily mortality and production and to evaluate the sensitivity (Se) of the thresholds for early detection of outbreaks.

Results

For broiler flocks, daily mortality > 0,3% or using a regression model for aberration detection would indicate infection with Se and Sp higher than 80%. Drops in daily feed or water intake greater than 7gr or 14 ml respectively are sensitive indicators of infection but have poor Sp. For breeders, mortality thresholds are very poor indicators of infection (low Se and Sp). However, a consecutive drop in egg production greater than 9% is an effective indicator of a HPAI outbreak. For both broilers and breeders flocks, cumulative average methods were also assessed, which had high Se but generated many false alarms (poor Sp).

Conclusions

The identified reporting thresholds can be used to inform policy and provide guidelines to farmers and veterinarians to notify suspicions of HPAI outbreaks in commercial duck flocks.

Financial Support
Ministry of Agriculture, Nature and Food Quality - Netherlands

Notes:

9 - Prevalence of severe fever with thrombocytopenia syndrome virus among wild animals in the Republic of Korea

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Session: Epidemiology -1, Dec. 5, 9:30 - 9:45 AM

Objective

Severe fever with thrombocytopenia syndrome (SFTS) is an emerging infectious disease and caused by *Dabie bandavirus*. It has been found in Asian countries including China, Japan, Korea, Pakistan, Vietnam, Taiwan, Thailand and Myanmar. SFTS virus (SFTSV) has been detected from various animals and humans. *Haemaphysalis longicornis* are known as the main vector of SFTSV. The aim of this study was to detect antigen and antibody against SFTSV from wild animals including wild boar, Korean water deer, and roe deer in the Republic of Korea (ROK).

Methods

From 2018 to 2020, 948 sera from wild boar, 388 sera from Korean water deer, and 23 sera from roe deer were collected in the ROK. For detection of SFTSV antigen, viral RNA was extracted from those sera and one-step RT-nested PCR was performed for amplifying the S segment of the SFTSV. Enzyme-linked immunosorbent assay (ELISA) was performed with above sera to detect antibodies of SFTSV. The optimal density was measured at 450 nm by a microplate reader.

Results

Of a total of 1,359 sera, 41 (4.3%) of 948 sera in wild boar, nine (2.3%) of 388 sera in Korean water deer, and one (4.3%) of 23 sera in roe deer were positive for SFTSV using RT-PCR. Fifty of 51 positive sera against SFTSV antigen were used in sequencing. The SFTSV sequences obtained in this study were included in genotype B2, B3, and D of SFTSV. In the result of ELISA, 768 sera of wild boar, 225 sera of Korean water deer, and 23 sera of roe deer were used to detect SFTSV antibodies. From these sera, 221 (28.8%) sera in wild boar, 47 (20.9%) sera in Korean water deer, and 6 (26.1%) sera in roe deer were seropositive for SFTSV.

Conclusions

The result of this study indicates that there is a possibility on circulation and transmission of SFTSV in wild animals such as wild boar, Korean water deer and roe deer. Thus, it is necessary to investigate the transmission routes in these animals and the strategies for prevention against SFTSV infection should be considered in wild animals.

Financial Support
Government-wide R&D Fund for Infectious Diseases Research (HG18C0021).

Notes:

10 - Serological survey of influenza A viruses in wild boar of the Korean peninsula

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Session: Epidemiology - 1, Dec. 5, 9:45 - 10:00 AM

Objective

Swine play an important role as a source of influenza viruses. Having common cellular receptors with birds and humans, swine provide opportunities for mixed infections and possibility for genetic reassortment between avian, human, and swine influenza. Unlike domestic pig, wild boars are free ranging and have many opportunities for exposure to IAVs through contact with migratory waterfowl, a natural reservoir for IAVs and animals of various habitats. As such, although the IAV that ultimately emerges from wild boars has the potential to be transmitted to humans, there are not many studies on the role of wild boars in IAV ecology.

Methods

7,781 wild boar were sampled from 25 November 2014 to 27 September 2019 across South Korea for serologic evidence of exposure to influenza. Wild boar samples were tested with the VDPro AIV Ab c-ELISA (Median Diagnostic, Korea) for serological screening and 105 ELISA-positive samples were tested again using the hemagglutination inhibition (HI) test for 8 subtypes (pandemic A(H1N1)2009, human H3N2, swine H1N1, avian H3N8, avian H5N3, avian H7N7, avian H9N2, HPAI H5N6).

Results

1.3%(105) of the samples were IAV positive by ELISA and 74(1.0%) serum was pandemic H1N1 positive, 23(0.3%) serum was human H3N2 positive, 41(0.5%) serum was swine H1N1 positive and all serum was negative for avian H3N8, avian H5N3, avian H7N7, avian H9N2, HPAI H5N6 by a HI test.

Conclusions

Our serological analysis indicates that Korean wild boars were exposed to several subtypes of swine influenza viruses and human influenza viruses. And some serum samples were cross-reacted with swine H1N1, human H3N2, suggesting that these wild boars may have been infected with more than one IAVs. Given these data, wild boar has a potential to become a novel mixing vessel, which will facilitate the adaptation of IAVs and allow to spillover to other hosts, including humans. Therefore, regular and frequent surveillance of circulating influenza viruses in wild boar population Is recommended as part of the prevention of the human influenza pandemic as well as the wild boar influenza epizootic.

Notes:

11 - Comparing the host response in the porcine mediastinal lymph node at day 3 post infection by PRRSV, influenza B or their coinfection

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Session: Pathogen-Host Interaction, Dec. 5, 10:30 - 10:45 AM

Objective

Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) and influenza B (IBV) cause natural infections in pigs. As a primary infection PRRSV can suppress the host immune system, leaving pigs susceptible to secondary infections such as IBV. One component of this immunosuppression is the ability to alter gene expression in the lymph nodes. This study investigates the host transcriptomic response in the mediastinal lymph node following PRRSV and IBV infections as well as their coinfection.

Methods

Seronegative pigs 3 to 4 weeks old were split into four treatment groups: control; intranasally infected with Type 2 PRRSV NPB strain; intranasally infected with B/Brisbane/60/2008 virus; or intranasally coinfected with both viruses. Three pigs from each of the four treatment groups were necropsied 3 days post-infection(dpi) and lymph node samples were collected for transcriptomic analysis. Differentially expressed gene (DEG) analysis was carried out using DeSeq2 based on the model treatment + E. Further analysis for over-enriched gene ontology (G.O.) terms and pathways was performed using the g:Gost function of g:Profiler.

Results

Comparison to the control samples resulted in 10 statistically significant (FDR <0.10) DEGs for IBV infected samples, 52 DEGs for the PRRSV infected samples, and 51 DEGs for the coinfected samples. The most significantly (FDR <0.05) over enriched GO terms for both IBV and coinfected samples were “establishment of protein localization to organelle”, “nitrogen compound transport”, and “intracellular protein transport”. These terms were not significant for PRRSV infected samples for which the most significantly over enriched GO terms were “antigen receptor-mediated signaling pathway”, “immune response-regulating cell surface receptor”, and “immune response-regulating signaling pathway”. These terms were also over enriched in the coinfected samples.

Conclusions

The differences in gene expression between treatment groups may indicate why PRRSV infections persist while, IBV infections clear, and provide a greater understanding pathology of these diseases.

Notes:

12 - Impact of porcine sapovirus on weaning weights in a herd with ongoing piglet diarrhea

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Session: Pathogen-Host Interaction, Dec. 5, 10:45 - 11:00 AM

Objective

Piglet diarrhea associated with porcine sapovirus was recently reported in the US. A diagnostic investigation in a herd with ongoing piglet diarrhea found fecal sapovirus PCR cycle times (CT) for litters with diarrhea at 12 days of age (DOA) were significantly lower than litters without diarrhea; 15.9 vs. 35.8, respectively. The objective of this survey was to characterize the reduction in weaning weights due to porcine sapovirus in this herd.

Methods

A total of 69 litters were weighed and fecal swabs collected 1-3 days prior to weaning (average 18 DOA; range 16-22 DOA). Three swabs per litter were pooled for sapovirus reverse transcription real-time PCR at Iowa State University Veterinary Diagnostic Laboratory. The positive/negative PCR cut-off value was 35.0. Entire litters were weighed and the average pig weight, adjusted for pig age and dam parity, was calculated by litter. This value was analyzed using the Kruskal-Wallis ANOVA to determine if weight differences between PCR positive and negative litters were significant ($P< 0.05$).

Results

Of the 69 litters evaluated, 57 (82.6%) had PCR positive feces and 12 (17.4%) were negative. In positive litters, the average, median and range of CTs were 20.1, 17.7 and 12.6-34.6, respectively. The average adjusted pig weight was 11.82 lb for the positive litters and 13.29 lb for the negative litters; a difference of 1.47 lb ($P = 0.012$). Using lower CT cut-offs (30 or 25) to classify positive/negative litter status resulted in similar results.

Conclusions

These findings support the clinical assessment of farm staff that the ongoing piglet diarrhea due to porcine sapovirus was reducing weaning weights by 1-2 lb per pig. Based on this survey, weaning weight reduction due to sapovirus in this herd could be estimated as the prevalence of positive litters times the pig weight difference or 82.6% times 1.47 lb, which equals 1.21 lb.

Financial Support
Merck Animal Health

Notes:

13 - A single amino acid mutation in porcine reproductive and respiratory syndrome virus glycoprotein 2 significantly impairs its infectivity in macrophages

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Session: Pathogen-Host Interaction, Dec. 5, 11:00 - 11:15 AM

Objective

Porcine reproductive and respiratory syndrome virus (PRRSV) has a strict tropism for cells of the monocyte/macrophage linage of swine. CD163 is a key receptor for the virus entry into cells. MARC-145, a monkey kidney cell line, has been routinely used for propagation of PRRSV *in vitro*. We passaged a PRRSV isolate for 95 passages in MARC-145 cells, followed by three consecutive rounds of plaque purification. We discovered that one of the plaque clones (designated NCV1-1) lost its infectivity in porcine alveolar macrophages (PAMs). The objective of this study was to identify viral genetics that are determinants for the virus infectivity in PAMs.

Methods

The NCV1 at different passage levels was sequenced and analyzed to identify potential mutations responsible for the loss of infectivity in PAMs. Reverse genetics was employed to manipulate these mutation sites to demonstrate their association with the virus infectivity in PAMs and porcine kidney cells stably expressing CD163 (PK15-CD163).

Results

Compared to other NCV-1 plaque clones, NCV1-1 carried a unique amino acid mutation from lysine (K) to isoleucine (I) in glycoprotein 2 (GP2) at position 160 (K160I). Alignment of GP2 sequences available in GenBank revealed that the K160 residue was highly conserved among PRRSV-2 isolates. Thus, we hypothesized that the K160I mutation found in GP2 of NCV1-1 is responsible for the impairment of infectivity in PAMs. To test this hypothesis, we made an infectious cDNA clone of NCV1-1 and changed the I at position 160 back to K. The resulting NCV1-1 I160K regained its infectivity in PAMs and PK15-CD163. Using an infectious cDNA clone derived from a wild-type PRRSV strain that infects PAMs efficiently, a K160I mutation in GP2 was performed. The resulting K160I mutant displayed a significant reduction in its infectivity in PAMs.

Conclusions

Collectively, these results clearly demonstrate that the K160 residue in GP2 of PRRSV is one of the determinants for virus infectivity in macrophages.

Financial Support
U.S. Department of Agriculture, Animal Health Formula Funds; U.S. Department of Agriculture, National Institute for Food and Agriculture

Notes:

14 - Mitochondrial function of porcine primary lung cells upon infection with respiratory viruses

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Session: Pathogen-Host Interaction, Dec. 5, 11:15 - 11:30 AM

Objective

Porcine Respiratory Disease Complex (PRDC) is a multi-microbial disease affecting ~30-70% of pigs upon break-out on a unit, where Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) and influenza virus (IV) are key players, impairing the normal function of the respiratory immune system. PRRSV is classified in two different species, Type 1 (European) and Type 2 (North American). The lung is the primary site of infection of this virus, known to have tropism for primary lung mononuclear phagocytes (MNP), particularly porcine alveolar macrophages (PAM) and pulmonary intravascular macrophages (PIM), which are key cells of the innate immunity. Mononuclear phagocytes' role is based on a cellular process known as respiratory burst, a rapid production of reactive oxygen species (ROS) during phagocytosis that inactivates the pathogen within the cell. Preliminary data has shown that PRRSV and IV infection directly affect mitochondrial function, which is linked to the development of respiratory burst.

Methods

Our study describes the interactions between IV, NC PRRSV-2 strains and different MNP: PAM, PIM and dendritic cells (DC) using a unique porcine ex vivo model.

Results

Preliminary data showed differences in ROS production between infected and non-infected MNP for both PRRSV and IV, as well as different inflammatory cytokine expression between DC and macrophages, in which immune responses were driven by PIM over PAM. Furthermore, in this study we assess the application of potential antivirals that could influence mitochondrial function and have an immunomodulatory effect on MNP.

Conclusions

We expect that the results of this study can be the stepping-stone to investigate the efficacy of alternative antiviral therapeutics against IV and PRRSV infection and its immunomodulatory capacity to reduce the burden of PRDC in pig production.

Financial Support
North Carolina State University

Notes:

15 - Replacing a risk microbiome by protective fecal microbiota transplant (FMT) decreased allergic airway disease

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Session: Pathogen-Host Interaction, Dec. 5, 11:30 - 11:45 AM

Objective

Previously, we showed that germ free mice transplanted with fecal microbiota from 3-month-old children that developed persistent eczema had higher responses to house dust mite (HDM) allergen than mice with mouse microbiota (^{Mo}microbiota). This human risk microbiota (^{Hu}microbiota) also impacted overall elastic properties of the airways in mice including resistance and elastance. We hypothesized that 1) an allergenic gut microbiome can be replaced by a protective microbiome, and 2) that, once established, this transplant could significantly improve lung function and decrease allergic responses after HDM challenge.

Methods

C57BL/6 mice carrying a human allergic risk microbiota (^{Hu}microbiota) acquired vertically from their parents were given antibiotic (ABX) treatment for 1 week, then they were either transplanted with fecal slurries from the "protective" mouse microbiota (^{Mo}microbiota) or sham inoculated with sterile saline to allow natural recovery of their gut microbiome after antibiotics were removed. Four weeks after transplant, all mice except for saline controls were given HDM allergen and tested for lung function and allergic airway responses. Six experimental groups were used: 1) ^{Mo}microbiota (no ABX, no transplant, saline), 2) ^{Mo}microbiota (no ABX, no transplant, HDM), 3) ^{Mo}microbiota (ABX, no transplant, HDM), 4) ^{Hu}microbiota (no ABX, no transplant, saline), 5) ^{Hu}microbiota (ABX, no transplant, HDM), and 6) ^{Hu}microbiota (ABX, ^{Mo}microbiota transplant, HDM).

Results

The protective ^{Mo}microbiota transplant successfully replaced the ^{Hu}microbiota risk microbiome. Lung function was improved in mice given the protective FMT. Protective FMT decreased the total number of inflammatory cells in the lung, but not the proportions. IgE levels were not significantly decreased in mice given protective FMT.

Conclusions

Thus, lung function and inflammatory indices responded differently to the presence of a protective FMT.

Notes:

16 - Intramammary lipopolysaccharide infusion alters the fatty acid profile in blood, but not in milk, in dairy cows

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Session: Pathogen-Host Interaction, Dec. 5, 11:45 - 12:00 PM

Objective

Mastitis is a costly disease in lactating dairy cattle, causing milk yield and quality losses and can adversely affect cow health. We hypothesized that intramammary lipopolysaccharide (LPS) infusion would alter the blood and milk lipid profiles of dairy cows. This study aimed to determine the changes in fatty acid compositions in the blood and foremilk of dairy cows challenged with intramammary LPS.

Methods

Ten cows were paired to two groups. The treatment group (T) received intramammary infusions of 50 µg *Escherichia coli* LPS in saline per quarter in one udder half and saline alone in quarters of the other half. The control group (C) received intramammary infusions of saline in quarters of one udder half and received no treatment in the other half. Foremilk and blood were collected from each gland and cow, respectively, at -1, 3, 6, 12 and 24h post-infusion. Blood lipids were separated into cholesterol, free fatty acids, phospholipids and triacylgycerides (TAG), and the fatty acid compositions of each fraction and of foremilk were analyzed. Statistical significance was determined using mixed models with Tukey's test.

Results

Principal component analysis showed that the fatty acid profiles of blood phospholipids and TAG started to diverge in T and C cows at 6h, but re-clustered together at 24h only for TAG. Specifically, at 6h, polyunsaturated fatty acids, mainly C18:2 9c, 12c, were more abundant in TAG fraction of T cows, along with C18:1 9c and C16:0, whereas there was a decrease in C18:0 percentage ($p<0.05$). In the phospholipid fraction, percentages of C16:0, C18:0 and C18:2 9c, 12c were decreased and C22:0 was increased ($p<0.05$). The blood TAG concentration did not vary between groups nor did the fatty acid composition of foremilk between treatments.

Conclusions

These findings showed that intramammary LPS alters the fatty acid profiles of TAG and phospholipid fractions in blood, without affecting the fatty acid profiles of foremilk, indicating that the impact of mastitis on lipid profile in the blood does not extend to the milk in the first 24h post LPS infusion.

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

Notes:

17 - Characterization of monoclonal antibodies against SARS-CoV-2 nucleocapsid protein: Implication in diagnostic assays for animal surveillance

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Session: Diagnostic Testing - 1, Dec. 5, 8:30 - 8:45 AM

Objective

The global pandemic of coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), poses a significant threat to public health. Besides humans, SARS-CoV-2 can infect certain animal species, including wild/domestic cats and white-tailed deer. Highly sensitive and specific diagnostic reagents and assays are urgently needed for rapid detection and implementation in quarantine and elimination of infected animals. The objective of this study is to develop and characterize a panel of specific monoclonal antibodies (mAbs) for SARS-CoV-2 diagnostics and surveillance in animals. An mAb-based ELISA (bELISA) was developed, which is designed to be applicable for testing the serum antibody response in the samples from all animal species and human.

Methods

In this study, recombinant protein of SARS-CoV-2 nucleocapsid (N) was expressed as the antigen for mice immunization, and splenocytes from immunized mouse were fused with mouse myeloma cells to generate hybridomas. SARS-CoV-2 specific mAbs from hybridoma culture were initially screened using in vitro expression system and then confirm the reactivity in SARS-CoV-2-infected cells. They were further characterized by various assays, and tested against the variants of SARS-CoV-2. To explore the potential use of mAbs for serological diagnostics, mAb #94-5-based bELISA was developed. The biotinylated mAb serves as the detection antibody after incubation of serum samples with immobilized N antigen. The bELISA conditions were optimized using internal control standards of negative serum from healthy cats and positive serum with spiked anti-N antibodies. Initial test validation was performed using 419 serum samples from uninfected domestic cats and 19 serum samples from white-tailed deer experimentally inoculated with SARS-CoV-2. Optimal cut-off value with best diagnostic sensitivity and specificity were determined by receiver operating characteristic (ROC) analysis.

Results

A panel of monoclonal antibodies against SARS-CoV-2 N protein was generated and characterized by various assays with SARS-CoV-2 from different lineages, including immunofluorescent assay, western-blot, immunoprecipitation, and ELISA. Epitope mapping identified specific epitopes localized in different regions, among which mAb #94-5 recognizes immunodominant C terminal of N protein. The mAb #94-5-based blocking ELISA was developed for serological test. The coefficient of variation of the internal quality control serum were determined as below 10%, including between-runs (8.65%), within-run (7.89%), and within-plate (7.42%). Initial test validation on 481 serum samples from uninfected domestic cats and 19 serum samples from white-tailed deer experimentally inoculated with SARS-CoV-2 revealed an optimal percentage of inhibition (PI) cut-off value as 49.26% with a diagnostic sensitivity of 94.7% and diagnostic specificity of 97.1%.

Conclusions

The panel of mAbs generated in this study provide valuable tool for SARS-CoV-2 diagnostics and research. The mAb-based bELISA has potential to be used as a serological test in aid of COVID-19 surveillance in animals.

Financial Support
University of Illinois; Cornell University

Notes:

18 - Rules for the pool: Optimizing testing for populations and individuals

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Session: Diagnostic Testing - 1, Dec. 5, 8:45 - 9:00 AM

Objective

Pooled testing can increase the efficiency of detecting cases of low prevalence diseases. Two-stage hierarchical strategies are most commonly used, but generally without optimization. More advanced strategies, such as array or with more stages, may enhance the accuracy and efficiency of case identification. Methods can account for testing error and detection of more than one pathogen, i.e. multiplex PCR. For surveillance, pooled prevalence estimation can be optimized, without case identification, allowing screening of more and diverse animals for the same cost. Greater awareness of pooling options and access to optimization software will allow epidemiologists and diagnosticians to select the best blend of efficiency, precision, and cost-effectiveness for disease surveillance.

Methods

Samples were collected from Virginia market cattle. Whole blood was individually tested with a duplex qPCR for *Theileria orientalis* (sensitivity and specificity, 100%) and *Anaplasma marginale* (sensitivity 96.97%; specificity, 100%). Prevalence of each pathogen was determined for singular and co-infections. Maximum pooling limit was based on the distribution of Ct values. The most effective pooling strategies for individual case identification and prevalence estimation were determined, based on minimizing (a) the average number of tests, (b) mean squared error, and (c) cost per unit information. Measures of optimality were calculated numerically using large-sample simulation and maximum likelihood estimation.

Results

Optimizing hierarchical pooling, with both pathogens present at about 8%, and a maximum pool of 10, the optimal strategy was 2-levels with a pool size of 3. For prevalence, based only on initial pools, optimal pool size was either 2, 3, 4, or 5 depending on which criterion was emphasized. Using this approach, prevalence for theileriosis and anaplasmosis were simultaneously estimated at 65% or more savings in testing cost without loss of precision.

Conclusions

Optimizing pool sizes and strategies, aided by user-friendly R programs, allows diagnosticians to improve detection and surveillance for disease.

Notes:

19 - Development of a PCR assay to quantify *Fusobacterium necrophorum* subsp. *necrophorum* and *funduliforme* in rumen fluid

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Session: Diagnostic Testing - 1, Dec. 5, 9:00 - 9:15 AM

Objective

Fusobacterium necrophorum, a Gram-negative anaerobe and a ruminal bacterium, is the primary causative agent of liver abscesses in feedlot cattle. Two subspecies, subsp. *necrophorum* and subsp. *funduliforme* have been described. Subsp. *necrophorum* is more frequently involved in liver abscesses than subsp. *funduliforme*. Although PCR assays have been developed for the quantification of *F. necrophorum*, none exists to quantify at the subspecies level. Therefore, our objectives were to develop and validate a quantitative PCR assay for the quantification of the species and the two subspecies of *F. necrophorum* in rumen fluid of cattle.

Methods

The assay targeted the species-specific gyrase subunit B gene, *gyrB*, and subspecies-specific promoter sequence of the leukotoxin gene, *lktA*. Assay specificity was validated with *F. necrophorum* strains and other *Fusobacterium* species. Assay sensitivity was determined with pure cultures and rumen fluid spiked with pure cultures of the two subspecies. The applicability of the assay to quantify *F. necrophorum* was determined with rumen fluid collected from slaughtered cattle.

Results

Species- and subspecies-specificities of the assay were confirmed. The detection limits of pure cultures were 7.6×10^3 CFU/ml for the species, 1.4×10^4 CFU/ml for subsp. *necrophorum* and 2.5×10^4 CFU/ml for subsp. *funduliforme*. In spiked-rumen fluid, the detection limits were 2.3×10^4 CFU/ml, 2.0×10^4 CFU/ml, and 1.9×10^4 CFU/ml, respectively. Application of the assay to ruminal fluid collected at slaughter from cattle with healthy liver (n=14) and from cattle with abscessed liver (n=10) indicated that all the samples tested were positive subsp. *funduliforme* with mean concentrations of 1.9×10^5 and 1.3×10^5 CFU/ml, respectively. Two of the 14 samples (14.3%) from cattle with healthy livers contained subsp. *necrophorum* (mean 2.8×10^4 CFU/ml) compared to 5 of 10 samples (50%) from cattle with abscessed livers (mean 3.8×10^4 CFU/ ml).

Conclusions

In conclusion, the novel assay allowed differentiation and quantification of the *F. necrophorum* subspecies in rumen fluid of cattle.

Financial Support
Micronutrients Inc.

Notes:

20 - Comparison of sampling and diagnostic techniques for recovery of *Mannheimia haemolytica* from feedlot cattle

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Session: Diagnostic Testing - 1, Dec. 5, 9:15 - 9:30 AM

Objective

Bovine respiratory disease (BRD) is caused by interactions between host, environment, and pathogens. The current standard for antemortem pathogen identification is deep-guarded nasopharyngeal swabbing, which is challenging, costly, and waste generating. The objective was to compare recovery rates of *Mannheimia haemolytica* (MH) by culture and real time-quantitative PCR (qPCR) using deep-guarded nasopharyngeal swabs (DG), 16-inch proctology swabs (PS), or 6-inch nasal swabs (NS).

Methods

Samples were collected from beef steers and bulls 14 days after arrival at a feedlot after being purchased from an auction market (n=120, mean weight=262.2 ± 12.5 kg). One nostril was sampled with each swab type for culture, MALDI-TOF-MS identification-genotyping, and antimicrobial susceptibility testing by broth microdilution. The other nostril was sampled for qPCR for the MH leukotoxin D gene (lktD).

Results

Culture yielded MH from 56% (201/360) of swabs (67 DG, 66 NS, 68 PS); all isolates were Genotype 2. Nearly all isolates (198/201) were resistant to ≥2 drug classes (MDR); 3 were pansusceptible to tested drugs. There was complete concordance in culture results for the 3 sampling methods for 77% of cattle (92/119); two concordant positive and 1 discordant negative result was found in 11% of cattle (13/119), and 1 discordant positive result was identified in the remaining 12% of cattle (14/119). Frequency of MH isolation and recovery of MDR isolates was not statistically different between swab types ($P>0.05$, McNemar's Chi-square). qPCR was positive for 75 % (268/356) of swabs (78 DG, 94 NS, 96 PS). Identification by qPCR were significantly different by swab type, with fewer DG positive compared to NS and PS ($P<0.05$, McNemar's Chi-square).

Conclusions

Different swab types provided comparable results for MH culture. Sensitivity of qPCR was greater than culture for all swabs, though somewhat lower for DG. Nasal swabs and proctology swabs provided comparable culture results and more sensitive qPCR results compared to DG with greater technical ease and less cost and waste than guarded nasopharyngeal swabs.

Financial Support
Texas A&M University

Notes:

21 - Whole genome sequencing and genotyping of *Mycobacterium bovis* directly from clinical tissue samples in research and diagnostics

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Session: Diagnostic Testing - 1, Dec. 5, 9:30 - 9:45 AM

Objective

To date, the genome of *M. bovis* could only be sequenced if the mycobacteria were cultured from the tissue. This requirement was due to the overwhelming amount of host DNA present when DNA was isolated directly from a granuloma. To overcome this formidable hurdle, we evaluated the usefulness of an RNA-based targeted enrichment method to directly sequence *M. bovis* from tissue samples. This system enriches target *M. bovis* DNA using custom biotinylated RNA probes that are designed to capture the whole *M. bovis* genome.

Methods

Initial validation experiments employed DNA from tissue samples spiked with 10^{-1} to 10^{-4} *M. bovis* BCG DNA. Next, the technique was used to sequence *M. bovis* from tissue samples from naturally infected animals with variable Ct values.

Results

Four replicate experiments achieved 99.1±0.07 % genome coverage (at 108±33.5X depth of coverage) and 98.8±0.1 % genome coverage (at 26.4±18.3X depth of coverage) for tissue samples spiked with BCG DNA at 10^{-1} (Ct: 20.3±0.6) and 10^{-2} (Ct: 22.9±0.3), respectively. Tissue samples spiked with BCG DNA at 10^{-3} (Ct: 28.2±0.9) and 10^{-4} (Ct: 30.2±0.4) achieved only 58.9±9.15 % genome coverage (at 1.4±0.4X depth of coverage) and 15.4±5.09 % genome coverage (at 0.2±0.09X depth of coverage), respectively.
The *M. bovis* genomes from all naturally infected tissue samples were successfully sequenced with mean genome coverage of 99.6% and mean depth of coverage of 18.35X. *M. bovis* was grown from the same tissues and the genomes sequenced. The genotyping information derived from sequencing DNA direct from the tissue samples matched that of the cultured isolates from the same sample.

Conclusions

We show that direct sequencing of tissue samples has the potential to provide *M. bovis* genotyping in one week which is significantly faster than whole-genome sequencing from cultures in research and diagnostic settings. We are currently working on workflow optimization to increase enriching *M. bovis* when fewer mycobacteria are present in the tissues.

Notes:

22 - Sources of variation in a commercial bovine immunoglobulin G radial immunodiffusion assay

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Session: Diagnostic Testing - 1, Dec. 5, 9:45 - 10:00 AM

Objective

The objective of this study was to determine the magnitude of variation in a commercial bovine immunoglobulin G (IgG) radial immunodiffusion (RID) assay.

Methods

Six calf sera were repeated 7 times on 4 plates from 2 test kit lots (28 observations per serum). Three standard sera of known IgG concentration were measured across 69 plates and 5 lots (75 observations per standard). After 24 hours, precipitin ring diameters were measured. IgG concentrations were calculated using a linear and logarithmic equation generated using all standards performed on the same day. Levene's test was used to test homogeneity of variance between serums.

Results

No difference in standard deviation (SD) for diameters of serum or standards was detected (serum SD=0.25mm, p=0.34; standard SD=0.25mm, p=0.14). No difference in the SD for IgG concentrations based on the linear equation was detected (212mg/dL, p=0.34) across serums. Based on the linear IgG concentration equation, 95% of samples for a given serum would have values expected to fall over a range of 848 mg/dL. The SD for IgG based on the linear equation differed across standards (p=0.0001). The logarithmic equation did not perform as well because 95% of the measured values of the middle standard fell between 1,536 and 1,968 mg/dL which did not encompass the known value (1,472 mg/dL).

Conclusions

The assay performed within a range of variance expected for RID assays. However, the test would allow misclassification of failed transfer of passive immunity status for calves with true IgG concentrations within 848 mg/dL of the cut-off point.

Financial Support
House officer grant, Office of Research and Graduate Studies, College of Veterinary Medicine, Mississippi State University

Notes:

23 - The role of the conserved alphaherpesvirus glycoprotein C in host-to-host transmission

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Session: Virology - 1, Dec. 5, 10:45 - 11:00 AM

Objective

Transmission from host-to-host is an essential component in the herpesvirus' life cycle. 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.

Methods

We will use chicken MDV, infectious laryngotracheitis alphaherpesvirus (ILTV), *Gallid alphaherpesvirus* 3 (GaHV3) (chicken), and turkey alphaherpesvirus (HVT) gC in our host-to-host transmission models to test the ability of mutant viruses to spread from bird-to-bird. We will also determine the role gC homologs play during replication in skin, the tissue in which human and avian alphaherpesviruses often disseminate into the environment. We have established that MDV expresses 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.

Results

We deleted gC in GaHV3 strain 301B and tested the gC-null virus in our natural infection model. Consistent with the essential role of MDV gC, 301B/1 gC was also required for natural infection of 301B/1 in chickens. Additionally, replacement of MDV gC in gC-null 301B/1 rescued this defect showing MDV gC can compensate for 301B/1 gC.
We also tested the ability of HVT to transmit in chickens and found that though HVT replicated efficiently in chickens when experimentally infected, it was unable to transmit to sentinel chickens. We also tested whether HVT expressing MDV gC would lead to transmission, but this virus also did not spread to sentinel contact chickens.
Studies examining the role VZV gC plays during infection were performed in cell culture and SOCs. VZV lacking gC replicated significantly less in SOCs indicating its role in infection of skin cells. In cell culture, VZV lacking gC replicated as well as wildtype in MeWo cells, but lacked the ability to generated infectious cell free virus. Thus, these results indicate VZV gC is required for cell free virus infectivity.

Conclusions

Our results suggest that alphaherpesvirus gC proteins did not necessarily evolve dependent on the host and is most likely evolved based on cellular tropism and pathogenesis. Importantly, we identified that MD vaccine 301B/1 required gC for transmission.

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

Notes:

24 - Marek's disease alphaherpesvirus UL13, virion protein US10, and cellular LY6E in horizontal transmission

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Session: Virology - 1, Dec. 5, 11:00 - 11:15 AM

Objective

We have identified potential targets for UL13 in horizontal transmission, specifically virion protein unique short (US) 10 (US10) and cellular lymphocyte antigen 6E (LY6E), thought to be involved in enhancing virus infection. Our objective is to delineate the mechanistic importance of UL13 in transmission through US10 and LY6E. This project is funded through the USDA-NIFA-AFRI grant no. 2020-67015-21399.

Methods

In Specific Aim 1, we will use recombinant MDV expressing fluorescent proteins and epitope-tagged viral proteins to study the importance of US10 in transmission and the interactions of US10 and LY6E using immunoprecipitation assays. In Specific Aim 2, we will sequence the LY6E gene from chickens genetically resistant or susceptible to MD and determine whether LY6E is incorporated into the MD virion using virus extraction and western blotting.

Results

Using an epitope-tagged US10 virus, we determined that US10 expression was completely absent in chickens infected with a UL13-mutant virus suggesting US10 is required for horizontal transmission. Therefore, we generated a mutant virus in which US10 was deleted from the viral genome and tested horizontal transmission. Interestingly, the US10-null virus transmitted like wildtype, showing that US10 is not required for transmission contrary to our original hypothesis.
Pure Columbian (PC) chickens are highly susceptible to MD, while white Leghorn (WL) chickens are more resistant. LY6E has been formally linked to genetic resistance of chickens; therefore, we sequence the LY6E genes from these chicken lines. Differences were identified, including regions in the 3' untranslated region of the genes formerly shown to be linked to genetic resistance.

Conclusions

Our current results show that US10 is not required for horizontal transmission of MDV, contrary to our original hypothesis. Thus, the abrogated expression of US10 in the UL13-mutant virus does not link US10 to the requirement of UL13 in horizontal transmission. Further studies are required to address the functional importance of UL13 during horizontal transmission including the potential role LY6E may play in this interaction.

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

Notes:

25 - Temperature-induced reactivation of Marek's disease alphaherpesvirus transformed CD4+ T cells

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Session: Virology - 1, Dec. 5, 11:15 - 11:30 AM

Objective

Marek's disease alphaherpesvirus (MDV) infects T cells in chickens where it integrates into the host telomeres and establishes latency. Latency is important for survival of MDV in the host to evade host immunity. However, MDV can reactivate during the life of the host due to numerous factors such as stress and immune suppression. The switch of reactivation is essential for MDV to replicate and transmit to another host. However, factors contributing to reactivation are not well understood. Here, we developed a mild but robust method for inducing reactivation of MDV-transformed cell cultures (MDCCs). Utilizing MDCCs, we established a reactivation assay *ex vivo* that can be utilized in future studies to examine the latency to lytic switch. The main objective of this project is to validate a novel temperature sensitive reactivation assay of MDCCs that is natural and less toxic than tradition drug treatment methods. The long-term goal is to utilize this system to understand cellular and viral genes involved in the latent to lytic switch in cells.

Methods

In order to easily identify reactivation of MDV in MDCCs, we used recombinant MDV engineered to express monomeric red fluorescent protein (mRFP) fused to the RLORF4 protein that has formerly been shown to be expressed during early lytic replication. This give us the ability to examine lives cells for RLORF4mRFP expression using immunofluorescence assays (IFAs), flow cytometry, and fluorescence-activated cell sorting (FACS). We utilized established reactivation assays in cell culture, as well as measure stages of virus replication during various cell treatments using flowcytometry, RT-PCR and western blot assays, as well as apoptosis assays to evaluate the degree of cell death induced by each treatment.

Results

We have established thirteen MDCCs from Pure Columbian (PC) chickens and characterized them *ex vivo*. Two MDCCs were used in our studies and analyzed at 27- and 60-weeks *ex vivo*. To validate these MDCCs reactivate MDV, they were seeded onto chicken embryotic cells (CECs). Our results showed an average of 4.9 ± 0.8 and 12.8 ± 5.5 PFU per 10,000 MDCCs after 27 weeks in culture. However, by 60 weeks in culture, the number of PFU was reduced to 0.3 ± 0.1 to 0.43 ± 0.3 PFU per 10,000 cells suggesting time in culture effects the ability of MDV to reactivate from MDCCs.

To test temperature-induced reactivation, we incubated MDCCs at 32°C for 8 to 24 h, while untreated MDCCs were kept in normal culture temperature of 41°C. Sodium butyrate was used at 3 μM as a positive control. Our results showed that both temperature-induction and sodium butyrate increased RLORF4mRFP+. However, analysis of live versus dead cells in each group showed there was a significant increase in dead cells in the sodium butyrate treatment group compared to untreated and temperature treatment groups. Analysis of apoptosis using the Annexin-V staining assay showed that among the RLORF4mRFP+ cells, there was a significant increase in necrosis and apoptosis in the sodium butyrate treatment group compared to both other groups.

Conclusions

Our results show that that low temperature can reactivate MDV in MDCCs as efficiently as drug treatment, but without the significant toxicity and apoptosis associated with non-specific drug treatment. In addition, temperature treatment is more physiologically relevant and may provide clearer answers to how cells and herpesviruses control the latent-lytic switch.

Notes:

26 - Experimental SARS-CoV-2 transmission and re-infection in cats

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Session: Virology - 1, Dec. 5, 11:30 - 11:45 AM

Objective

SARS-CoV-2 is the causative agent of COVID-19 and responsible for the current global pandemic. In addition to humans, SARS-CoV-2 can infect several animal species including felines. In the presented studies, we conducted in-depth investigations of SARS-CoV-2 infection, disease, transmission and re-infection in domestic cats.

Methods

In two independent studies, cats were challenged with SARS-CoV-2 via intranasal/oral routes. At 1-day post challenge (DPC), 2 naïve sentinels were introduced. All cats were monitored for clinical signs, viral shedding and antibodies. Necropsies were performed at 4, 7, 21 DPC and tissues examined for gross and histologic lesions as well as the presence of viral RNA. To study re-infection, cats recovered from SARS-CoV-2-infection were re-challenged with SARS-CoV-2 at 21 DPC, and two naïve sentinels introduced at 1-days post-secondary challenge (DP2C). Clinical signs, viral shedding and antibody responses were evaluated, and necropsies performed at 4, 7 and 14 DP2C.

Results

Viral RNA was transiently shed via the nasal, oropharyngeal and rectal cavities of primary infected cats and sentinels. Both primary challenged cats and sentinels developed neutralizing antibodies. Viral RNA and antigen were detected in the respiratory tract of the primary SARS-CoV-2-infected cats at early DPCs but was absent at 21 DPC. Following re-infection, viral RNA was transiently shed via the nasal, oropharyngeal and rectal cavities of re-challenged cats; viral antigen was absent in the respiratory tract at all DPCs. Naïve sentinels co-housed with the re-challenged cats did not shed viral RNA or seroconvert.

Conclusions

Together, our results indicate that cats are highly susceptible to experimental SARS-CoV-2 infection, and capable of transmitting SARS-CoV-2 to contact sentinels. Cats previously infected with SARS-CoV-2 can be experimentally re-infected; however, the levels of virus shed was insufficient for transmission to co-housed naïve cats. We conclude that SARS-CoV-2 infection in cats induces immune responses that provide partial, non-sterilizing immune protection against re-infection.

Notes:

27 - NF-κB activation and proinflammatory cytokine productions by ORF7a protein of SARS-CoV-2

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Session: Virology - 1, Dec. 5, 11:45 - 12:00 PM

Objective

The novel coronavirus disease emerged in 2019, and SARS-CoV-2 was identified as the etiologic agent for COVID-19. According to the data from the GISAID, four major clades of SARS-CoV-2 have been identified and subsequently named clade L (prototype virus Wuhan-Hu-1), clade G (D614G variant of the spike protein), clade V (G251V variant of ORF3a), and clade S (L84S variant of ORF8). In April 2020, five tigers and three lions at the Bronx zoo tested positive for COVID-19. By sequencing, the viruses infecting tigers and lions belonged to different clades. ORF3a sequences from affected tigers belong to the clade L, ORF3a from affected lions belong to clade V. Severely ill COVID-19 patients exhibit the elevation of proinflammatory cytokines, and such unbalanced hyperproduction of cytokines is linked to acute respiratory distress syndrome. In the present study, we aimed to identify the molecular mechanism of SARS-CoV-2 proteins that modulated the inflammatory cytokine expressions.

Methods

SARS-CoV-2 viral protein coding sequences for ORF3a, M, ORF7a, and N were individually cloned and expressed in the HeLa, A549, or 16HBE14o cells. The luciferase reporter assays were conducted to examine viral modulation of NF-κB pathways. RT-qPCR was used to determine mRNA transcriptions for various cytokines and chemokines in viral gene expressing cells.

Results

The SARS-CoV-2 ORF3a, M, ORF7a, and N proteins were NF-κB activators. Comparing the clade L and clade V ORF3a, G251V mutation did not change the NF-κB activation. Among the four NF-κB activators, the ORF7a protein induced the NF-κB-related proinflammatory cytokines, including IL-1α, IL-1β, IL-6, IL-8, IL-10, TNF-α, and IFNβ. The ORF7a protein also induced IL-3, IL-4, IL-7, IL-23. Of 15 different chemokines, CCL11, CCL17, CCL19, CCL20, CCL21, CCL22, CCL25, CCL26, CCL27, and CXCL9 were significantly upregulated by ORF7a.

Conclusions

This study demonstrates that four SARS-CoV-2 proteins as the NF-κB activators. Of these four, ORF7a was the most potent NF-kB inducer and thus proinflammatory cytokine producer. Our study suggests that ORF7a is one of the virulence factors for the unbalanced overproduction of inflammatory cytokines during SARS-CoV-2 infection.

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

Notes:

29 - Immunization with a mucosal, postfusion F/G protein-based polyanhydride nanovaccine protects against BRSV infection in neonatal calves

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Session: Immunology - 1, Dec. 5, 8:45 - 9:00 AM

Objective

Previously, immunization with a single intranasal dose of a post-fusion F/G nanovaccine induced partial protection from a BRSV challenge in calves with preexisting maternal antibodies (McGill et al. 2019, McGill et al. 2018). Knowledge gained from these previous studies was used to optimize the nanovaccine by incorporating CpG-ODN and to investigate the impact of a prime-boost regimen using both mucosal and systemic vaccination routes.

Methods

36 neonatal, mixed-sex, Holstein calves were divided into 6 groups. Group 1 received a mucosal prime/systemic boost of saline and group 2 a mucosal prime/mucosal boost of the 'empty' nanovaccine CPG. Groups 3 and 4 received a mucosal prime/mucosal boost of the BRSV post-fusion F/G nanovaccine ± CpG, while groups 5 and 6 received a mucosal prime/systemic boost of the nanovaccine ± CpG. Vaccine-induced immune responses were monitored and 6 weeks after the booster, all calves were challenged via aerosol with BRSV. Nasopharyngeal swabs were collected for viral shedding and calves monitored for clinical signs and euthanized on day 7 after infection. Lungs were scored for gross pathology. Blood, nasal secretions and lung tissue samples were analyzed for BRSV specific immune responses.

Results

Both heterologous and homologous immunization schedules induced potent virus-specific IgA responses in the nasal and lung mucosa. Calves that received the vaccines incorporating the CpG adjuvant had significantly less lung pathology and less viral burden in the lungs compared to nonvaccinated control calves.

Conclusions

These results indicate that vaccination with the post-fusion F/G + CpG nanovaccine can induce virus-specific IgA in the mucosa and protect neonatal calves from BRSV infection.

Financial Support
U.S. Department of Health and Human Services; U.S. National Institute of Child Health and Human Development

Notes:

30 - Modulation of inflammatory IL-17A signaling affects *Mannheimia haemolytica* pathogenesis

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Session: Immunology - 1, Dec. 5, 9:00 - 9:15 AM

Objective

Mannheimia haemolytica (MH) is a common commensal bacteria found in the nasopharynx of healthy cattle, where it is generally well contained and immunologically tolerated. Following immunological stressors, MH can migrate into the lungs and develop into a lower respiratory tract infection or pneumonia. Severe MH infections are often characterized by cytotoxic neutrophil infiltration into the lungs, and pathogenesis is further exacerbated by inflammatory IL-17A signaling that polarizes and activates other immune cells into a pro-inflammatory state. Therefore, to better understand how IL-17A signaling may be impacting immune responses and disease severity following MH infection, this study employed the use of an IL-17 inhibitor, ursolic acid (UA).

Methods

Two independent experiments were performed using 4-week old male Holstein calves. Study 1 included 32 animals that were divided into four treatment groups: non-challenged and untreated, MH-challenged and untreated, non-challenged and UA treated, or MH-challenged and UA treated. Study 2 included 16 animals that were divided evenly into two MH-challenged groups with or without UA treatment. Serum samples, whole blood, and nasal swabs were collected throughout the course of the study, while bronchoalveolar lavage fluid, tissue sections, and lung isolates were collected at the time of necropsy. Lung tissue samples were analyzed for expression of inflammatory markers using RT-qPCR.

Results

UA treatment appears to modulate inflammatory IL-17A signaling and subsequently alters disease progression following MH infection, including changes in lung pathology, decreased bacterial burden, increased expression of innate defensins, and changes in genes associated with lung-tissue remodeling.

Conclusions

Modulating inflammatory IL-17A signaling may reduce disease severity following MH infection. However, dampening protective inflammatory responses can have both beneficial and deleterious effects for the host, so additional studies are needed to better understand the effects of this altered signaling.

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

Notes:

31 - The use of blood myeloid and lymphoid cell profiles to predict metritis in dairy cows

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Session: Immunology - 1, Dec. 5, 9:15 - 9:30 AM

Objective

The objective was to evaluate the peripheral blood myeloid and lymphoid cell profile of cows at calving as predictors of metritis.

Methods

Holstein cows (n=102) had blood collected at calving. Parity, gestation length, bodyweight change (BWC) prepartum, body condition score (BCS), and temperature were recorded. Cows having dystocia, twins, stillbirth, vaginal laceration, or retained placenta were classified as having a risk factor for metritis. Cows with a red-brownish, watery, fetid vaginal discharge were diagnosed with metritis. Flow cytometry was used to evaluate the percentage of myeloid and lymphoid cells, and extracellular markers of myeloid and lymphoid cell adhesion and activation. Cell markers for monocytes (CD172a+/CD14+), granulocytes (CD172a+/CD14-), B cells (MHC2+/CD21+), T-helper cells (CD4+), cytotoxic T cells (CD8+), and gamma delta T cells (γδTCR+) were evaluated. CD62L- and CD11b+ were markers of cell activation. Data were analyzed by logistic regression, and the model included parity, risk factor for metritis, BWC, and immune markers with $P \leq 0.2$.

Results

Live cell%, activated Granulocytes, Monocytes, and B cells were predictors of metritis, in addition to parity, risk factors, and BWC. For every 1-unit increase in the live cell%, the odds of developing metritis tended to decrease by 85% (OR = 0.54; 95% CI = 0.25, 1.12; P = 0.11). For every 10-unit increase in PMNCD62L-% the odds of developing metritis tended to increase by 40% (OR =1.40; 95% CI = 0.96, 2.12; P = 0.09). For every 100-unit increase in the MøCD11bMFI, the odds of developing metritis tended to increase by 8% (OR = 1.08; 95% CI = 1.01, 1.18; P = 0.06). For each 10-unit increase in the BcellsCD62L-% the odds of developing metritis increased by 2.5-folds (OR =2.52; 95% CI = 1.29, 5.55; P = 0.01). The model including parity, risk factor, BWC and immune markers had Se of 69%, Sp of 92%, PPV of 90%, NPV of 75%, AUC of 87%, and overall accuracy of 80%.

Conclusions

In summary, markers of live cells, granulocyte, monocyte, and B cell activation were predictors of metritis.

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

Notes:

32 - Regulation of bovine Th2 differentiation in vitro

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Session: Immunology - 1, Dec. 5, 9:30 - 9:45 AM

Objective

The helper T cell subtype-2 (Th2) cell is critical for controlling the extracellular pathogens including most parasites, which cause billions of dollars in losses annually in the cattle industry. It is unclear if bovine CD4+ T cells differentiate into effective Th2 cells in vitro in a way similar to mice and humans, which is important information to understand how to induce appropriate Th2 responses to control extracellular pathogens in cattle.

Methods

Naïve CD4+ T cells, sorted from PBMCs of grass-fed beef cattle, were stimulated with anti-bovine CD3 under Th2 differentiation conditions with or without *Ostertagia ostertagi* (OO) protein extract. Th1 differentiation conditions were included for comparison. The effect of recombinant bovine IL-4 (rbIL-4) and weakening TCR signal strength on Th2 differentiation were also tested. Flow cytometry, qPCR, and proteomic assay were performed to analyze the differentiated cells.

Results

The majority of differentiated cells expressed IFNγ (a hallmark cytokine for Th1) and a small percentage of cells expressed both IFNγ and IL-4 (a hallmark cytokine for Th2), indicating the presence of Th0 cells, as previously reported in cattle. While weakening TCR signal strength reduced Th2 cell expansion, adding rbIL-4 or OO protein extract enhanced Th2 cell expansion but without significant changes in IFNγ and IL-4 expression. Proteomic data predicted that, as in mice and humans, bovine Th2 differentiation results from three upstream stimulation with CD3, CD28, and IL-4, which were inhibited when OO protein extract was added into the Th2 differentiation media. Importantly, protein profiling indicated that the addition of rbIL-4 enhanced IL-4 signaling but inhibited IL-12 signaling. We are planning to explore if other cytokines like IL-10 can be applied to optimize this Th2 differentiation soon.

Conclusions

Naïve bovine CD4+ T cells differentiate into a mixed population containing a high percentage of IFNγ producing cells and a small percentage of Th0 cells. Furthermore, bovine Th2 differentiation is sensitive to regulation such as by OO or rbIL-4.

Financial Support
U.S. Department of Agriculture

Notes:

33 - Oleic acid improves insulin sensitivity in dairy cow adipose tissue through PPARα and PLIN5

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Session: Immunology - 1, Dec. 5, 9:45 - 10:00 AM

Objective

Excessive adipose tissue (AT) lipolysis around parturition in dairy cows is associated with impaired AT insulin sensitivity (IS) and increased incidence of metabolic diseases. We have previously demonstrated that supplementing oleic acid (OA) can promote lipid accumulation in AT. In the liver, OA promotes lipid droplet formation by activating PPARα and perilipin 5 (PLIN5), however it is unknown if this mechanism occurs in AT. We hypothesize that OA promotes lipogenesis and enhances IS in AT of periparturient dairy cows by signaling through PLIN5 and activating PPARα.

Methods

Multiparous Holstein cows (n=12) were infused abomasally following parturition with 60 g/d of saline (CON) or OA for 14d. Intravenous glucose tolerance test was performed on d14. Subcutaneous AT samples were obtained at 11±3.6d before calving (PreP), and 6±1d (PP1) and 13±1.4d (PP2) after parturition. Adipocyte morphometry was performed on H&E stained sections. Isoproterenol (ISO, 1μM) stimulated lipolysis and insulin (1μg/L) inhibition of ISO was determined using an in vitro explant culture by measuring glycerol release. PPARα and PLIN5 expression were determined by RT-qPCR and capillary electrophoresis. Statistical analyses were performed using a mixed effect linear model in JMP.

Results

Compared to CON, OA reduced plasma glucose concentration peak (188 vs 173±5.8, $P<0.001$) and the clearance rate (2.06 vs 1.89±0.04, $P<0.01$). At PP2, compared with CON, OA reduced AT response to ISO and increased AT sensitivity to insulin ($P<0.01$). Postpartum lipolysis increased the % of smallest (<3000μm^2) and reduced that of the largest (>9001 μm^2) adipocytes at PP1 and PP2 compared to PreP ($P<0.001$). Moreover, OA limited adipocyte size reduction postpartum. Compared to CON, OA tended to have a higher PPARα content at PP1 (0.006 vs 0.002±0.001; $P<0.10$). Additionally, OA increased PLIN5 protein expression at PP2 compared to CON (0.007 vs 0.002±0.001; $P<0.05$).

Conclusions

Our results provide initial evidence that OA may limit lipolysis by improving IS and enhancing lipogenesis through the activation of PPARα in a PLIN5 dependent manner.

Notes:

35 - A critical evaluation of *Mycobacterium bovis* pangenomics, with reference to its utility in outbreak investigation

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Session: Omics, Dec. 5, 10:30 - 10:45 AM

Objective

The increased accessibility of next generation sequencing has allowed enough individuals from a given bacterial species to be sequenced to describe the distribution of genes in the pangenome, without limiting analyses to genes present in reference strains. Although some taxa have thousands of whole genome sequences available on public databases, most genomes were sequenced with short read technology, resulting in incomplete assemblies. Studying pangenomes could lead to important insights into adaptation, pathogenicity, or molecular epidemiology, however given the known information loss inherent in analyzing contig-level assemblies, these inferences may be biased or inaccurate. In this study we describe the pangenome of a clonally evolving pathogen, *Mycobacterium bovis* and examine the utility of gene content variation in *M. bovis* outbreak investigation.

Methods

We constructed the *M. bovis* pangenome using 851 *de novo* assembled genomes. We tested the assumption of strict clonal evolution by studying evidence of recombination in core genes and analyzing the distribution of accessory genes among core monophyletic groups. To determine if gene content variation could be utilized in outbreak investigation, we carefully examined accessory genes detected in four *M. bovis* outbreaks.

Results

We found significant errors in both recombination detection and accessory gene classification. After accounting for these errors, we show that *M. bovis* has a much smaller accessory genome than previously described, and provide evidence supporting ongoing clonal evolution, with little gene content variation generated over outbreaks. We also identified frameshift mutations in multiple genes, including a mutation in glpK, which has recently been associated with antibiotic tolerance in *Mycobacterium tuberculosis*.

Conclusions

A pangenomic approach enables a more comprehensive analysis of genome dynamics than is possible with reference-based approaches; however, without critical evaluation of accessory gene content, inferences of transmission patterns employing these loci could be misguided.

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

Notes:

36 - Assessment of presence of a uterine microbiome at birth and at 60 days of life in cattle

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Session: Omics, Dec. 5, 10:45 - 11:00 AM

Objective

The time of establishment of the uterine microbiome in cattle is not known. The objective of this project is to determine when the uterine microbiome is established in cattle.

Methods

Holstein heifers were euthanized 4.6 ± 2.3 hours after birth (n=14) and at 62.9 ± 1.5 days of age (n=14) via non-penetrating captive bolt and exsanguination. The uterus, vagina, and vulva were aseptically excised and tissue samples were cultured in BHI broth, Brucella and Mycoplasma agar in aerobic and anaerobic conditions. Swabs of the uterine serosa were taken as negative controls. Positive cultures were speciated by 16S rRNA gene sequencing. Quantitative PCR was performed to quantify bacterial DNA presence in the sampled tissues. Data were log10 transformed prior to performing two-way mixed ANOVA to compare bacterial copy number differences between the groups.

Results

Only 1 Day-60 uterine sample and 1 Day-60 vaginal sample were culture-positive for bacterial growth of *Staphylococcus simulans* and *Corynebacterium glutamicum*, respectively. All vulvar samples were culture positive from which 316 isolates were identified, predominantly composed of Firmicutes, Fusobacteria, Bacteroidetes, and Actinobacteria. Bacterial DNA was identified at significantly higher abundance in the vulva (4.35±0.51, $P<0.01$) and vagina (4.21±0.13, $P=0.04$) than in the uterus (3.91±0.33). There were no significant differences in bacterial copy number per mg of tissue between Day-0 and Day-60 samples. Negative control genomic DNA concentrations were below the detection level for the Nanodrop, therefore, copy number per milligram could not be determined.

Conclusions

These results demonstrate abundant presence of bacterial DNA in the bovine uterus, vagina, and vulva immediately after birth, which persisted up to 60 d of age. Nonetheless, bacteria were consistently cultured only from the vulva. Therefore, future metagenomics-guided culture studies will be needed to confirm the presence of a uterine microbiome at birth.

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

Notes:

37 - Holstein cow genotype affects mammary skin microbiome during experimentally induced *E. coli* mastitis

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Session: Omics, Dec. 5, 11:00 - 11:15 AM

Objective

The bovine teat epithelium harbors a diverse microbial population that may influence susceptibility to invading pathogens. Previous research indicates host genetics can influence establishment and dynamics of host-associated microbiomes. Our objective was to investigate the temporal composition of the bovine teat epithelial microbiome before, during, and after intramammary *E. coli* challenge in Holstein cows with contemporary genetics, and a unique population of unselected Holstein cows with genetics preserved from the 1960s.

Methods

Five primiparous unselected and seven primiparous contemporary Holstein herdmates were subjected to the same management and production practices to reduce potential confounders. An individual sterile gauze was used to swab the distal 1/3 surface of each individual teat of each cow at -12, 0, 12, 24, 36, 48, and 72 h relative to the challenge. DNA was extracted from the individual quarter-level samples and subjected to 16S rRNA gene (v4 region) library preparation and sequencing. Amplicon sequence variants (ASVs) were identified using the DADA2 (Divisive Amplicon Denoising Algorithm) pipeline. Permutational multivariate analysis of variance (PERMANOVA) was used to assess effects of cow genotype and sampling time on the microbiome.

Results

Overall microbial composition varied throughout the sampling times and between genotypes (PERMANOVA $P < 0.05$) but remained similar across quarters (PERMANOVA $P = 0.3$). Microbial population counts were lower in unselected than in contemporary cows prior to the challenge, while *Enterobacteriaceae* family-level abundance was higher in contemporary than in unselected cows at 12 h post-challenge.

Conclusions

Results indicate mastitis susceptibility may be influenced by a complex interplay of host genetics and teat epithelial microbiome composition and highlight the need to incorporate microbiome analysis into future studies of mastitis epidemiology and pathophysiology.

Financial Support
Minnesota Rapid Agricultural Fund; Minnesota Agricultural Experiment Station, Saint Paul and a grant from the Rapid Agricultural Response Fund program of the Minnesota State Legislature and Minnesota Agricultural Experiment Station

Notes:

38 - The effect of fecal microbiota transplantation on *Campylobacter jejuni* colonization in young broiler chicks using a seeder-bird infection model

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Session: Omics, Dec. 5, 11:15 - 11:30 AM

Objective

Campylobacter jejuni, a frequent cause of foodborne illness, is commonly found in chicken flocks. Previously, we showed that the level of *Campylobacter* colonization in the ceca of broilers receiving fecal microbiota transplantation (FMT) from *Campylobacter*-free adult birds was significantly reduced compared with the control group using a direct oral challenge model, along with significant differences in the cecal microbiota compositions between the groups. The purpose of this study was to determine if a seeder-bird infection model that better mimics natural bird-to-bird transmission will produce similar results.

Methods

A fecal microbiota transplantation (FMT) experiment was performed to evaluate the effect of cecal microbiota of *Campylobacter*-negative adult broilers from a commercial farm on the colonization of young broilers by *C. jejuni*. FMT was performed on the day of hatch prior to chicks were given feed; control group received PBS orally. Two weeks after the FMT, chickens in both groups were co-mingled with *C. jejuni* positive seeder-birds (1 seeder-bird/10 chickens) and cecal contents at necropsy were collected periodically for 2 weeks to determine *Campylobacter* colonization levels via culture and for microbiota (16s rRNA gene-based) analysis.

Results

Campylobacter colonization levels in the FMT group did not differ significantly from those of the control group for any of the sampling points. Microbiota analysis indicated that the overall alpha diversity of the FMT group was noticeably higher than the control group. Significant temporal shifts in the bacterial community structure were observed throughout the experiment in the control group. The composition of the cecal microbiome was significantly different between the FMT group and the control group. Taxonomic analyses at the phyla level showed that *Firmicutes* (75.6%), *Campilobacterota* (7.5%), *Bacteroidetes* (7.4%), and *Cyanobacteria* (6.2%) were the top four most abundant taxa in the control group, while *Firmicutes* (57.3%), *Bacteroidetes* (15.6%), *Campilobacterota* (7.5%) and *Proteobacteria* (7.6%) were predominant in the FMT group.

Conclusions

FMT using the cecal contents of *Campylobacter*-free adult commercial broilers significantly affects the subsequent temporal development of the gut microbiota composition and diversity. However, FMT did not have a measurable inhibitory effect on *Campylobacter* colonization in young broilers using the seeder bird transmission model.

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

Notes:

39 - Detection of polyadenylated mRNA in *Leptospira* transcriptome using Nanopore based RNA sequencing

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Session: Omics, Dec. 5, 11:30 - 11:45 AM

Objective

Leptospira, a spirochete bacterium, causes life-threatening diseases in humans and animals. In this study, we applied Oxford Nanopore Technologies (ONT) to sequence and characterize the *Leptospira* transcriptome.

Methods

Total RNA was extracted from *Leptospira interrogans* serovar Copenhageni and was subjected to two methods, the Direct cDNA and Direct RNA sequencing protocol, followed by analysis using a suite of RNA analysis software programs.

Results

The composition of the transcriptome was dominated by several known virulence gene transcripts related to flagella and lipoproteins and hypothetical gene transcripts. Our analysis serendipitously identified the presence of Poly A tailed mRNA within the *Leptospira* transcriptome. However, the relative proportion of Poly A tailed mRNA reads was lower compared to the total transcriptome reads. The length of the Poly-A tail mapped to the reference transcriptomes as calculated by the software Tailfindr ranged from ~3- ~252bps with a median of ~23bps. Several reads of polynucleotide adenylyltransferase *PcnB* encoding for poly(A) polymerase (PAPI), a homolog of the gene which is involved in the addition of adenine bases to the 3‘end of mRNA in *E. coli*, were observed in the *Leptospira* transcriptome. Upon constructing the pangenome of *Leptospira interrogans*, we identified a coding sequence of the *PcnB* gene as a core gene, existing in all 440 *L. interrogans* genomes included in the analysis.

Conclusions

Polyadenylated mRNA is considered a characteristic of eukaryotic mRNA, and are known to improve the stability of mRNA, promoting their translation to protein. Whereas in *E. coli*, the PAPI gene adds “A” residues to the 3’ end of many RNA species targeting it for degradation, and the overproduction of such transcripts is detrimental to the survival of bacteria. To our knowledge, this is the first report to describe the presence of polyadenylated mRNA in a non-model bacterium other than *E. coli.* Further studies are underway to characterize the native RNA composition of *Leptospira* and the role of polyadenylated RNA in shaping *Leptospira* biology.

Notes:

40 - MinION-based sequencing rapidly identifies several putative new avian paramyxovirus species

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Session: Omics, Dec. 5, 11:45 - 12:00 PM

Objective

The emergence of new viruses, and the need to differentiate pathogenic from nonpathogenic (and vaccine) strains confound current PCR-based diagnostic assays. Collectively, these features create a need for virus characterization methods that efficiently classify known and unknown viruses. Wild animals are known reservoirs for RNA viruses, such as avian influenza and avian paramyxoviruses (APMVs). Avian influenza virus epidemiologic studies typically rely on egg culturing samples, followed by hemagglutination testing and then virus-specific PCRs. The objective of this study was to determine if MinION sequencing could characterize known viruses, unknown viruses, and mixed virus infections in hemagglutination-positive, influenza-virus-negative samples.

Methods

Archived egg-cultured samples from swabs of water birds in the United States were used. Total RNA was extracted from 37 egg-cultured, influenza-negative, hemagglutination-positive samples. Multiplexed MinION libraries were prepared using our previously described random strand-switching approach. Raw reads were basecalled, trimmed, demultiplexed, and taxonomically classified, and genome sequences were built using a reference-based consensus building method or a de-novo assembly on a desktop PC.

Results

Complete APMV polymerase coding sequences were obtained from 35 samples. These were speciated using the paramyxovirus ICTV phylogenetic methodology, which identified APMV-1, -4, -6, -8, and several putative novel species in the samples. Additionally, co-infections of APMVs were identified in a subset of the samples. Complete genomic coding sequences from all putative species and all other APMV species detected in this study were also obtained, allowing for analysis of the fusion gene for genotyping.

Conclusions

Specifically, this study expands the knowledge of APMV diversity. More broadly, this study demonstrates the utility of adding MinION sequencing to current cultured-based virus screening methods, which can be applied to future avian and non-avian studies to better identify and characterize known and unknown viruses.

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

Notes:

41 - Effect of antimicrobial-based anaplasmosis control on *E. coli* chlortetracycline susceptibility from pastured cattle

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Session: Antimicrobial Resistance/Use - 1, Dec. 5, 8:30 - 8:45 AM

Objective

Anaplasmosis is a hemolytic, economically important, tick-borne disease caused by *Anaplasma marginale* which can cause clinical anemia and death in cattle. Current control options are limited, and FDA-approved antimicrobial control options do not have a defined duration of use. A practical and routinely used anaplasmosis control method involves feeding free-choice chlortetracycline (CTC)-medicated mineral to pastured cattle for several months. Constant antimicrobial use poses the risk of expediting the development and dissemination of antimicrobial resistance in off-target commensal bacteria in the bovine gastrointestinal tract. The objective of this study was to determine the CTC-susceptibility of *Escherichia coli* isolated from anaplasmosis endemic beef cattle herds provided different FDA-approved free-choice CTC-medicated mineral formulations.

Methods

A closed-herd, comprised of Hereford-Angus cows, naturally endemic for anaplasmosis, were grazed in five different pastures with one herd serving as an untreated control group. The other cattle herds were randomly assigned to one of the four FDA-approved CTC-medicated mineral formulations (700, 5,000, 6,000, 8,000 g/ton) labeled for 'the control of active anaplasmosis' and provided their respective CTC-medicated mineral formulation for five consecutive months. Fecal samples were collected monthly from a subset of cows (n=6 or 10) per pasture. Fecal samples were cultured for *E. coli* isolates and the minimal inhibitory concentration of CTC determinations.

Results

Baseline CTC-susceptibility of *E. coli* was variable among all treatment and control groups. Despite prolonged administration of free-choice CTC-medicated mineral, susceptibility of *E. coli* isolates were not significantly different between study herds over the treatment period (p-value 0.075).

Conclusions

Investigation of how open-ended antimicrobial use for anaplasmosis control is important to determine if current dosing indications require refinement to reduce the potential for exacerbating antimicrobial resistance in off-target microbial species of public health concern.

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

Notes:

42 - Viable bacteria from cattle feedyard dust: Phenotypic and genotypic characterization

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Session: Antimicrobial Resistance/Use - 1, Dec. 5, 8:45 - 9:00 AM

Objective

Antimicrobial use in food animals selects for antimicrobial-resistant (AMR) bacteria, which may reach humans via the food chain, runoff, crop fertilizer, and dust. A study found AMR genes in cattle feedyards dust but didn't investigate whether those genes were in viable bacteria. Our aim was to isolate and characterize *Salmonella* and *E. coli* isolated from fugitive dust from cattle feedyards.

Methods

Six samplings have been conducted in 3 commercial feedyards in Texas. *E. coli* and *Salmonella* were isolated using CompactDry™ and selective media with and without antibiotics at CLSI/NARMS breakpoints. *Salmonella* (n=27) and *E. coli* (n=28) isolates were confirmed by MALDI-TOF and further characterized by Sensititre® to test antimicrobial susceptibility. *Salmonella* and *E. coli* serotypes, sequence types, plasmids, and antibiotic resistance genes were identified by sequencing.

Results

Fourteen *Salmonella* (51.8%) were pan-susceptible, 7 (25.9%) were resistant to 1 or 2 antibiotic classes, and 6 (22.2%) were multidrug-resistant (≥3 classes). Eight *Salmonella* sequence types (ST) were found, the most prevalent ST413 serotype Lubbock (n=7;25.9%) and ST64 serotype Anatum (n=6;22.1%). The most prevalent AMR genes in *Salmonella* were fosA7 and *tet*(A). Aminoglycosides and sulphonamides genes were also detected. One *E. coli* (3.5%) was pan-susceptible, 10 (35.7%) were resistant to 1 or 2 antibiotic classes, and 17 (60.7%) were multidrug-resistant. There were 18 STs in *E. coli,* ST278 (n=5;17.9%) and ST20 (n=3;10.7%) were the most frequent. The most common AMR genes in *E. coli* were *aph(3")-ib*, *aph(6)id*, and *tet*(A). Genes encoding amphenicols, beta-lactamases, ESBLs, phosphonic acid, macrolides, sulphonamides were also identified. In *Salmonella*, the most common incompatibility plasmid types were I1 (46%) and X1 (23.1%), while in *E. coli* were FIA (40%), FIB (64%), FICFII and Y (24%).

Conclusions

The presence of resistance determinants in viable bacteria from cattle feedyard dust was found. Further research is needed to better understand the impact beyond the feedyard of viable, AMR bacteria in fugitive bioaerosols.

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

Notes:

43 - Evaluation of *Salmonella* serovar dynamics in beef cattle and the feedlot environment using whole genome sequencing

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Session: Antimicrobial Resistance/Use - 1, Dec. 5, 9:00 - 9:15 AM

Objective

This study assesses the relatedness of *Salmonella* serovars and antimicrobial resistance (AMR) genes between isolates from the feedlot environment, cattle feces and lymph nodes. Results will provide insight on *Salmonella* dynamics and the effectiveness of environmental feedlot interventions to mitigate *Salmonella* in beef cattle and therefore beef products.

Methods

Beef cattle (n=360) and feedlot pens (n=30) were sampled on a monthly basis from June-December 2019 at the West Texas A&M University Research Feedlot in Canyon, TX. The selected pens were evenly distributed geographically throughout the feedlot. Longitudinal samples of freshly voided beef cattle feces, feedlot pen composite manure pack, feedlot pen composite dry, water, feed and cattle subiliac lymph nodes were collected and selectively cultured for *Salmonella*. Isolate identity was confirmed via MALDI-TOF. All *Salmonella* isolates from pen composite manure pack (n=162), water (n=34), feed (n=13) and subiliac lymph node (n=96) samples and one randomly selected fecal *Salmonella* isolate per pen per month (n=189) were selected for DNA extraction and whole genome sequencing.

Results

Salmonella was present in 60% of all sample types, specifically 78% for pen composite manure pack and 42% for cattle lymph nodes. A multilevel mixed effect logistic regression model suggests prevalence varies significantly by sample type ($P<0.005$), collection month ($P<0.005$) and feedlot pen($P=0.029$). *Salmonella* serovars Anatum (20.12%, n=100), Cerro (8.20%, n=42), Derby (0.2%, n=1), Kentucky (30.66%, n=150), Lubbock (11.33%, n=58), Montevideo (28.52%, n=138), Senftenberg (0.2%, n=1) and Virginia (0.78%, n=4) were identified. Sequence type was consistent for all isolates within each serovar. All isolates, except a single Senftenberg isolate, did not harbor AMR genes that confer phenotypic resistance and therefore were considered pan-susceptible.

Conclusions

Observing dynamics between cattle and *Salmonella* in the feedlot environment will help identify environmental treatments necessary to reduce or shift *Salmonella* from AMR to pan-susceptible serovars.

Financial Support
Texas A&M University; National Cattlemen's Beef Association - Beef Checkoff; U.S. Department of Agriculture, Food Safety Inspection Services

Notes:

44 - Characterization of *Salmonella enterica* serotype Infantis from animals in the United States

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Session: Antimicrobial Resistance/Use - 1, Dec. 5, 9:15 - 9:30 AM

Objective

Salmonella enterica is a pathogenic bacterium that causes foodborne illness in humans. Poultry is the most common source of *Salmonella* Infantis, although it has also been found in other sources. Historically, *S.* Infantis has been highly susceptible to most classes of antimicrobials, but multi-drug resistant (MDR) isolates have emerged associated with poultry in recent years. The aim of this study was to determine antimicrobial resistance and relationships in *S.* Infantis from different animal species.

Methods

Isolates were obtained from National Veterinary Services Laboratories submissions (2014-2017) for a total of 206 geographically diverse isolates from different animal species in the U.S. Antimicrobial susceptibility testing was performed on microdilution plates using NARMS interpretive criteria. Isolates were sequenced and analyzed for the presence of resistance genes, plasmids and phylogenetic relationships.

Results

Sixty-nine (33.5%) of the 206 isolates were resistant to at least one antibiotic, and most of these isolates were MDR. Phenotypic resistance was most common against streptomycin (23.3%), tetracycline (20.4%), sulfisoxazole (17.5%) and ampicillin (17%). Resistance to ceftriaxone was found in 12% (n=26) of isolates, most often mediated by blaCMY-2. However, we found 10 isolates with extended spectrum β-lactamase genes (ESBLs); these genes confer resistance to clinically important third generation cephalosporins, which are often used to treat invasive salmonellosis. The ESBL blaCTX-M-65 gene was found in 9 isolates from chickens (3), horses (3), turkeys (2) and cattle (1) during the years 2016 and 2017. Eleven MDR isolates, including the blaCTX-M-65 gene positives, carried several resistance genes, a MDR (IncFIB(K)1Kpn3) plasmid and presented a gyrA mutation, consistent with MDR isolates associated with human cases and the pESI plasmid.

Conclusions

This dataset captures the emergence of MDR strains in 2016 in different animal species and represents an opportunity to observe that process and potentially apply to predicting or preventing future emergence.

Financial Support
U.S. Department of Agriculture

Notes:

45 - Resistance to extended spectrum cephalosporins in enteric bacteria from selected national surveillance program

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Session: Antimicrobial Resistance/Use - 1, Dec. 5, 9:30 - 9:45 AM

Objective

Extended-spectrum cephalosporins (ESC) are categorized by World Health Organization as critically important antimicrobials with limited therapeutic alternatives for the treatment of severe bacterial infections. Preserving the effectiveness of ESC requires continuous monitoring of resistance and comparison of associated data across national surveillance programs in the face of globalization.

Methods

In this ecological study, we compared ESC resistance in *Escherichia coli* and *Salmonella enterica* isolated from food-producing animals (cattle, chicken, pig, and turkey) from 2003-2019 between nine countries (Canada, Denmark, Finland, Japan, Netherland, Norway, Sweden, United Kingdom, and the United States).

Results

Using the beta-regression model, non-selective ESC-R *Salmonella* spp. was less likely isolated in other eight countries compared with Canada (Odds ratio range: 0.07-0.76). We observed an interaction between the country and the year with a significantly decreased proportion ($P<0.05$) of non-selective ESC-R *Escherichia coli* from the Netherland, the United Kingdom, and the United States compared to Canada over the years. There was a linear correlation between non-selective ESC-R *Escherichia coli* and ESC use from Netherland (Spearman's $\rho = 0.91$, $P<0.0001$). For the six European countries, the interaction between the country and year showed a significant decrease in the proportion of selective ESC-R *Escherichia coli* over the years for the Netherland compared to Denmark ($P=0.002$). While there were variations in the proportion of beta-lactamase genes reported over the years, *bla*$_{\text{CTX-M}}$ and *bla*$_{\text{CMY-2}}$ genes were commonly detected among the selective ESC-R *Escherichia coli*.

Conclusions

This study reveals variability in the recovery of ESC-resistant bacteria among the countries, probably influenced by the individual country policy on the use of critically important antimicrobials and resistance surveillance programs. However, there is a need for harmonization and consistency in food animal sources of bacterial isolates used in surveillance programs within and between the countries for easy comparability.

Notes:

46 - Conjugative plasmid-mediated transfer of antibiotic resistance genes to commensal and multi-drug resistant bacteria

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Session: Antimicrobial Resistance/Use - 1, Dec. 5, 9:45 - 10:00 AM

Objective

Many antibiotic-resistant (AR) bacteria carry antibiotic resistance genes (ARGs) on conjugative plasmids transferable to commensals and other pathogens. We hypothesized that different pairings of donor and recipient strains influence plasmid transfer frequency and that fluorescent donor and recipient bacterial strains and plasmids could be used to quantify these effects.

Methods

We engineered red fluorescent-labeled *E. coli* MG1655 and commensal *E. coli* LM715-1 donor strains carrying a green fluorescent protein (GFP)-labeled broad host range RP4 plasmid. Donor strains chromosomes were constructed to carry antibiotic resistance markers (*kan*R, *cam*R) distinct from the plasmid (*amp*R, *tet*R and *kan*R). Recipient bacteria included laboratory, commensal and pathogenic strains *E. coli* DH5α, *E. coli* LM697-2, *Citrobacter rodentium, Pseudomonas aeruginosa, Klebsiella pneumoniae, Salmonella typhimurium,* and *Enterococcus faecium* carrying rifampicin resistance. We performed *in vitro* filter matings using different combinations of donor and recipient strains and determined conjugation frequencies using culture and flow cytometry. Transconjugant colonies were confirmed using fluorescent microscopy and colony PCR with primers for ARGs and GFP.

Results

We observed a difference in plasmid transfer frequency (TF) between donor *E. coli* MG1655 and recipient *E. coli* DH5α (0.11) and commensal donor *E. coli* LM715-1 and recipient *E. coli* DH5α (TF=0.042). Using the commensal LM715-1 RfmR as the recipient, we observed different plasmid frequencies for the donor strains *E. coli* MG1655 (TF=0.0035) and *E. coli* LM715-1 (TF=0.021). Moreover, the RP4 plasmid was transferred to *Citrobacter rodentium, Salmonella typhimurium, Pseudomonas aeruginosa* and *Klebsiella pneumoniae* recipient strains at different frequencies by donor *E. coli* LM715-1. An assessment of plasmid stability in the recipient bacteria will be performed using growth curve and fitness assays.

Conclusions

Thus, identities of donor and recipient strains influenced plasmid transfer frequency even within a single species of bacterium.

Notes:

47 - Effect of chicken IgY on *Staphylococcus aureus* growth in vitro

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Session: Antimicrobial Alternatives, Dec. 5, 10:30 - 10:45 AM

Objective

Mastitis is one of the most common health problems affecting the animal industry. A major causative agent, Staphylococcus aureus (SA) is often treated with antibiotics. Staphylococci rapidly develop antimicrobial resistance. Recent studies have shown IgY as a potential alternative to antibiotics to mitigate emerging antimicrobial resistance. This study aims at determining the effect of IgY concentration on SA inhibition in relation to the duration of incubation.

Methods

A 2-fold serial dilution of SA was made in nutrient broth. IgY from Rhode Island Red (RIR) chicken eggs was purified using the water dilution method, and standardized to a concentration of 1mg/mL in PBS; commercial chicken anti-SpA IgY from Exalpha (EA) was used for comparison. Two tubes per replication each containing SA (7.5 x 10^3 CFU) and nutrient broth were used. The IgY treatments were given as follows: no IgY, 5 IgY µg/mL, 25 IgY µg/mL, and 125 IgY µg/mL. Ten µL of inoculum were used in plating. The plates were set in duplicates and incubated at 37°C for 24 hours, and then CFU count was determined. To determine the inhibition in relation to duration of incubation, the above-mentioned tubes were incubated for 4, 8 and 12 hours, respectively after which were plated on tryptic soy agar for 24 hours.

Results

Significant inhibition ($P<0.0001$) of SA was noted in EA IgY concentration of 5 µg/ml, 25 IgY µg/mL, and 125 IgY µg/mL. Significance ($P<0.0001$) in RIR IgY was seen in concentration of 5 IgY µg/mL and 25 IgY µg/mL. On the whole, there was a significant difference between RIR IgY and EA IgY.

Conclusions

The results indicated both EA and RIR IgY can significantly inhibit SA growth in vitro. Overall, IgY inhibits SA; however further studies are required to research into duration and concentration interaction.

Financial Support
Agricultural Competitiveness Improvement Project - Azerbaijan; The Health and Human Services Center of Excellence (HHS COE) Veterinary Scholar Program (COE-VSP), Tuskegee Veterinary Scholars Program

Notes:

49 - Synergistic enhancement of disease resistance of chickens by butyrate, forskolin, and lactose

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Session: Antimicrobial Alternatives, Dec. 5, 11:00 - 11:15 AM

Objective

The rising concern of antimicrobial resistance highlights a need for effective alternatives to antibiotics for livestock production. Butyrate, forskolin, and lactose are three natural products known to induce the synthesis of host defense peptides (HDP), a critical component of innate immunity.

Methods

In this study, the synergy among butyrate, forskolin, and lactose in enhancing innate host defense, barrier function, and resistance to necrotic enteritis and coccidiosis was investigated.

Results

Our results indicated that the three compounds synergistically augmented the expressions of multiple HDP and barrier function genes in chicken HD11 macrophages. The compounds also showed an obvious synergy in promoting HDP gene expressions in chicken jejunal explants. Dietary supplementation of a combination of 1 g/kg sodium butyrate, 10 mg/kg forskolin-containing plant extract, and 10 g/kg lactose dramatically improved the survival of chickens from 39% to 94% ($P < 0.001$) in a co-infection model of necrotic enteritis. Furthermore, the three compounds largely reversed growth suppression, significantly alleviated intestinal lesions, and reduced colonization of *Clostridium perfringens* or *Eimeria maxima* in chickens with necrotic enteritis and coccidiosis ($P < 0.01$).

Conclusions

Collectively, dietary supplementation of butyrate, forskolin, and lactose is a promising antibiotic alternative approach to disease control and prevention for poultry and possibly other livestock species.

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

Notes:

50 - *Bacillus* fed microbial reduces the pathogenic synergy of a co-infection with *Salmonella enterica* serovar Choleraesuis and PRRS virus

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Session: Antimicrobial Alternatives, Dec. 5, 11:15 - 11:30 AM

Objective

We examined the impact of providing a *Bacillus*-based direct-fed microbial (DFM) on the syndrome resulting from orally infecting pigs with either *Salmonella enterica* serotype Choleraesuis (*S.* Choleraesuis) alone, or in combination with an intranasal challenge, three days later, with porcine reproductive and respiratory syndrome virus (PRRSV).

Methods

Weanling pigs (n=11 per group) received *Bacillus*-based DFM (Provent ECL) in their diet for two weeks before being challenged orally with *S. enterica* Choleraesuis either alone, or in combination with an intranasal challenge, three days later, with PRRSV virus.

Results

Nine days after the bacterial challenge, *Salmonella* was isolated from ileocecal lymph nodes of all challenged pigs regardless of DFM treatment. Compared to the single bacterial challenge, the dual challenge with *Salmonella* and PRRSV resulted in a pathogenic synergy exhibited by a higher rate of colonization of the lung by *Salmonella*, a more extensive and severe interstitial pneumonia, and peritoneal exudation. Provision of DFM to dually challenged pigs, reduced the rate of lung colonization by *Salmonella*, eliminated or reduced the presence PRRSV in the blood and lung, reduced the extent and severity of gross lung pathology, and the frequency of pigs with peritoneal exudate. Dually challenged pigs that received DFM had increased concentrations of IL-1 and IL-8 in lung lavage fluids, accompanied by increased expression in their blood cells of nod-like receptor 2 (NOD2) and triggering receptor expressed in macrophages 1 (TREM1) molecules.

Conclusions

The increased expression of NOD2 and TREM1 and changes in pulmonary cytokine production suggest that the DFM exerted a systemic modulating effect on innate immunity. These observations are consistent with the notion that tonic stimulation by gut-derived microbial products can poise the innate immune system to fight infections distally.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture; United Animal Health

Notes:

51 - Novel therapeutic leads: Demonstration of efficacy, safety, and applicability of anti-APEC molecules in chickens

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Session: Antimicrobial Alternatives, Dec. 5, 11:30 - 11:45 AM

Objective

Avian pathogenic *E. coli* (APEC), causes colibacillosis in chickens which results in severe economic losses and the control of APEC infections relies on antibiotic medication and vaccination. The emergence of antibiotic-resistant APEC and diverse APEC serotypes causing disease limits their use. Goal of this study is to demonstrate the efficacy, safety, and mass applicability of two anti-APEC small molecules (SM), GI-7 and QSI-5 (alone and in combination) in conditions simulating the natural settings, which are the key parameters to assess the suitability of these antimicrobials for commercial application in poultry.

Methods

We evaluated the efficacy and safety of two anti-APEC SMs (GI-7: growth inhibitor and QSI-5: quorum sensing/virulence inhibitor) in chickens. The combination of GI-7 and QSI-5 was also evaluated with an aim to induce a better anti-APEC effect in chickens. The efficacy (reduction in mortality, APEC lesions, and APEC load) of these SMs was evaluated under conditions mimicking the natural settings. The safety of these SMs was measured by assessing the impact of SMs treatment on body weight gain (BWG) and feed conversion ratio (FCR) of chickens as well as by quantifying the SMs residue in chicken's tissues.

Results

The efficacy of GI-7 and QSI-5 either alone or in combination was superior to currently used antibiotic sulfadimethoxine (SDX). The SMs treatment did not affect the BWG and FCR of chickens indicating no adverse effect of these SMs in chickens. Further, the residue accumulation of GI-7 and QSI-5 in chicken's tissues is below the FDA permissible limits set for currently used antibiotics suggesting no risk for human consumption. The pharmacokinetic (PK) profile of these SMs was also studied which revealed that the GI-7 and QSI-5 were readily absorbed upon oral delivery but have a shorter half-life compared to SDX.

Conclusions

These studies facilitate submitting new animal drug application (NADA) to FDA Center for Veterinary Medicine as well as to advance these SMs for industrial application. Our goal is to promote sustainable poultry production worldwide together with improved food security and food safety.

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

Notes:

52 - Trans-cinnamaldehyde as an antimicrobial feed additive to control and prevent enteric septicemia of catfish

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Session: Antimicrobial Alternatives, Dec. 5, 11:45 - 12:00 PM

Objective

Edwardsiella ictaluri is the causative agent of enteric septicemia of catfish (ESC) and one of the most significant pathogens of US catfish aquaculture. Mortalities resulting from ESC have been reported in over 78% of all operations, with outbreaks reported from 42% of catfish production ponds. The current therapeutic strategies to prevent ESC have their limitations, and catfish operations continue to suffer significant losses due to ESC. The problem is exacerbated by the increasing emergence of *E. ictaluri* multidrug-resistant (MDR) strains. The objective of this study is to find alternative intervention strategies to conserve antimicrobial use.

Methods

The Minimum Inhibitory Concentrations (MIC) of trans-cinnamaldehyde (TC) was assessed against *E. ictaluri* 93-146 using broth microdilution. Morphologies of *E. ictaluri* in response TC were observed using scanning electron microscopy (SEM) and transmission electron microscopy (TEM). An efficacy trial was conducted to evaluate the effectiveness of TC to treat *E. ictaluri* infection in channel catfish.

Results

TC inhibited the growth of *E. ictaluri* at concentrations of 20 µg/ml. After *E. ictaluri* challenge, a significantly higher survival was found in catfish that received dietary-TC at the levels of 15 and 20 mg/kg compared to the control group (49.12% and 65.52% survival vs 11.11% survival). Bacterial concentrations in the spleen and anterior kidney were significantly lower in fish fed with TC (20 mg/kg diet) compared to control at 5-day post-infection. Results indicate that supplementation of catfish feed with TC reduces *E. ictaluri* infection.

Conclusions

A safe and efficacious alternative to antimicrobials will reduce the emergence and spread of antimicrobial-resistant strains. Application of TC to treat MDR *E. ictaluri* strains is promising, and novel aspect of this application reduce costs and the consequences associated with spread of MDR strains.

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

Notes:

53 - Microbiota-directed complementary foods for treating childhood undernutrition

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Session: CRWAD Council Keynote & Special Symposium, Dec. 5

Human postnatal development is typically viewed from the perspective of our 'human' organs. As we come to appreciate how our microbial communities are assembled following birth, there is an opportunity to determine how this microbial facet of our developmental biology is related to healthy growth as well as to the risk for and manifestations of disorders that produce abnormal growth. We are testing the hypothesis that perturbations in the normal development of the gut microbiota are causally related to childhood undernutrition, a devastating global health problem whose long-term sequelae, including stunting, neurodevelopmental abnormalities, plus metabolic and immune dysfunction, remain largely refractory to current therapeutic interventions. The journey to preclinical proof-of-concept, and the path forward to clinical proof-of-concept emphasize the opportunities as well as the experimental and analytic challenges encountered when developing microbiota-directed therapeutics

Financial Support
Bill and Melinda Gates Foundation; U.S. National Institutes of Health

NIH National Institutes of Health
Turning Discovery Into Health

Notes:

54 - USDA-NIFA programs on precision livestock farming and concerns for meeting animal health standards

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Session: CRWAD Council Keynotes and Special Symposium, Dec. 5, 2:00 - 2:45 PM

Adaptive control and mitigation strategies have advanced in an effort to improve animal health by early detection of disease, enhancement of animal comfort, and the ability to detect behavioral changes. The USDA National Institute of Food and Agriculture has several grant programs with primary focus on these and ancillary areas. Highlighted programs include Interdisciplinary Engagement in Animal Systems (IDEAS), NIFA-NSF Cyber-Physical Systems (CPS), NIFA-NSF National Robotics Initiative (NRI), Engineering for Agricultural Production Systems, and Diseases of Agricultural Animals. Foci for competitiveness and sustainability of animal management in small and medium sized farms will be outlined within the Agriculture Economics and Rural Communities (AERC): Small and Medium-Sized Farms program. Description of USDA's role in risk analysis to meet animal health standards and as a key component of trade are examined.

Notes:

55 - Application of precision technologies on livestock farms

D. Pitta Department of Clinical Studies, School of Veterinary Medicine, University of Pennsylvania. dpitta@vet.upenn.edu
Session: CRWAD Council Keynote & Special Symposium, Dec. 5

Objective

Modern livestock operations can be considered a "superorganism" where the animals within the herd share resources for living and are exposed to environmental changes and diseases as a group. A better understanding of health and performance at the superorganism level is needed to inform precision management decisions that lead to improved productivity, profitability, and sustainability of modern herds. Precision livestock farming technologies offer the opportunity to monitor the health, reproductive status, activity, and production of individual cows as well as the superorganism. These technologies along with high-throughput sequencing technologies have the potential to generate new knowledge and identify novel phenotypes for various responses in livestock. Better management, handling, and analysis of the voluminous data ('big data') generated by these automated technologies is critically needed to maximize the use of precision technologies.

Methods

To accomplish this, lateral integration of data-driven automated technologies coupled with the use of statistical modeling, machine learning tools, and artificial intelligence is needed to transform these different types of data and generate new actionable items to inform producers. Notable examples of precision technologies include accelerometers that are used to track animal behaviors such as eating, rumination, standing, lying, and movement within the barn and have huge potential to predict rumen function and ultimately phenotypic responses in ruminant species.

Results

Recently, our group has gathered evidence that there is a natural variation among dairy cows for rumination time and microbiota and that deciphering the link between the two metrics may help us identify healthier and more efficient animals.

Conclusions

Such efforts will lay the foundation to expand the scope of precision technologies in identifying novel phenotypic traits. However, multi-disciplinary collaborations between academia, industry, and producers are needed to generate new hypotheses that lead to development of better algorithms for smarter livestock operations.

Notes:

56 - Precision livestock farming: From where we come, to where we go?

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Session: CRWAD Council Keynotes and Special Symposium, Dec. 5, 4:45 - 5:30 PM

Objective

Precision Livestock Farming is a tool for management of livestock by continuous automated real-time monitoring of production/reproduction, health & welfare and environmental impact. A tool means that this technology does not replace experts like farmers, veterinarians, feed experts, etc. but to support people in their decision taking by objective measurement on the animals. The objective is to give an overview of where it started, where we are today and where to go.

Methods

We used results from the work we did since 1991 and the literature on this topic published in peer reviewed publications and in many conference proceedings.

Results

When starting this research in 1991 with a more fundamental question on predictability of the responses of living organisms, we started on insects and mussels. It soon became clear that animals, like humans, are so called C.I.T.D. systems: Complex, Individually different, Time-varying in their responses and dynamic. Then we did experiments on bees, fish, mice, rats, chicken, pigs, cow, horses, dogs to from 2001 also work on humans. Results will be shown in videos and graphs. The research trajectory has given principles on how to develop the technology and to bring it implementable products. The pickup in the field however goes far too slowly and that is where we must put more efforts. Finally, we will show where to go with this technology to create a real impact for many people and animals worldwide.

Conclusions

Today the European ECPLF2022 conference does the 10th version of the two-yearly conference, in USA the USPLF2023 conference will be held and ACPLF2019 was the second big PLF conference in Asia. The PLF concept takes off worldwide and will help us to create a more sustainable livestock sector which is so much needed. We need to deliver more animal product with less feed input, less manure and environmental impact and improve animal welfare and health. PLF shows high potential to help us create these solutions.

Notes:

57 - Pathogens from the host point of view: A journey with microbes, hosts, data, & learning

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Session: ACVM - Microbiology & Disease Pathogenesis Featured Speakers, Dec. 6, 8:30 - 9:15 AM

Receiving the American College of Veterinary Microbiologists designated 2021 Distinguished Microbiologist award clearly recognizes the decades long contributions of dedicated graduate students, post-doctoral fellows and collaborators converging research on pathogens, hosts, pathogenesis, vaccines, diagnostics and therapeutics. Data will be converged from our and other's experiments to paint a multi-level landscape of host-pathogen interactions. The presentation will be focused on the broad vision of generating, converging and analyzing information from multiple sources to more fully understand perturbed biological systems and their consequences on pathogens, hosts and environments. How will we apply the data streams, spatial multi-omics, global sampling, genomic sequencing, integrative artificial intelligence platforms, systems biology and systems vaccinology to reliably forecast outbreaks, spillover and spillback events while rapidly developing effective diagnostics and vaccines? Will we learn to collaborate developing far more coherent and robust nationally integrated systems of large cohorts of private veterinary medical records to accelerate disease prediction, detection, treatment and advanced precision veterinary medicine? Focusing on the pursuit of this ambitious vision promises future improved health and well-being for animals, humans and the environment.

Notes:

58 - Coronaviruses and cats and humans: Where are feline coronaviruses and SARS-CoV-2 alike?

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Session: ACVM - Microbiology & Disease Pathogenesis Featured Speakers, Dec. 6, 9:15 - 10:00 AM

Comparative medicine can be a powerful approach to help refine our understanding of coronavirus pathophysiology, emergence and response. With the ongoing COVID-19 pandemic it has become apparent that many other species can be infected with SARS-CoV-2, but also harbor their own readily transmissible coronaviruses; e.g., feline coronavirus, which causes feline infectious peritonitis (FIP) in cats. While both FIP and COVID-19 are caused by coronaviruses, these are in distinct families. However, there are notable similarities in the spike (S) proteins of these distinct viruses, with commonalities that may translate into the infection process, and with implications for viral tropism, transmissibility and diagnostics. This presentation provides an update on FCoV and FIP in cats in the context of the ongoing SARS-CoV-2 pandemic, with a focus on the viral spike protein—its role in infection and implications for viral tropism, transmissibility and diagnostics.

Notes:

59 - *Campylobacter jejuni* invasion of the intestinal epithelium triggers activated neutrophil recruitment

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Session: ACVM - Microbiology & Disease Pathogenesis Featured Speakers, Dec. 6, 10:30 - 11:15 AM

Campylobacter jejuni is a foodborne pathogen that binds to and invades the epithelial cells lining the intestinal tract. One of the hallmarks of *C. jejuni* intestinal infection is a robust host inflammatory response. We hypothesized that the ability of *C. jejuni* to invade intestinal epithelial cells drives the recruitment of neutrophil infiltration immune cells to the infection site. In vitro and in vivo models were used to examine the interaction of a *C. jejuni* wild-type strain and *C. jejuni* ciaD deletion mutant with cultured epithelial cells and gut epithelia, respectively. In vitro assays revealed that CiaD binds to the host cell protein IQGAP1 (a Ras GTPase-activating-like protein), thus displacing RacGAP1 from the IQGAP1 complex. This, in turn, leads to the unconstrained activity of the small GTPase Rac1, which is known to have roles in host cell actin reorganization and internalization of *C. jejuni*. In vivo assays, using a novel porcine ligated intestinal loop model, revealed that the ability of *C. jejuni* to invade the intestinal epithelium triggers the release of the chemokine IL-8, prompting neutrophil recruitment and activation. In contrast, a *C. jejuni* ΔciaD mutant was attenuated in its ability to promote intestinal inflammation. In summary, we have demonstrated that the ability of *C. jejuni* to invade the intestinal epithelium is, in part, responsible for the recruitment of activated neutrophils and the initial pathology observed in the intestine.

Notes:

60 - A novel epitope- and structure-based vaccinology platform empowers broad-spectrum and precision vaccine development

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Session: ACVM - Microbiology & Disease Pathogenesis Featured Speakers, Dec. 6, 11:15 - 12:00 PM

Vaccine is regarded a more cost-effective prevention against infectious diseases. One major challenge in vaccine development is virulence heterogeneity, since pathogens constantly evolve genetically and immunologically to adopt divergent hosts or to escape host immune protection. To achieve broad protection, cocktail vaccine, epitope vaccine, chimeric or fusion antigen and recently structural vaccinology strategies have been attempted to overcome challenge of heterogeneity among isolates or strains; yet, we do not have licensed vaccines for many diseases. Like many other pathogens, enterotoxigenic E. coli (ETEC) bacteria are immunologically heterogeneous; they produce different virulence factors, mainly adhesins or fimbriae (K88, K99, 987P, F41, F18; CFAs and CSs) and enterotoxins (LT, pSTa, STb, Stx, hSTa), to cause diarrhea in young animals (neonatal diarrhea, post-weaning diarrhea), children from developing countries (children's diarrhea) and international travelers (travelers' diarrhea). Adhesins promote ETEC colonization at small intestines and enterotoxins disrupt host intestinal epithelial cell homeostasis and stimulate fluid hyper-secretion. As an ETEC strain carries one adhesin and one enterotoxin can cause diarrhea, an effective ETEC vaccine would need to induce broadly protective antibodies against all these adhesins and toxins; this has been a major road block in ETEC vaccine development over 50 years. Assisted with structural biology and computational biology, we combined epitope vaccinology and structural vaccinology concept and developed an epitope- and structure-based multiepitope-fusion-antigen (MEFA) vaccinology platform to construct polyvalent immunogens and to develop broadly protective and precisely targeted vaccines. This MEFA platform identifies a backbone immunogen, integrates functional antigenic domains or epitopes from heterogenous virulence determinants on backbone, maintains epitope native antigenicity, and constructs a polyvalent MEFA immunogen for broadly protective multivalent vaccines against heterogeneous strains or pathogens.

Notes:

61 - Prevalence and risk factors for *Anaplasma marginale* seropositivity in cattle in California between 2010 and 2019

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Session: Epidemiology - 2, Dec. 6, 8:30 - 8:45 AM

Objective

The objective of this study was to examine the prevalence of *Anaplasma marginale* infection in cattle in California. The specific goal was to identify potential risk factors for infection and the effects of weather on seroprevalence.

Methods

A total of 3154 *Anaplasma marginale* ELISA test results from the California Animal Health and Food Safety Lab recorded between 2010 and 2019 were used in this study. True prevalence was calculated based on the sensitivity and specificity of the ELISA test. Univariable and multivariable regression models were used to analyze risk factors, including sex, age, type of cattle, region, and year. Furthermore, mixed effect models were built to examine the effects of weather (maximum/minimum temperature and precipitation in dry/wet seasons) on seropositivity in cattle with "region" as a random effect. Considering effects of tick ecology on the disease, the weather data from the current year to 3 years before data collection were analyzed in the mixed effect models.

Results

The overall true prevalence of *Anaplasma* infection was 20.6% (95% CI: 19.1-22.2). In a multivariable model, risk factors for seropositivity identified were sex (P=0.008), production type (P=0.025), age (P=0.004), region (P<0.001), and year (P<0.001). A mixed effects model that takes into consideration the correlation of weather data measured within a region, found maximum temperature and precipitation in wet seasons to be positively associated with seropositivity and maximum temperature in dry seasons and minimum temperature in wet seasons negatively associate with seropositivity while controlling for age and production type.

Conclusions

The results suggested that Anaplasma infection in cattle in California is associated with animal and environmental characteristics. Weather effects on infection may be related to tick population dynamics.

Notes:

62 - Within herd seroprevalence of bovine anaplasmosis in Tennessee, 2020-2021

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Session: Epidemiology - 2, Dec. 6, 8:45 - 9:00 AM

Objective

Recently, statewide seroprevalence of bovine anaplasmosis (BA) in Tennessee (TN) was estimated using slaughterhouse or diagnostic lab data. However, the severity of this economically important disease within TN herds has not been evaluated. Thus, this study was aimed at determining the within herd seroprevalence of BA across the 3 regions (East, Middle, and West) of TN.

Methods

In an active screening for BA using competitive ELISA, 1529 beef cattle from 24 beef herds across the 3 regions of TN were sampled between April 2020 and January 2021. Samples from 1081 of the 1529 cattle were also screened with PCR test for confirmation of cELISA results.

Results

Of the 30,462 beef herds in TN, 24 beef herds (8 from East, 11 from Middle, and 5 from West) were selected because either they had a history of BA (Middle and West TN herds) or they were part of routine veterinary care (East TN herds). The sample size per herd ranged 20 – 111 (East TN), 17 – 178 (Middle TN), and 17 – 256 (West TN). Using cELISA, BA positive herds came from 3 of the 8 East TN herds (37.5%), 7 of the 11 Middle TN herds (63.6%), and 4 of the 5 West TN herds (80%). Among BA positive herds, within herd apparent seroprevalence ranged 2.70 — 56.25% in East TN, 0.65 —64.3% in Middle TN, and 58.09 — 91.43% in West TN. Regionally, counties with the greatest prevalence were Jefferson (East TN), Montgomery (Middle TN), and Weakley (West TN).

Conclusions

Although BA is prevalent throughout the 3 regions of TN, it appeared to be most prevalent in West TN and within herd prevalence in affected herds is quite high. Future prevention and control measures for BA in TN should preferentially target counties in West TN compared to other regions of the state.

Financial Support
Foundation for Food and Agricultural Research; Tennessee Beef Promotion Board

Notes:

63 - Comparison of statewide and herd level seroprevalence of *Anaplasma marginale* antibodies in Florida cattle

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Session: Epidemiology - 2, Dec. 6, 9:00 - 9:15 AM

Objective

The objective of this case report is to assess within-herd seroprevalence of *Anaplasma marginale* (*Am*) antibodies across 12 Florida beef cattle herds that experienced disease outbreak and compare to statewide seroprevalence.

Methods

Twelve Florida cattle herds were surveyed and ranged in size from 160 to 456 adult *Bos taurus* - *Bos indicus* cattle. Statewide *Am* seroprevalence relied on blood samples obtained at slaughter from 201 Florida cattle. Screening relied on competitive enzyme linked immunosorbent assays.

Results

Data indicate that surveyed Florida cattle herds impacted by anaplasmosis experienced increased mortality (up to 17.8%) and abortions. Up to 29.2% of cows aborted late in gestation in 2 herds that included many cattle introduced from Texas. Among 1,085 cattle tested in the 12 herds, seroprevalence of *Am* varied from 2.6% to 85%, with an overall seropositive rate of 50.3%. Cattle in open herds were 6.23 (95% confidence interval: 4.26-9.17) times more likely to experience mortality, and 3.10 (95% confidence interval: 2.39-3.98) times more likely to abort than animals in closed herds. Average mortality (12%) and abortion (16.3%) among open herds were significantly ($P < .05$) higher than mortality (1.9%) and abortion (5.3%) among closed herds. Overall seropositivity among affected herds was higher than the apparent statewide seroprevalence of 20.32%.

Conclusions

This survey provides estimates of seropositivity among Florida cattle and reports the absence of uniform herd immunity in an area considered endemic for bovine anaplasmosis. These data highlight unrestricted cattle movement and environmental conditions that favor vector-borne disease transmission as risk factors for disease outbreaks even in regions that are considered endemic for bovine anaplasmosis.

Financial Support
American Association of Bovine Practitioners Foundation; USDA-NIFA

Notes:

64 - Case-control study to identify management practices associated with illness or death from bovine anaplasmosis in Mississippi cow-calf herds

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Session: Epidemiology - 2, Dec. 6, 9:15 - 9:30 AM

Objective

The objective of this study was to identify management practices associated with illness or death from bovine anaplasmosis (BA) in Mississippi cow-calf herds.

Methods

Mississippi veterinarians were solicited for case and control herds. Cases had clinical BA diagnosed within the past year. Controls were any herd under the care of the same practice with no clinical BA diagnosed in the past year. Interviewers blinded to herd status conducted telephone surveys of producers. Management factors were tested for association with herd status using a generalized linear mixed logistic regression model with veterinary practice included as a random variable.

Results

Forty-seven cow-calf herds, 22 cases and 25 controls, from 6 veterinary practices across Mississippi were interviewed, representing 22 different counties in Mississippi. The average case herd size was 131 breeding females, while the average control herd size was 135. Across all herds, the average number of times cattle were caught for processing each year was 2.4, and the average number of injections administered during each processing event was 2.9. Twenty of 22 case herds and 13 of 25 control herds fed chlortetracycline (CTC) medicated mineral or feed. Whether or not the producer fed CTC was associated with case herd status (OR=9.2, 95%C.I.=1.7,50.7).

Conclusions

Feeding CTC was common. The association between case herds and feeding CTC might be explained by herds that experienced BA previously being more likely to feed CTC, or herds that were feeding CTC being more likely to experience clinical BA due to chemosterilization increasing susceptibility to BA.

Financial Support
House officer grant, Office of Research and Graduate Studies, College of Veterinary Medicine, Mississippi State University

Notes:

65 - Investigating the impact of intranasal vaccine against IBR, PI3 and BRSV on calf mortality and average daily gain

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Session: Epidemiology - 2, Dec. 6, 9:30 - 9:45 AM

Objective

A relationship between intranasal vaccination against IBR, PI3 and BRSV (IN) and increased mortality and decreased gain among commercially raised high-risk stocker cattle was detected from health records. However, vaccine allocation decisions and season may have influenced bovine respiratory disease (BRD) risk-status. The objective of this study was to use additional records to investigate the effect of IN compared to modified-live BRD virus injectable vaccination (INJ) on mortality and gain in high-risk stocker calves.

Methods

Data were collected September through February from 2016-2019 on 41,660 beef calves from 496 lots on 12 stocker operations in Missouri. Mortality was modeled in a multilevel, multivariable logistic regression GLMM model (alpha=0.05). Average daily gain (ADG, including deads), was modeled by linear mixed model. In both models, clustering by lot within site was defined by random effect variables.

Results

Factors associated with mortality were year (2016: OR=2.6; 2017: OR=2.4; 2018: OR=2.7; compared to 2019), steer lots (OR=1.4; compared to heifer lots), weight class (154-204kg: OR=1.8; 205-249kg: OR=1.2; 250-294kg: OR=0.9; compared to 295-340kg), and arrival month (January: OR=1.0; February: OR=0.6; September: OR=2.2; October: OR=1.7; November: OR=1.3; compared to December). Factors associated with ADG were INJ (0.19kg/day, 95%C.I.=0.07,0.3; compared to IN), year (2016: 0.06kg/day, 95%C.I.=-0.09,0.22; 2017: -0.16kg/day, 95%C.I.=-0.31,-0.01; 2018: -0.08kg/day, 95%C.I.=-0.25,0.08; compared to 2019), and pen size (≥100 head: 0.27kg/day, 95%C.I.=0.17,0.37; 75-99 head: 0.03kg/day, 95%C.I.=-0.06,0.12; 60-74 head: -0.07kg/day, 95%C.I.=-0.18,0.04; 50-59 head: -0.6kg/day, 95%C.I.=-0.16,0.04; compared to <50 head).

Conclusions

After accounting for other factors, IN was associated with decreased ADG, but we failed to find a relationship with mortality.

Notes:

66 - Retrospective analysis of porcine circovirus 2 and porcine circovirus 3 in the United States swine industry

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Session: Epidemiology - 2, Dec. 6, 9:45 - 10:00 AM

Objective

Porcine Circovirus 2 (PCV2) and 3 (PCV3) are single-stranded DNA viruses. PCV2 was described in the 1990s associated with wasting disease and has become an economic burden for the swine industry. A PCV2 vaccine became commercially available in 2006. PCV3 was discovered in 2016 and has been associated with reproductive failure and multisystemic inflammation. However, the prevalence of PCV2/3 coinfection has not been retrospectively assessed. Therefore, the objective was to conduct a retrospective prevalence and genotypic evaluation of PCV2 and PCV3 from 2000, 2006, and 2012.

Methods

In total, 2,684 serum samples collected during 2000, 2006, and 2012 representative of 179 farms were evaluated from the National Animal Health Monitoring System serology archive. Per farm, 15 serum samples were divided into 3 pools of 5 serums for PCV2 and PCV3 qPCR detection (ISU VDL). In addition, subsets of PCV2 and PCV3 positive samples were analyzed by genomic sequencing and phylogenetic analysis (ISU).

Results

PCV2 prevalence decreased dramatically from 2006 to 2012; however, PCV3 prevalence remained similar from 2000 to 2012. Although the coinfection rate in 2000 was high and widespread through 19 swine production states, double-positive farm proportion declined from 2006 to 2012. The percentage of PCV2-single infected farms was approximately 20%, and this proportion remained stable through the study while the PCV3-single infected farm proportion increased from 2000 to 2012. No PCV2/PCV3 double-negative farms were detected in 2000 and 2006, while in 2012, 20% of farms were double negative. In 2006, PCV2a, PCV2b, PCV3a1, and PCV3a2 were the most common subtypes in circulation.

Conclusions

PCV2 was highly prevalent in the US swine industry before 2006 and was frequently accompanied by a PCV3 coinfection. Thus, PCV3 was highly prevalent and geographically widespread in the US swine industry before the first description in 2016. After PCV2 vaccine development, the percentage of coinfected farms decreased while the percentage of farms positive only for PCV3 increased from 2000 to 2012. Although PCV2 vaccines are not effective against PCV3, PCV2/PCV3 coinfection may hamper PCV2 vaccine clinical protection. PCV2a and PCV2b were the most prevalent subtypes before vaccine development. PCV3 subtypes detected in 2006 have high homology with current circulating subtypes.

Notes:

67 - Prevalence of severe fever with thrombocytopenia syndrome virus in dogs and cats from the Republic of Korea

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Session: Epidemiology - 3, Dec. 6, 10:30 - 10:45 AM

Objective

Severe fever with thrombocytopenia syndrome (SFTS) is caused by a novel tick-borne *Dabie bandavirus* in the family *Phenuiviridae* in China, the Republic of Korea (ROK), Japan and Vietnam. SFTS is mainly characterized by fever, depression, leukopenia, and thrombocytopenia in human. The objective of this study was to investigate the prevalence of SFTS virus in dogs and cats hospitalized at veterinary clinics in the ROK.

Methods

A total of 560 serum samples of dogs (n = 448) and cats (n = 112) were collected from April 2019 to December 2020 in the ROK between January and December 2019. Viral RNA was extracted from sera using viral RNA extraction kit and one-step RT-nested PCR was performed to amplify the S segment of the SFTSV. The sequence data were analyzed using Chromas and were aligned using CLUSTAL X. The phylogenetic analysis was constructed using the maximum-likelihood method using MEGA7. In addition, sera were tested for IgG antibody against SFTSV-NP by enzyme-linked immunosorbent assay (ELISA).

Results

The 14 (3.1%) out of 448 dogs and six (5.4%) out of 112 cats were positive for SFTS virus by PCR analysis. The 72 (19.3%) out of 374 dogs and five (4.8%) out of 105 cats were seropositive for ELISA. The SFTSV sequences obtained in this study were included in genotype B (subgenotype B-1, B-2, B-3), D, and F.

Conclusions

These results highlight a concern about secondary infection in humans from animals infected with SFTSV, therefore the status of SFTSV infection in companion animals means a great importance for public health.
*Acknowledgement: This work was supported by Korea Institute of Planning and Evaluation for Technology in Food, Agriculture and Forestry (IPET) through Animal Disease Management Technology Development Program, funded by Ministry of Agriculture, Food and Rural Affairs (MAFRA) (321014-01).

Notes:

68 - Apparent and true prevalence of feline leukemia virus and feline immunodeficiency virus in northern Mississippi shelter cats

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Session: Epidemiology - 3, Dec. 6, 10:45 - 11:00 AM

Objective

Feline leukemia virus (FeLV) and feline immunodeficiency virus (FIV) are important infectious viruses of cats. Shelters often make euthanasia or adoption decisions based on the results of these tests but given the low estimated prevalence of these diseases and imperfect test performance, this might not be a good practice. The Zoetis Witness FeLV-FIV Rapid ImmunoMigration test has reported sensitivity and specificity of 92.9% and 96.5% for FeLV antigens and 97.5 and 94.4% for FIV antibodies, respectively. The objective of this study was to determine the apparent prevalence of FeLV and FIV in apparently healthy shelter cats in Mississippi and estimate the true prevalence of infection.

Methods

Blood samples (n=150) were collected from apparently healthy cats (> 6 months of age) selected from five shelter locations across northern Mississippi. The Witness test was performed on each serum sample. Median age was 24 and interquartile range was 24. Cats were categorized as FeLV and FIV test-positive or -negative.

Results

Two cats (1.3%; 95% CI 0.16%, 4.7%) tested FeLV-positive and 5 (3.3 %; 95% CI 1.1%, 7.6%) tested FIV-positive. No cats tested positive for both viruses concurrently. True prevalence estimates, based on reported test performance were -2.4% (95% CI -3.7, 1.4%) and -2.5% (95% CI -4.7%, 1.9%) for FeLV and FIV, respectively, and could be consistent with the absence either virus in the population.

Conclusions

True prevalence is at likely to be extremely low, possibly approaching zero, and, therefore, it is appropriate to question the predictive value of a positive test result for these two viruses.

Financial Support
House officer grant, Office of Research and Graduate Studies, College of Veterinary Medicine, Mississippi State University

Notes:

69 - The association of hematological variables and data collected at diagnosis with metritis cure in dairy cows

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Session: Epidemiology - 3, Dec. 6, 11:00 - 11:15 AM

Objective

Predicting metritis cure in dairy cows will be instrumental for the development of a selective therapy strategy and to reduce antimicrobial use in dairy farms. Hence, the objective of this study was to investigate the association of hematological variables and information collected at diagnosis with metritis cure.

Methods

A total of 358 cows diagnosed with metritis between January 2020 and May 2021 were enrolled in the study. Cows were housed in farms located in TX (n = 117), CA (n = 106), and FL (n = 35) and were randomly allocated into receiving subcutaneous injections of 6.6 mg/kg of ceftiofur crystalline-free acid at diagnosis and 72 h later (n = 176) or to remain untreated (n = 182). Information collected at diagnosis such as days in milk (DIM), parity, vaginal laceration (VL), body condition score (BCS), and rectal temperature were recorded. Blood samples were collected at metritis diagnosis and submitted to hematological analysis. Metritis cure was defined as absence of metritis 14 days after initial diagnosis. Univariable analysis and multivariable logistic models were fitted to the data.

Results

The distribution of parity, DIM to metritis diagnosis, BCS at enrollment, rectal temperature, VL, and white blood cells counts (WBC) did not differ between treatment groups ($P > 0.15$). In a multivariable logistic regression model, treatment, parity, DIM at enrollment, and WBC were associated with cure risk. Ceftiofur-treated cows were at 1.97 greater odds of cure than untreated controls ($P < 0.01$). Additionally, multiparous cows were more likely to be cured than primiparous counterparts (OR = 1.74, $P < 0.03$). Moreover, metritis cure was positively associated with DIM at diagnosis ($P < 0.01$) and negatively associated with WBC ($P < 0.01$).

Conclusions

As previously reported, therapy with ceftiofur increased cure of metritis in dairy cows. In addition, parity, DIM and WBC at diagnosis were also associated with metritis cure, indicating that some of these variables can potentially be used in a selective strategy for metritis therapy.

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

Notes:

70 - Association between bacterial group and persistence in the mammary gland in early lactation primiparous cows

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Session: Epidemiology - 3, Dec. 6, 11:15 - 11:30 AM

Objective

The objective of this study was to assess the association between bacterial group and the persistence of intramammary infections (IMI) in dairy cows raised under organic certification.

Methods

We enrolled 503 primiparous cows from 5 organic dairy farms in a prospective cohort study. Quarter milk samples were collected weekly during the first 5 weeks of lactation. Milk samples were pooled into a composite sample for bacterial culture. Isolates were identified using MALDI-TOF MS. A total of 1,669 samples from 391 cows were used in the analysis, after exclusion of contaminated samples and those from animals with less than 3 samples. The association between bacterial group and IMI-persistence (harbor the same microorganism for ≥ 2 weeks) was analyzed using mixed logistic regression. Cow and farm were used as random effects.

Results

Staph. aureus had higher odds of persistence compared to other bacterial groups (Adj. Prob. (SE): 0.88 (0.05), $P<0.05$), except when compared to *Staph. chromogenes* (Adj. Prob. (SE): 0.74 (0.06), $P=0.27$) and *Strep. uberis* which tended to be higher (Adj. Prob. (SE): 0.37 (0.20), $P=0.10$). *Staph. chromogenes* showed significantly higher odds of persistence compared to Non-*aureus Staph*. non-*chromogenes* (Adj. Prob. (SE): 0.24 (0.06), *Strep.*-like organisms (Adj. Prob. (SE): 0.16 (0.06)) and unspeciated members of the *Strep.* genus (Adj. Prob. (SE): 0.32 (0.10), $P<0.05$) but not when compared to *Strep. dysgalactiae* (Adj. Prob. (SE): 0.53 (0.10)) or *Strep. uberis* ($P>0.05$).

Conclusions

Our results indicate that different bacterial species may exhibit divergent epidemiological behavior in terms of persistence in the mammary gland. Specifically, *Staph. aureus* showed higher odds of persistence than other bacterial groups, except for *Staph. chromogenes* and *Strep. uberis*. This may have implications for our understanding of the epidemiology of mastitis-causing microorganisms, prognosis of infected quarters and decision-making in dairy farms. These results are especially crucial for organic-certified dairy farms in which antibiotics are not used for herd-level management of mastitis.

Financial Support
U.S. Department of Agriculture

Notes:

71 - Examining the canine infectious disease risks associated with importing dogs from Asia into Canada

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Session: Epidemiology - 3, Dec. 6, 11:30 - 11:45 AM

Objective

Every year thousands of dogs are imported into Canada. With minimal regulations controlling this practice, imported dogs have been shown to contribute to the introduction of foreign or uncommon pathogens. The 2017 outbreak of canine influenza virus A(H3N2) in Ontario was traced back to several dogs imported from South Korea and demonstrates the imminent threat posed by canine importation. The objective of this study was to estimate the prevalence of selected zoonotic and canine-specific pathogens in dogs recently imported from Asia.

Methods

Nasal swabs, fecal and serum samples were collected from dogs in Ontario that had been imported from Asia within the last two weeks. Samples were tested for a variety of pathogens including vector-borne pathogens, *Brucella canis*, extended spectrum beta lactamase (ESBL) producing Enterobacteriaceae, canine distemper virus, canine influenza virus, canine parainfluenza virus, canine adenovirus type 2, and canine herpes virus.

Results

To date, samples have been collected from 59 dogs. Majority of the sampled dogs (96.6%) were from South Korea. Preliminary review of the data illustrates that a third of dogs sampled were carrying at least one infectious pathogen. 13 dogs (22.4%, n=58) were positive for the parasite *Dirofilaria immitis*. 2 dogs (3.9%, n=51) tested positive for the zoonotic pathogen *Brucella canis*. These dogs came from a larger shipment, and further cases of canine brucellosis in Canada and the United States were then identified. 9 dogs (37.5%, n= 24) tested positive for carrying ESBL producing bacteria.

Conclusions

Under current import regulations, dogs imported from Asia are a source of infectious pathogens. Diseases identified in imported dogs from this study are a concern for both animal and human health. Further sampling and expansion of this project to other regions is necessary to understand the infectious disease risks posed by importing dogs from various global regions.

Financial Support
Zoetis; Ontario Animal Health Network; OVC Scholarship

Notes:

72 - Prevalence of bacteremia in clinically healthy dairy calves

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Session: Epidemiology - 3, Dec. 6, 11:45 - 12:00 PM

Objective

Diarrhea is a leading cause of death in pre-weaned dairy calves, which is often a result of intermittent bouts of septicemia. Antimicrobial use recommendations were based on a high prevalence of bacteremia in diarrheic calves; however, in preliminary research focusing on heifer calves with diarrhea, 9.3% (10/108) of diarrheic calves and 14.8% (4/27) of clinically healthy calves were bacteremic. This finding may suggest that clinically healthy calves experience intermittent bouts of bacteremia due to high intestinal permeability early in life. The objective of this study was to determine if clinically healthy neonatal calves experience bouts of bacteremia. We hypothesized that younger calves would be more likely to experience bouts of bacteremia due to higher intestinal permeability when compared to older calves.

Methods

Healthy calves were enrolled based on health scoring criteria including temperature, dehydration, navel score, fecal score, and depression. Aseptic jugular and saphenous venous blood samples were collected from the same calves at 2-7 days of age and again at 21-27 days of age. Isolates from positive blood culture bottles were evaluated with mass spectrometry to determine bacterial species.

Results

Bacterial isolates recovered from calves aged 2-7 days included *Bacteroides fragilis, E. coli, Enterococcus faecalis, Streptococcus uberis* and *Trueperella pyogenes*. Isolates at 21-27 days of age included *Bacteroides fragilis, Bacteroides pyogenes, Campylobacter fetus*, *Fusobacterium necrophorum*, and *Trueperella pyogenes*. There was not a significant difference between the prevalence of bacteremia in healthy calves at 2-7 days of age is 22.2% (10/45) and at 21-27 days of age is 12.5% (5/40) ($P = 0.5$).

Conclusions

This study demonstrated that healthy neonatal dairy calves do experience bouts of bacteremia. Calves were not more likely to be bacteremic at 2-7 days of age when compared to 21-27 days of age. However, further research is needed to better understand the mechanism of bacteremia in clinically healthy dairy calves.

Notes:

73 - The canine host serves as a sentinel species for *Anaplasma*, *Ehrlichia*, and *Borrelia* species infections in people

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Session: Public Health, Dec. 6, 8:30 - 8:45 AM

Objective

Tick-borne diseases continue to threaten the health of people and dogs. In the USA, human Lyme disease cases caused by *Borrelia burgdorferi* are the highest followed by diseases resulting from *Ehrlichia* and *Anaplasma* species pathogens. We investigated the prevalence of these diseases in dogs and compared with the human data available at the CDC.

Methods

Clinically suspected dog blood samples from across the US were assessed for pathogen-specific antibodies. An ELISA was performed for *B. burgdorferi*, while IFAs were performed for *E. chaffeensis, E. canis,* and *A. phagocytophilum* antibodies.

Results

A total of 1,340 samples were assessed. Two hundred and eighty six (21.3%) samples tested positive for *A. phagocytophilum*, 228 (16.9%) for *E. chaffeensis,* 233 (17.3%) for *E. canis,* and 366 (27.2%) for *B. burgdorferi*. Some of the *Ehrlichia* positives are likely the result of antigenic cross-reactions between the two species. Similarly, some of *A. phagocytophilum* positives may represent cross-reactions with the *A. platys* positives. Co-infections with both *Anaplasma* species and *E. chaffeensis* were observed in 65 dogs; 64 dogs tested positive for both *Anaplasma* species and *E. canis*; 76 dogs were double-positive for *Anaplasma* species and *B. burgdorferi*; 34 for *Ehrlichia* species and *B. burgdorferi*; and 8 dogs positive for all three species. We observed a significant overlap in the geographical distribution for the tick-borne disease prevalence in dogs with those reported for people.

Conclusions

Our data suggest the occurrence of tick-borne diseases in dogs is very similar to documented human cases. Thus, monitoring canine infections has important implications for both human and companion animal health.

Financial Support
Abaxis Inc. (Zoetis), Union City, CA

Notes:

74 - Initial data on the molecular epidemiology of cryptosporidiosis in humans and cattle in Akkar, Northern Lebanon

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Session: Public Health, Dec. 6, 8:45 - 9:00 AM

Objective

Cryptosporidium apicomplexan protozoa are ubiquitous intracellular agents affecting humans and animals worldwide. However, little is known regarding the transmission and maintenance of these pathogens at the wildlife-livestock interface. To improve our understanding of the epidemiology of cryptosporidiosis in Lebanon, the main aim of this study was to determine the prevalence and the genetic diversity of *Cryptosporidium* in both human and cattle populations living in the rural region of Akkar in North Lebanon.

Methods

Fecal specimens were collected from 100 randomly selected hospitalized patients in different medical departments of two hospitals (El-Youssef Hospital Center and Rahal Hospital), and from 153 Holstein cattle from 33 farms or barns, in the Akkar governorate. Samples were analyzed by both microscopical observations of Ziehl-Neelsen stained slides and 18S rRNA nested PCR. To identify *Cryptosporidium* species, positive PCR products were purified and sequenced. Specimens genotyped as *C. parvum* or *C. hominis* were further subtyped using a second nested PCR, which amplifies a fragment of the 60 kDa glycoprotein (gp60) gene.

Results

The overall prevalence of *Cryptosporidium* spp. infection obtained by molecular analysis was 5% and 8% in humans and cattle, respectively. Among *Cryptosporidium* isolates in humans, 80% were identified as *C. hominis*, while 20% were identified as *C. parvum*. In cattle, *C. andersoni* was predominant (50%) followed by *C. bovis* (33%) and *C. parvum* (17%). After analysis of the *gp60* locus, *C. hominis* IdA19, a rare subtype, was found to be predominant in humans. Two *C. parvum* subtypes were found in common among humans and cattle: IIaA15G1R1 and IIaA15G2R1.

Conclusions

This study presents the first molecular epidemiological data on *Cryptosporidium* spp. infection in cattle livestock in Lebanon. Sequencing revealed the presence of three different *Cryptosporidium* species. Even if the molecular evidence predicts that anthroponotic transmission is important in the Akkar governorate, livestock seems to play a role as a zoonotic reservoir of this parasite.

Notes:

75 - *Clostridioides difficile* on dairy farms and potential risk to dairy farm workers

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Session: Public Health, Dec. 6, 9:00 - 9:15 AM

Objective

Clostridioides difficile causes severe colitis in people and is a significant enteric pathogen in many species of animals, including swine, horses, and potentially cattle. *C. difficile* is shed in feces, and transmission occurs horizontally via the fecal-oral route. Livestock has been suggested as a potential reservoir for *C. difficile*, and while studies have shown that swine and farm workers can be colonized with identical clones of *C. difficile*, the zoonotic transmission of *C. difficile* from livestock to people has not been definitively demonstrated. The goal of this study was to determine whether dairy calves and dairy farm workers harbored genetically similar isolates of *C. difficile*.

Methods

First, we validated a glove juice protocol for detecting *C. difficile* on farm workers' hands. We then visited 23 farms and collected 1) fecal samples from 92 dairy calves, 2) hand rinsates from 38 dairy farm workers, and 3) fecal samples from five of the dairy farm workers who were willing to submit them. All samples underwent anaerobic culture and qPCR to detect *C. difficile*.

Results

C. difficile was detected on 15 of the farms (65.2%, 95% confidence interval (CI)I 42.7%-83.6%) and in 28 calves (30.4%, 95% CI 21.2-40.9%) but in none of the hand rinsates (0%, 95% CI 0.0-92.2%) or human fecal samples.

Conclusions

The zoonotic transmission of *C. difficile* on dairy farms could not be demonstrated, and dairy farmers did not appear to be at increased risk of acquiring C. difficile via the fecal-oral route.

Notes:

76 - Isolation and characterization of enteric bacteria from abattoir effluent in Abuja, Nigeria

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Session: Public Health, Dec. 6, 9:15 - 9:30 AM

Objective

Effluent discharges from abattoirs in Nigeria are mostly not treated before being released into water bodies thereby constituting grave danger to communities depending on them as sources of water supply. This study was carried out to determine and characterize the enteric bacteria status of the abattoir effluent and it's receiving nearby surface water in Abuja, Nigeria.

Methods

A total of 106 samples were collected from two selected abattoirs (A and B) and their receiving water bodies from three points namely; 100 meters before the discharge of effluent, at the point of discharge and 100 meters after the discharge. The samples were analyzed using colony counter to determine the total bacterial counts (TBC). Bacteria species were then isolated, characterized and identified using standard microbiological and biochemical techniques. Confirmation of the presence of the enteric organisms was conducted using PCR.

Results

The average total bacteria count on abattoir A was 2.8×10^2 cfu/ml at the 100-meter point before the discharge; 3.6×10^6 cfu/ml at the point of discharge and 3.0×10^6 at the 100-meter point after discharge. For abattoir B, the average TBC was 2.0×10^2 cfu/ml at the 100-meter point before the discharge; 4.3×10^6 cfu/ml at the point of discharge and 3.8×10^6 at the 100-meter point after discharge. The isolates reacted to standard biochemical tests typical of enteric bacteria. The organisms identified through cultural analysis were *E. coli* O157, *Salmonella spp* and *Shigella spp*. Further characterization using PCR confirmed *E. coli* and *Salmonella spp* as enteric organisms in the effluent.

Conclusions

The result provided evidence that enteric pathogens present in abattoir effluent can contaminate water bodies and environment as a result of discharge. There is need for proper treatment and safe disposal of abattoir effluents in the study area.

Notes:

77 - Safety first: Needs assessment reveals adoption of COVID-19 mitigation strategies in the US food industry

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Session: Public Health, Dec. 6, 9:30 - 9:45 AM

Objective

The objectives of this study were to determine what is needed by the United States (US) food industry to control COVID-19 impacts in the work environment and what mitigation strategies are being implemented.

Methods

A web-based needs assessment survey was implemented in Qualtrics and distributed from January 19 to April 6, 2021, through 13 food industry organizations and two social networks. The survey targeted management professionals at labor-intensive farms and food processing companies with facilities in the US. Non-parametric tests were used to identify predictors associated with the outcomes of interest, namely (i) the self-reported adoption of mitigation strategies against COVID-19 in participant's facilities and (ii) perceived needs of the food industry regarding COVID-19. Responses to open-ended questions were analyzed using thematic analysis.

Results

A total of 145 responses were received with 79 considered to be usable, including 38 (48%) from the dairy industry sector, 17 (22%) from fresh produce, and 24 (30%) from other food industry sectors. Only two usable responses were from the beef/pork sector and none from the poultry sector. Findings revealed that several social distancing, biosecurity, and surveillance mitigation strategies against COVID-19 have been commonly implemented in the participants' facilities, but how frequently they are implemented is influenced by facility size and industry sector. Participants indicated that collaboration between the food industry and government agencies, the establishment of contingency plans and appropriate training, and the development and implementation of new technologies are needed to control COVID-19 in the food industry.

Conclusions

Although affected by a low response rate, findings from this study suggest that the US food industry sectors represented in the survey are prepared to safeguard their workers and businesses through the COVID-19 pandemic and in the event of a similar future disaster. However, appropriate infrastructure needs to be developed to ensure coordination and compliance before and during the disaster event.

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

Notes:

78 - The application of artificial intelligence to detect disease outbreaks in swine farms

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Session: Public Health, Dec. 6, 9:45 - 10:00 AM

Objective

The introduction of a pathogen into a population can cause devastating losses in swine farms. Thus, early detection of disease plays an essential role in limiting the effects of a pathogen introduction. While the use of routine lab-based molecular testing can aid in rapid detection, the number of samples required, and their expense make frequent use unrealistic. For these reasons, researchers continue to develop additional tools to aid in disease detection. Statistical process control utilizes production data to detect disruptions in farm performance as a signal of disease. The sensitivity of this method is low because of the inherent biological variation present in swine production. This project proposes a predictive tool that overcomes the limitations of inherent biological variation by utilizing machine learning to detect disease quickly and accurately within a population.

Methods

This project describes a tool that facilities sensitive syndromic surveillance for sow farms by applying machine learning to sow production records to predict the outcome of a breeding event. The tool predicts which events will yield piglets and subsequently monitors the outcome of the breeding event. If more breeding events result in failure than expected, the model signals a disruption. To compare the sensitivity of our tool to the established SPC approach, retrospective data from two sow farms that experienced a PRRSv introduction were assessed.

Results

While both the machine learning tool and SPC detected PRRSv introduction on each farm, average detection was 1&3 weeks before the farm reported a disease event using the machine learning-based method and 2 weeks after and 1 week before using SPC. The machine learning approach also identified production disruption resulting from changes to the electronic sow feeding system. SPC failed to identify this disruption.

Conclusions

These two test cases demonstrate that our novel machine learning-based method maybe more sensitive for the surveillance of swine farms for disruptions to average production compared to previously described approaches.

Notes:

79 - Photocatalytic oxidation decontamination of indoor air, a brief review

L. Pantaleon. lucaspantaleon@gmail.com
Session: Biosecurity & Infection Control, Dec. 6, 10:30 - 10:45 AM

Objective

Clean air is a basic requirement for life. Indoor air is a complex media and obtaining good indoor air quality requires a multipronged approach. In people and animals, airborne disease spread is influenced by season, temperature and humidity, ventilation system, number of people and animals, and how often doors are opened or people move. Mitigation strategies such as ventilation, filtration or photocatalytic oxidation, decontaminate the air reducing the concentration of pathogens and making indoor spaces safer.

Methods

Literature review

Results

Titanium dioxide is a photocatalytic material and when irradiated with ultraviolet light leads to the promotion of electrons from the valence band to the conduction band leaving positive holes behind. The positive holes and electrons can be trapped by adsorbed molecules of oxygen and water forming the high reactive hydroxyl radicals and superoxide radicals. These highly reactive oxygen species induce oxidative stress to microorganisms and death.

Conclusions

Last generation PCO technologies that are safe and effective can add an additional layer of protection to decontaminate the indoor air and surfaces. Photocatalytic oxidation was shown to lower infective viral particles in the air, this likely minimizes the chances for virions to be inhaled, contact mucous membranes or contaminate surfaces are reduced.

Notes:

80 - Efficacy of a photocatalytic oxidation device in decreasing airborne pathogens

L. Pantaleon. lucaspantaleon@gmail.com
Session: Biosecurity & Infection Control, Dec. 6, 10:45 - 11:00 AM

Objective

Aerosol transmission of pathogens indoor is an important source of infection for humans and animals. Decreasing the concentration of respiratory pathogens, as part of a layered set of infection prevention strategies, is key to minimize contagion. Improving indoor air quality via engineered controls such as ventilation, filtration and air ionization devices can make indoor areas safer.
Except for human hospitals, most building's current ventilation and filtration standards are not designed for infection control. It is currently recommended to have 4 to 6 air exchanges per hour in order to reduce transmission of SARS-CoV-2 indoors, however this could be difficult to achieve.
Photocatalytic oxidation (PCO) is a type of air decontamination technology that generates highly reactive oxygen species and ions that are released into the air inducing irreversible microbe damage and death.

Methods

MS2 bacteriophage was aerosolized in a sealed stainless-steel chamber containing the PCO device. Hourly air samples were obtained for 7 hours to quantify MS2 reduction rate of the PCO device. The ion concentration in the chamber was also measured. Statistical analysis was not performed for this small study.

Results

The PCO device yielded a net log reduction for MS2 of 2.23 (99.4% reduction). The ion concentration was 400 ions/cm^3.

Conclusions

The study demonstrated that PCO could be an effective component of a layered strategy to decrease airborne pathogens.

Financial Support
Ogena Solutions

Notes:

81 - Feed dust as a novel diagnostic sample type for detection of African swine fever virus: Proof of concept

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Session: Biosecurity & Infection Control, Dec. 6, 11:00 - 11:15 AM

Objective

African swine fever virus (ASFV) is stable in feed ingredients and there is evidence supporting the role of feed in ASFV introduction risk and transboundary spread. However, diagnostic tools for surveillance and detection of ASFV in feed remains an industry challenge. Feed dust has demonstrated sensitivity for detection of other bacterial and viral pathogens, such as Salmonella and PEDV. Hence, the objective of our study was to evaluate feed dust as a novel diagnostic sample type for ASFV detection in experimentally contaminated feed.

Methods

Feed dust samples were collected opportunistically during four independent *in vivo* studies investigating the efficacy of feed additives for reducing ASFV infectivity to pigs during natural consumption. Moist swabs were used to collect dust from creep feeders after natural consumption of feed inoculated with 3.1–5.4 $\log_{10}$ $TCID_{50}$/g ASFV Georgia 2007 in the presence and absence of antimicrobial feed additives. Swab samples were processed and supernatant from each sample were tested for the presence of viral DNA and infectious virus.

Results

Detection of ASFV on PCR and VI in dust swabs collected from ASFV-inoculated feed varied across the four studies depending on inoculation dose, environmental exposure, and antimicrobial feed additives inclusion. ASFV genome was detected in all dust swabs collected between 30 and 480 minutes after ASFV inoculation of feed (5.1-5.4 $\log_{10}$ $TCID_{50}$/g) indifferent to the presence or absence of feed additives. However, infectious ASFV was only detected in dust swabs collected 30 min after ASFV inoculation of feed (5.4 $\log_{10}$ $TCID_{50}$/g) in the absence of feed additives. ASFV titres of the positive dust swab supernatants ranged between 2.6 and 2.9 $\log_{10}$ $TCID_{50}$/ml.

Conclusions

Results validate the potential use of feed dust swabs as a novel diagnostic surveillance tool for detection and quantification of viral nucleic acid and infectious virus titre in ASFV-contaminated feed.

Financial Support
State of Kansas; State of Kansas National Bio and Agro-defense Facility Fund and Purina Animal Nutrition and Kemin Industries

Notes:

82 - Evaluation of air filters in swine farms as a surveillance method to assess the spread of airborne viruses

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Session: Biosecurity & Infection Control, Dec. 6, 11:15 - 11:30 AM

Objective

Porcine reproductive and respiratory syndrome virus (PRRSV) and influenza A virus (IAV) are two top respiratory pathogens affecting pigs in the US. In the Midwestern US, air filtration of incoming air is being used to effectively reduce the incidence of airborne PRRSV infections in breeding herds. The objective of this study was to evaluate the use of air filters as a surveillance strategy to monitor the regional spread of PRRSV and IAV, and to enhance our understanding of the epidemiology and control of airborne diseases.

Methods

We selected 7 breeding herds from high pig density areas which were either PRRSV negative or stable at the beginning of the study. Twenty brand new air filters were installed in each farm, and five filters were removed each time at approximately 6, 8, 11- and 14-months post installation. Five samples of six square inch were cut from each filter, and these samples were processed and tested by real time RT-PCR for PRRSV and IAV. A filter was considered positive if at least one sample tested positive. Samples positive for PRRSV or IAV were further analyzed by whole genome sequencing.

Results

A total of 136 air filters were received. Filters were installed in July 2019 at the earliest, and removed successively until October 2020. These filters were cut into 680 samples for testing. Out of the 136 filters, ten (1.5%) samples corresponding to seven (5%) filters from three farms tested positive for PRRSV. During the study, PRRS outbreaks were reported in four farms, however, only one positive filter originated from farms that had PRRSV positive filters. In contrast, sixty-five (47.8%) filters from all seven farms tested positive for IAV, with a total of 131 samples positive (19.3%). Six IAV positive samples were sequenced and one sample was successfully subtyped as H3N2 human-like influenza virus. In addition, multiple lineages were identified for the influenza internal genes from different samples.

Conclusions

Testing of used air filters in swine farms did not result in enhanced surveillance methods for airborne PRRSV. However, used filters for influenza surveillance should be further evaluated. Overall, detection of PRRSV and IAV in the air filters showed some potential evidence of airborne transmission for these viruses, but additional investigations are needed to better understand the factors that contribute to airborne transmission of these viruses.

Financial Support
National Pork Board

Notes:

83 - Development of capture antigens and functionalization procedures for sensor-based bovine health screening

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Session: Biosecurity & Infection Control, Dec. 6, 11:30 - 11:45 AM

Objective

This research seeks to develop antibody capture assays suitable for adaption to cost-effective electronic (potentiometric and electrochemical impedance) sensor-based systems allowing for on-farm screening and detection of bovine infections of key economic importance.

Methods

Immuno-dominant surface protein antigens for a range of infectious bovine viruses were recombinantly expressed for application in serologic diagnostic assays. Expressed proteins were designed based on consensus sequences constructed from reported virus strain genes. Western blot and ELISA assays were used to characterize the efficacy of recombinant protein antigen synthesis and to assess immune specificity. Multiplexed biosensors were designed and fabricated for use in electronic biosensing with chronoamperometry used to electrodeposit gold foam nanostructures on the surface of working electrodes. Biosensors were characterized using Cyclic Voltammetry (CV), Atomic Force Microscopy (AFM), and Scanning Electron Microscopy (SEM). Surface Plasmon Resonance (SPR) platforms were used to develop capture antigen functionalization approaches amenable for use in electronic biosensing.

Results

Recombinant expression produced sufficient levels of viral protein antigens (BPI-3 HN, BVDV NS3, BRSV gF, BHV-1 gE/gB, BLV gp51) at required purity to allow for incorporation into developed electronic biosensor assay platforms. Terminal His-tag regions were incorporated within recombinant viral protein sequences to facilitate assay sensor development, with covalent attachment of capture antigen via amine coupling or oxidation stabilized cobalt-NTA interaction shown to enable stable functionalization of sensors and potential to develop reusable assay systems.

Conclusions

SPR has been used to develop immobilization procedures to enhance the level of capture antigen on the sensor surface and will be translated to electrochemical biosensors. The fabricated sensor design increases the biosensing operational efficiency by enabling multiple immunologic assays to be run on single chips. The porous gold electrodes show an increase in the electroactive surface area which amplifies the measured current density, thereby increasing biosensor sensitivity.

Financial Support
U.S. Department of Agriculture, Animal and Plant Health Inspection Services

Notes:

84 - Biofilm forming abilities of *Salmonella* serovars isolated from clinically ill livestock at 48 and 168 hrs

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Session: Biosecurity & Infection Control, Dec. 6, 11:45 - 12:00 PM

Objective

Biofilm formation is a microbial strategy for environmental survival, and increased biofilm density has been correlated with decreased biocide efficacy in Gram-negative pathogens. Although *Salmonella* biofilms have received attention in food processing environments, little is known regarding the biofilm forming capabilities of a somewhat distinct population of *Salmonellae* responsible for illnesses in livestock and humans. Indeed, evaluation of cleaning and disinfection in preharvest environments has found little success in eradicating *Salmonella* biofilms to date. Disrupting the environmental survival of *Salmonella* via biofilm removal, particularly for virulent strains with abundant biofilm growth, will be critical to reducing carriage in livestock reservoirs and the risk of foodborne illness in humans. Therefore, the objective of this study is to characterize the biofilm forming abilities of *Salmonellae* relevant to livestock and human health. We hypothesized that biofilm density varies between serovar and will increase from 48 to 168 hrs.

Methods

Sixty isolates from 8 serovars (*S.* Typhimurium, Heidelberg, Montevideo, Agona, Newport, Dublin, I 4,[5],12:i:-, Enteriditis) were sourced from clinically ill poultry, cattle, swine, and equine. Isolates were grown in 24-well microplates in tryptone soy broth at ambient temperature. Crystal violet assays were used to quantify biofilm density at 48 and 168 hrs.

Results

All isolates formed a biofilm by 168 hrs., with 70% (42/60) of isolates categorized as strong biofilm formers and 11.7% (7/60) considered weak, of which six were *S.* Dublin. The median biofilm density was greater at 168 hrs. relative to 48 hrs. ($p < 0.005$).

Conclusions

These results suggest inconsistent (i.e., weekly) cleaning may allow for the establishment of biofilms in on-farm environments. This study provides baseline data necessary to inform the development of evidence-based cleaning and disinfection protocols effective against the most prolific biofilm forming strains of virulent *Salmonella.*

Notes:

85 - Sustained systemic immune response following subcutaneous administration of a solid phase fat-encapsulated vaccine

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Session: Vaccinology - 1, Dec. 6, 8:30 - 8:45 AM

Objective

We assessed the efficacy of solid-phase fat-encapsulated vaccines administered once under the skin in conjunction with a soluble vaccine to stimulate antigen-specific immunity in mice.

Methods

In Trial 1, mice were injected with either PBS administered subcutaneously (s.c.; control group) or with Cy5- OVA with Emulsigen-D (*OVA-Em; sOVA). Two groups were also administered a solid-phase vaccine under the skin consisting of *OVA-Em coated with 80 µl of melted palmitate (PA-*OVA-Em) or stearate (SA-*OVA-Em), respectively, which immediately solidified.
In Trial 2, mice were injected s.c. with either PBS (control group) or with *OVA+Em + Poly I:C (*OVA-Adj); sOva). Another control group received a second s.c. *OVA-Adj vaccine 2 weeks later (sOva2X). Two groups were administered PA and SA-coated vaccines under the skin with the new adjuvants (PA-*OVA-Adj and SA-*OVA-Adj).
Blood samples were taken on days 0, 15, 30, 45 and 60 to estimate the anti-OVA IgG1 and Ig2a titres. Mice were imaged weekly to detect *OVA. On day 60, all mice were humanely euthanized to collect their spleens to measure IFNγ levels.

Results

Trial 1. In comparison to sOva group, we observed a significant increase in anti-OVA IgG1 in PA-*OVA-Em group at days 45 and 60 but only at day 60 in SA-*OVA-Em. However, anti-OVA IgG2A titres were significantly increased at day 45 in both the PA-*OVA-Em and SA-*OVA-Em groups.
Trial 2. Mice from both PA-*OVA-Adj and sOVA2X groups had higher anti-OVA IgG1. A significant increase in anti-OVA IgG2A was only observed in the SA-*OVA-Adj group at day 60.
IFNγ recall response was evident in PA-*OVA-Em group (Trial 1) and fat pellet and sOva2X groups (Trial 2), suggesting induction of cell-mediated immunity. Imaging showed that the *OVA was visible in the pellets under the skin for at least 52 days post insertion.

Conclusions

Our results indicate that coupled with s.c. injection of the same vaccine, a solid-phase fat-encapsulated vaccine inserted under the skin prolonged the release of the vaccine and triggered a comparable immune response to the mice that received the liquid two injections.

Financial Support
Natural Sciences and Engineering Research Council of Canada

Natural Sciences and Engineering Research Council of Canada | Conseil de recherches en sciences naturelles et en génie du Canada

Notes:

86 - Nanoplatform vaccine for mucosal and systemic immunogenicity by intranasal route against infectious disease.

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Session: Vaccinology - 1, Dec. 6, 8:45 - 9:00 AM

Objective

Nasal delivery provides many benefits over parenteral approaches to vaccine injection. Mucosal immunization by intranasal administration offers a more effective defense against human and animal pathogens infected via mucosal pathways than traditional immunizations. These include ease of vaccination without needles and offer high immune responses both in the humoral and cellular immunity.

Methods

We synthesized a mucosal vaccine system based on nanovaccines (NVs) for enhancing mucus delivery of antigens (Ags) of respiratory viruses. Combinations of NVs–Ags were administered intranasally two times at 2-week intervals to female BALB/c mice. Virus-specific antibody enhancement was assessed by ELISA and SN test.

Results

Amine groups of NVs with Ags induce strong interaction with sialic acid-rich mucin, resulting in sufficient time for Ag mucus delivery and improved Ag-presenting cells delivery efficiency. Vaccine formulated with NVs induces high Ag-specific antibody-mediated immune responses *in vivo.*

Conclusions

Nanoplatform based Ag delivery systems, therefore, have potential as mucosal adjuvants and merit further research.

Financial Support
National Research Foundation of Korea

Notes:

87 - Evaluation of immune responses of vaccines administered to mice uteri

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Session: Vaccinology - 1, Dec. 6, 9:00 - 9:15 AM

Objective

Our lab has shown that vaccines administered to uterus can trigger an immune response but the mechanism for how this is achieved is not clear; the immune system of the upper reproductive tract lacks a mucosal-associated lymphoid tissue normally considered critical for immune induction. We want to understand precisely how antigens introduced to the uterus are taken up and presented to lymphocytes to promote the adaptive immune response and the location where it takes place. The objective of this study is to establish the timing of migration of vaccines across the uterine wall and which adjuvants promote the vaccine movement to draining lymph nodes.

Methods

We will administer 20µL of Cy5-labelled OVA mixed with different adjuvant combinations of; PolyIC, PolyIC Chitosan Nanoparticles and a triple combination of PolyIC, HDP, Polyphosphazene (1:2:1), into the uterine horn of mice to determine whether OVA moves to draining lymph nodes or remain in the uterine, and which adjuvants contribute to increase movement out of the uterine lumen. Both the uterine tissues and lymph nodes will be harvested for immunohistochemistry and will also be digested into single cell suspension for flow cytometry analysis. Cells will be stained for DC cells, macrophages and B cells for flow cytometry analysis.

Results

We anticipate that OVA will not be present in the epithelial cells lacking MHCII, but will be present in the dendritic cells. We predict that macrophages and B cells will play minor roles in antigen presenting.

Conclusions

This experiment will allow us to understand precisely how antigens introduced to the uterus are taken up and presented to lymphocytes to promote the adaptive immune response and the location where it takes place. This knowledge will allow us to formulate vaccines to target these cells, which will help tailor an intrauterine vaccine platform that will protect against infectious diseases.

Financial Support
Natural Sciences and Engineering Research Council of Canada; VIDO receives operational funding from the Government of Saskatchewan through Innovation Saskatchewan and the Ministry of Agriculture and from the Canada Foundation for Innovation through the Major Science Initiatives for its CL3 facility.

Natural Sciences and Engineering Research Council of Canada | Conseil de recherches en sciences naturelles et en génie du Canada | VIDO

Notes:

88 - Enterobactin-based immune interventions against Gram-negative bacterial infections in chickens

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Session: Vaccinology - 1, Dec. 6, 9:15 - 9:30 AM

Objective

A novel Enterobactin (Ent) conjugate vaccine was recently developed with significant potential to prevent and control various Gram-negative bacterial infections in livestock. In this study, using both *in vitro* and *in vivo* systems, we comprehensively evaluated the efficacy of different Ent-based immune intervention strategies against *Escherichia coli* and *Campylobacter* infections in chickens.

Methods

The inhibitory effects of specific anti-Ent antibodies on the *in vitro* growth of diverse *E. coli* (n=27) and *Campylobacter* strains (n=6) were examined in conjunction with in-depth metabolomics and genomics analyses of the tested strains. Using different vaccination regimens, the immunogenicity and protective efficacy of the Ent conjugate vaccine against *C. jejuni* and avian pathogenic *E. coli* (APEC) were evaluated. Laying hens were immunized with the Ent conjugate vaccine to produce anti-Ent egg yolk IgY. The hyperimmune egg yolk powder was supplemented in chicken feed (2%, w/w) to assess the effects of passive immune protection against *C. jejuni*. The stability of IgY in chicken gastrointestinal tract was examined using both *in vivo* and *ex vivo* systems.

Results

The Ent-specific antibodies significantly suppressed the growth of each tested strain under iron-restricted conditions. Both intramuscular and subcutaneous vaccinations consistently induced strong Ent-specific immune responses (up to 1,024 fold increase) when compared to control group in five independent trials. Consistent with the strong immune responses upon vaccination, *C. jejuni* colonization was significantly reduced by 3-4 $\log_{10}$ units in the cecum and the lesions in different chicken organs caused by APEC were significantly alleviated. Large amount of specific anti-Ent egg yolk was successfully produced. However, the hyperimmune egg yolk powder failed to confer passive immune protection against *C. jejuni* colonization due to substantial degradation of the IgY in gizzard.

Conclusions

The Ent-based immune intervention is a novel and effective strategy to control *C. jejuni* and APEC, the two pathogens significant in poultry health and food safety.

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

Notes:

89 - Influenza, COVID-19 and the quest for better vaccines.

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Session: Vaccinology - 1, Dec. 6, 9:30 - 9:45 AM

Objective
Outbreaks of influenza A virus can have devastating effects in poultry flocks and swine herds due to high morbidity and mortality resulting in substantial economic costs and threatening of food security. Seasonal and pandemic influenza strains are constant reminders of the significance of influenza infections in humans. Diversely, the COVID-19 pandemic caused by the newly emerged SARS-CoV-2 virus has resulted in millions of human deaths and declining economies around the world. Understanding how these different viruses cause disease is essential to developing more efficient interventions. The major objectives of the Perez lab are to better understand the molecular mechanisms of pathogenesis elicited by influenza viruses and SARS-CoV-2 using natural and laboratory animal models of disease and develop and test alternative vaccines and antivirals.

Methods
Discussion will be focused on the development of influenza viruses with rearranged genomes (RANS, RAM, and RAM42) as potential live attenuated virus vaccines. Reverse genetics was used to generate vaccine candidates in avian- and swine/human-origin internal gene segment backbones carrying a chimeric segment 2 encoding the polymerase subunit PB1 and either the NS2/NEP, the M2, or the M42 genes. Modifications were also introduced in either segment 8 or 7 to prevent expression of NS/NEP or M2/M42, respectively. The rearranged strategies complement our previously developed att backbone. In addition, the HA and NA segments have been modified to carry molecular markers and/or immunomodulatory adjuvants with the aim of promoting improved mucosal immunity. Furthermore, a recombinant influenza virus was produced encoding the RBD region of the S glycoprotein of SARS-CoV-2. The RBD region is a major target of neutralizing responses against SARS-CoV-2. The recombinant influenza-RBD virus is aimed as a dual purpose vaccine. The above has been complemented with studies of the microbial environment within the oropharyngeal and fecal microbiome of broiler chickens infected with an H9N2 subtype influenza virus and the lung and cecum microbiome of K18-hACE2 mice infected with a SARS-CoV-2 virus.

Results
Vaccine candidates of the H5N8 and H9N2 subtype were obtained in the RANS, RAM, and RAM42 platforms. Efficient rescue of RAM-H1N1 and RAM-H3N2 viruses was also observed. In addition, highly stable attenuated viruses were produced carrying the gene encoding the IgA-inducing protein (IGIP) in the att background. These viruses were stable and grew to similar levels in comparison to the wild type (wt) viruses. Mice vaccinated with either the IGIP-H1att or the RAM-H1N1 strain were completely protected from challenge with a 10,000-mouse lethal dose 50 of a prototypic pandemic 2009 H1N1 strain. Serum and bronchoalveolar lavage samples from the IGIP-H1att group showed trends towards increased stimulation of IgG and IgA responses compared to other control samples. Fecal microbiome analyses revealed similar composition among infected and not infected chickens at 21 days post-infection but comparatively different among earlier days post-infection. Similarly, the intestinal microbiome of K18-hACE2 mice infected with SARS-CoV-2 showed decreased Shannon and Inv Simpson diversity index correlating with infection dosage and a difference of Bray-Curtis dissimilarity distances among control and infected mice.The lung microbiome of SARS-CoV-2 infected mice showed limited diversity changes but a shift from Bacteroidetes to increased Firmicutes and Proteobacteria.

Conclusions
The findings support the goal of generating LAIVs based on genome rearrangement in addition to other attenuation strategies. Microbiome analyses identified changes in two different animal models throughout infection with distinct respiratory viral pathogens. A better understanding of microbiome changes and evaluation of natural adjuvants in the context of live attenuated viruses will aid in the development of safe and highly efficacious vaccines.

Financial Support
U.S. Department of Agriculture; U.S. National Institute of Allergy and Infectious Diseases; Caswell S. Eidson Endowment Funds, University of Georgia

Notes:

90 - Transcriptomics of a ranavirus reveals non-coding regulatory role of intergenic regions

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Session: Vaccinology - 1, Dec. 6, 9:45 - 10:00 AM

Objective

Ranaviruses of the family *Iridoviridae* infect amphibians, reptiles and fish, posing threats on ecological biodiversity and aquaculture production. Previous studies on ranaviral genomes have mainly focused on coding genes and rarely addressed non-coding regulatory role of intergenic regions. In this project, we aimed to characterize transcription of non-coding regulatory elements encoded in intergenic regions of a ranavirus FV3 genome and infer their potential regulatory role in virus-host interaction.

Methods

We conducted a whole transcriptomic analysis of samples containing both viral and cellular transcripts from frog tissues infected with frog virus 3 (FV3), a prevalent ranavirus pathogen serving as representative model. RNA-Seq library prepared from RNA samples were processed using the Illumina pipeline. Further bioinformatic analyses were used to identify transcripts reads non-coding regulatory elements in the intergenic regions.

Results

Using a whole transcriptomic analysis, we detected virus-specific reads mapping in non-coding intergenic regions. We also identified multiple cis-regulatory elements (CREs) in intergenic regions neighboring coding genes. These CREs include a TATA-Box promoter and viral mimics of CREs which are critical for regulation of cellular immunity and cytokine responses. Our study suggests that intergenic regions immediately upstream of highly expressed FV3 genes have evolved to bind interferon (IFN)-regulatory factors (IRFs), NF-κB, and STATs. We also found an enrichment of microRNA sequences in more than five intergenic regions. A fraction of these viral miRNAs targets the 3'-UTR regions of *Xenopus* genes involved in IFN-dependent responses.

Conclusions

Using FV3 as model, this study provides a first unbiased RNA-seq analysis and genome-wide characterization of non-coding regulatory mechanisms adopted by ranaviruses to epigenetically regulate both viral and host gene expressions. These findings provide new knowledge that guide ranaviral screening and antiviral intervention in aquaculture.

Financial Support
U.S. National Science Foundation; U.S. Department of Agriculture, National Institute for Food and Agriculture; U.S. National Institutes of Health

Notes:

91 - Modeled impacts of rapid and accurate cattle tracing in a Foot-and-Mouth Disease outbreak in the US

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Session: Modeling - 1, Dec. 6, 10:30 - 10:45 AM

Objective

Implementing an electronic cattle identification (EID) and tracing system in the United States (US) would help support consumer demand for transparency in beef product origins and could aid in control of foreign animal disease introductions. Our objective was to evaluate the impacts of rapid and accurate tracing of cattle direct contacts during a Foot-and-Mouth Disease (FMD) outbreak in the US.

Methods

We used InterSpread Plus, a spatially explicit disease transmission model, to simulate FMD outbreaks using a national livestock population. Scenarios specified the location of the index farms, the day the first infected premises (IP) was detected, and the direct contact tracing performance. Index farms represented FMD introduction via beef or dairy cattle in four regions of the US. The first IP was detected 8, 14, or 21 days after introduction. Tracing levels were a probability of a successful trace and the time delay to trace completion. Performance levels were developed to represent the current tracing performance in the US, an estimated partial implementation of an EID system, and the ideal use of an EID system. All scenarios were monitored for convergence.

Results

Number of IP's in the outbreak varied with location of the index farms. Early detection of the first IP (day 8) led to smaller outbreaks under all tracing conditions and index farm locations. For outbreaks detected at day 14 or 21, improved traceability reduced the number of IPs in large outbreaks (95th percentile) but had minimal impact on the median number of IPs within index farm regions. Similarly, early detection and improved traceability decreased the number of farms that were impacted by the outbreak, as estimated by the number of farms in the 0 to 10 km control area and 10 to 20 km surveillance zone around IP's.

Conclusions

These results are consistent with previous tracing studies. Practically, they support the value of early detection and improved animal traceability to control an FMD outbreak. Further development of animal traceability in the United States is necessary to achieve the modeled ideal EID system results.

Financial Support
U.S. Department of Agriculture, Animal and Plant Health Inspection Services; USDA Veterinary Services; USDA Center for Epidemiology and Animal Health

Notes:

92 - Using information from network meta-analyses to optimize the power and sample allocation of a new two-arm trial

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Session: Modeling - 1, Dec. 6, 10:45 - 11:00 AM

Objective

To increase power or reduce the sample size needed for a new two-arm trial, network meta-analysis (NMA), a quantitative method used to combine the results of multiple studies, is applied and evaluated by simulation.

Methods

The new trial compares treatments A and B. Prior studies have compared A or B to other treatments. Therefore, indirect evidence of A compared B exists from an NMA. Under the above conditions the simulation process was:
- From the NMA, the risk of treatment j is estimated as p_j. The risk of B is calculated from the risk of A and the effect size, log(OR).
- For each group with treatment j and total sample size $n_{s,j}$ in study s of the NMA, replace the number of events $r_{s,j}$ with a number generated from Binom($n_{s,j}$,p_j).
- Consider the new trial with the existing network, n_A, n_B is solved by optimization.
- Data representing the new study is generated by simulating r_i from Binom(n_i, p_i), i belongs to {A, B} and analyzed using logistic regression. The hypothesis is tested that the effect size (log(OR)) is equal to 0. An indicator 1 is used if the null is rejected, and 0 if not (significant level = 0.05).
- The new study is added to the simulated existing NMA data to obtain the NMA effect size (log(OR). An indicator variable is generated again.
- Re-generate the sample size for each treatment, adding the constraint of even allocation to the optimization problem. Repeat analysis and indicator variable generation steps.
- Repeat 10,000 times and calculate the proportion of the indicator equal to 1 to get the simulation power.

Results

Leveraging evidence from NMA increases power when the sample size was fixed or reduced the required sample size when power is fixed. In some scenarios, doubling the power is available. Gains in power or reduction in sample size were small when uneven allocation to the treatment group was compared to even allocation.

Conclusions

Borrowing information from an NMA is resource-saving, but the effect of optimizing sample size allocation for each treatment is minimal.

Notes:

93 - When we shouldn't borrowing information from the existing network for planning a new trial

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Session: Modeling - 1, Dec. 6, 11:00 - 11:15 AM

Objective

Randomized clinical trials (RCTs) are commonly designed for estimating the relative effect between two or more treatments. To achieve more power for a new trial, methods based on updating a network meta-analysis(NMA) have been proposed by researchers. However, this approach has the potential to be misinterpreted. Leveraging evidence from the existing network requires the assumption that the decision to conduct the "new trial" was not informed by knowledge of the estimates in the network i.e. the trial as of interest is independent of the outcome of the NMA. If there is evidence showing the relative effect size between two treatments is significantly not equal to 0 at a predefined level, investigators tend to include them in future trials. We aim to address the potential problems if the decision to conduct a new trial depends on the result of a significant finding of the existing network.

Methods

We use simulation to evaluate the scenario that a new trial will be conducted when a significant difference between two treatments is noticed from the existing network, and compare it to the traditional scenario: conduct the new trial regardless of the result of the existing network. Two analysis methods were applied to each simulation scenario: with existing network and without.

Results

For the traditional scenario, the type I error rate is controlled. For the scenario that the new trial will be conducted only when a significant finding is indicated by the existing network, the type I error is controlled when analyzing the trial without the existing network. However the type I error risk increased dramatically when analyzed with existing network. In our example dataset, the type I error rate increases to 35.7%.

Conclusions

The decision that a new trial is performed should not depend on a statistically significant finding indicated by the existing network.

Notes:

94 - Hyperketonemia classification in dairy cows with milk Fourier-transform infrared spectroscopy and deep learning

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Session: Modeling - 1, Dec. 6, 11:15 - 11:30 AM

Objective

Detection of hyperketonemia (HYK) in dairy cows guides the treatment of metabolic disorders, and better HYK detection improves animal welfare and producer profitability. Fourier transform Infrared Spectroscopy (FTIR) of milk, has demonstrated moderate prediction accuracy for HYK. Previous research has utilized small, similar sourced datasets with contemporary modeling options, i.e. partial least squares and artificial neural network (ANN). We employed a larger and more diverse dataset to fit contemporary and deep learning models, hypothesizing that deep learning models would exhibit improved performance compared to our baseline models.

Methods

We obtained a dataset of 20,060 milk and blood samples collected from 2015 to 2021 in Germany and the USA. Using milk FTIR data (FOSS) as input features and HYK (BHB $\geq$ 1.2 mmol/L) as the outcome variable we fit the data to three contemporary and three deep learning models. The contemporary models used were linear discriminant analysis (LDA), ElasticNet (ENET), and shallow ANN; and deep learning models including deep neural network (DNN), one-dimensional convolutional neural network (1DCNN), and two-dimensional neural network (2DCNN). For the 2DCNN we converted each FTIR sample into a 53x20 pixel grayscale image to facilitate the use of transfer learning with a pretrained image classification model. We evaluated a variety of performance metrics using cross-validation to compare the models.

Results

The best performing model determined by AUC-ROC was 1DCNN 0.859 (95%CI: 0.856-0.862). Contemporary models exhibited similar performances (LDA AUC:0.854 (95%CI: 0.849-0.858).

Conclusions

We demonstrated the ability to use a large, diverse dataset to produce HYK predictions models with performance comparable to the published literature. Deep learning-based models exhibited performance similar to other contemporary models.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture; U.S. National Institutes of Health; Federal Ministry of Food and Agriculture (BMEL); LKV Bayern e. V. (Dairy Herd Improvement Association of Bavaria); MPR Bayern e.V. (Bavarian Association for Raw Milk Testing)

Notes:

95 - Early detection of ketosis in dairy cows using computer vision and machine learning

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Session: Modeling - 1, Dec. 6, 11:30 - 11:45 AM

Objective

Computer vision systems (CVS) can generate high-throughput animal-level phenotypes that can be used to monitor potential health problems, animal growth, and welfare. The development of CVS to monitor minute changes in a cow's body shape in the prepartum could be an effective alternative to early-detect cows with a high risk of metabolic disorders. In this study, we aimed to develop a CVS to process 3D images collected from Holstein cows in the prepartum period and early-detect ketosis events.

Methods

A total of 27,300 top-down 3D images from 76 Holstein cows were individually collected 21, 14 and 7 days prior to calving. Background pixels were removed in all images; then, three biological features were extracted: the number of pixels containing the cow, its estimated surface area, and the projected volume of the cow's body. A total of 1,024 features were extracted from the image dataset using a Convolutional Neural Network (CNN) previously trained to predict body condition score. The features extracted using CNN were combined with the biological features for each point and used as inputs to predict ketosis during the first 15 days after calving. The dataset was randomly split into training (85%) and testing set (15%), and this procedure was repeated 10 times. Gradient Boosting Decision Tree (GBDT) and a Partial Least-Squares Discriminant Analysis (PLS-DA) were used as predictive approaches. Hyperparameters were selected using a 5-fold cross-validation (training set) to maximize F1-score.

Results

The GBDT achieved a precision of 0.65, recall of 0.91, and F1-score of 0.75, and the PLS-DA achieved a precision of 0.63, recall of 0.91 and F1-score of 0.74.

Conclusions

The results of this study are very promising, especially considering that ketosis incidence can be affected by several other factors that are not always expressed through changes of body tissue mobilization. Our results suggest that CVS can generate integrated animal-level phenotype to acquire information from individual animals in large dairy operations, allowing the development of preventive practices to improve animal health.

Financial Support
U.S. Department of Agriculture; Dairy Innovation Hub

Notes:

96 - Comparative analysis of computer vision algorithms for the real-time detection of digital dermatitis in dairy cows

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Session: Modeling - 1, Dec. 6, 11:45 - 12:00 PM

Objective

Digital dermatitis (DD) is responsible for ulcerative lesions on the interdigital space of the hoof. DD is associated with massive herd outbreaks of lameness and influences welfare and production. Therefore, early detection can lead to prompt treatment, reduce costs, and decrease lameness. Computer vision (CV) provides a unique opportunity to improve early detection of DD. The study aims to build an application for the real-time detection of DD in dairy cows. We compared various CV models for scoring using performance metrics and inference time and then automated the best model for detection using pre-recorded and live streaming video.

Methods

Images were collected from commercial dairy farms facing the rear foot of the interdigital space of the hoof. Images were scored for M-stages of DD by a trained investigator using the M-stage DD classification system. Overall, the library of images includes 1,177 M0 and 1,050 M2 images and the corresponding annotations. Models were trained to detect M0 and M2 lesions. Accuracy, precision-recall, and mean average precision (mAP) were used for performance measures to compare between the predictions made by the CV models and a trained investigator (ground truth).

Results

All models performed well compared to the ground truth as well as a baseline using ResNet-18. All models achieved a mAP between 96.4% to 99.8% and an accuracy between 94.0% to 99.0%. Overall, YOLOv4 and YOLOv4-tiny outperformed all other models with an accuracy of 99.0%, precision of 98.0%, and recall of 100.0%. Tiny YOLOv4 outperformed all other models with respect to inference speed where the model processes images at 333 FPS, followed by YOLOv4 at 65 FPS.

Conclusions

The CV models were able to identify and classify DD lesions on a commercial dairy farm with high accuracy. The models were able to detect DD lesions in a milking parlor with high prediction speed as well. This result is a small step in applying CV algorithms to veterinary medicine and implementing real-time detection to dairy farms. The proposed CV tool can be used for early detection and prompt treatment of DD in dairy cows.

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

Notes:

97 - Monoclonal antibody development for horses: An update

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Session: Immunology - 2, Dec. 6, 8:30 - 8:45 AM

Objective

Specific monoclonal antibodies (mAbs) for equine immune molecules enable basic and clinical research to improve research on infectious diseases, development of vaccines, and testing of new treatments for acute and chronic inflammatory diseases. In this project, we are developing new mAbs for the analysis of the immune system of the horse.

Methods

Recombinant equine immune target proteins are expressed in mammalian cells, purified for immunization of mice, and hybridoma technology is performed for mAb production. All mAbs are tested for their specificity to the target protein and it is also confirmed that the mAbs recognize the corresponding native immune proteins of the horse.

Results

New mAbs with confirmed specificity to the native equine target that were developed and/or finally characterized in the past year. These include mAbs against equine IL-8, TNF-a, IL-1b, IgD, and IL-6. In addition, several new equine mAbs are in the development pipeline and directed against SLPI, granlysin, and various T-cell memory and effector cell markers. A complete list of mAbs specific for equine immune molecules is available at (https://courses2.cit.cornell.edu/wagnerlab/research/reagents.htm). This list currently includes a total of 31 mAbs for cytokines and chemokines, 12 mAbs for immunoglobulin isotypes, and 8 for CD markers that have been developed during the course of the equine immune reagent project at Cornell. Utilizing these mAbs, we also developed multiplex assays that are available through AHDC at Cornell (https://www.vet.cornell.edu/animal-health-diagnostic-center) and used by the equine research community. In addition to cytokine (IFN-a, IFN-g, IL-4, IL-10, IL-17) and sCD14 assays developed previously, a new chemokine assay for quantification of TNF-a, IL-1b, CCL2, CCL3, CCL5 and CCL11 is now available to analyze immune responses of the horse.

Conclusions

The mAbs and assays developed during the horse immune reagent development project provide valuable new tools for immunological research. They have been used in clinical studies and basic research applications to improve the evaluation of host immunity during infectious and inflammatory diseases of the horse.

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

Notes:

98 - Peripheral IgE+ plasmablasts secrete IgE and correlate to allergic disease severity

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Session: Immunology - 2, Dec. 6, 8:45 - 9:00 AM

Objective

Production and secretion of IgE by B cells, plasmablasts and plasma cells is a central step in the development and maintenance of allergic diseases. Serum IgE can bind to its low-affinity receptor CD23, which is constitutively expressed on all peripheral B cells. We characterized CD23+/IgE+ plasmablasts in horses to identify the roles of these cells during the allergic response.

Methods

We developed a novel acid wash approach to characterize IgE+ plasmablasts using flow cytometry, bead-based assay, *in vitro* assay and qPCR. We then compared the presence of IgE+ plasmablasts in peripheral blood of allergic and healthy horses using a model of naturally occurring seasonal allergy, *Culicoides* hypersensitivity. The model allows the comparison of immune cells both during periods of clinical allergy and also when in remission and clinically healthy.

Results

Equine IgE+ plasmablasts readily secrete biologically active IgE, upregulate specific mRNA transcripts (IRF4, XBP1, CD138, TACI), and exhibit highly differentiated morphology, all consistent with plasmablast differentiation. Allergic horses had significantly higher percentages of IgE+ plasmablasts and IgE secretion during clinical allergy than healthy horses. Allergy severity and IgE secretion were both positively correlated to the frequency of IgE+ plasmablasts in peripheral blood.

Conclusions

These results provide strong evidence for the identification of a novel population of peripheral IgE-secreting plasmablasts and provide a missing link in the mechanism of IgE up-regulation during allergy.

Financial Support
Harry M. Zweig Memorial Fund for Equine Research; U.S. Department of Agriculture, National Institute for Food and Agriculture

Notes:

99 - IgE-binding monocytes promote allergic inflammation through IL-8 production

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Session: Immunology - 2, Dec. 6, 9:00 - 9:15 AM

Objective

Allergic diseases depend upon the recruitment of inflammatory cells, such as basophils and mast cells, to the site of allergen exposure. This mechanism of disease is incompletely understood and involves both innate and adaptive immune cells. We have identified IgE-binding monocytes as an important source of IL-8, a pro-inflammatory chemokine, in allergic individuals. We developed a novel equine IL-8 monoclonal antibody for future study of IL-8 in different inflammatory equine diseases.

Methods

We characterized IgE-binding monocytes using flow cytometry, *in vitro* assay, RT-PCR, and confocal microscopy. We then compared IgE-binding monocytes in peripheral blood of healthy horses and horses with *Culicoides* hypersensitivity, a recurrent, seasonal allergy. We developed a monoclonal antibody to measure equine IL-8 chemokine production in both horse groups by flow cytometry.

Results

IgE-binding monocytes comprise about 6% of peripheral monocytes in both allergic and healthy horses. IgE crosslinking, which activates the same intracellular signaling pathway as an allergen, induces significantly higher percentages of IL-8+ IgE-binding monocytes in allergic horses. However, IgE-binding monocytes in all horses bind similar densities of IgE on their cell surface, which suggests a pro-inflammatory bias in the context of allergy.

Conclusions

These results demonstrate how the initial innate immune response following allergen exposure is connected and leads to IgE-mediated clinical allergy. IL-8 production by IgE-binding monocytes in allergic horses provides a link between allergen-specific IgE and innate immune cell-mediated inflammation.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture; Harry M. Zweig Memorial Fund for Equine Research

Notes:

100 - The effect of intra-articular corticosteroids on the systemic mRNA response in an equine acute synovitis model

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Session: Immunology - 2, Dec. 6, 9:15 - 9:30 AM

Objective

Given the regularity with which various anti-inflammatory drugs are used in equine athletes, additional research is needed to investigate the effect of these drugs on the inflammatory mRNA response. Thus, the goal of this study was to determine the effect of intra-articular (IA) corticosteroids on an acute synovitis model.

Methods

Five mixed-breed, 2 year-old horses were randomly allocated to an IA treatment of the radiocarpal joint with 9 mg of either triamcinolone or betamethasone. Two weeks following treatment, horses were injected with 1 µg of lipopolysaccharide (LPS) diluted in 1 mL of saline. Following treatment, horses were crossed-over two weeks later and treated with the other drug followed by a subsequent LPS injection. Blood samples were collected using Tempus Blood RNA Tubes® pre-injection and at 2, 4, 6, 9, 12, 24, 48, 72, and 144 hours post-injection, followed by RNA isolation and RT-qPCR for 22 different genes. Samples were also collected for serum amyloid A (SAA) determination and lameness was subjectively scored at each time point. Additional injections with saline-only or LPS-only were conducted as negative and positive controls, respectively.

Results

Two-way repeated measures analysis of variance was used to analyze all data. Evidence of an anti-inflammatory effect 14 days post-treatment with triamcinolone was identified, most notably based on increased expression of *IL-6* and *PTGS1* (cyclooxygenase-1), as well as a reduction in lameness severity beyond 6 hours LPS injection and decrease in SAA concentration at later time points. A similar anti-inflammatory effect with betamethasone was not noted.

Conclusions

Evaluation of mRNA expression following intra-articular corticosteroid administration in an acute synovitis model provides a means to determine the potential timeframe for the effects of these drugs on the inflammatory response in horses in training and racing. In the future, this information and experimental model could be used to further refine regulatory withdrawal guidelines for intra-articular corticosteroids.

Financial Support
Zoetis; Schlaikjer Endowment

Notes:

101 - IgD monoclonal antibody development for phenotyping maturating B-cell populations in horses

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Session: Immunology - 2, Dec. 6, 9:30 - 9:45 AM

Objective

IgD preservation in evolution from fish to human supports its importance for B-cell development. IgD is highlighted as a marker of activated naïve B-cell populations in humans. Existence of a complete genomic equine IGHD gene supports the presence of IgD in horses. Additionally, IGHD mRNA can be amplified from equine PBMC, suggesting that IgD might be expressed by equine B-cells. Furthermore, the equine IGHD gene lacks a switch region suggesting an alternative splicing mechanism for IGHD gene expression, implying simultaneous expression of IgM and IgD. We generated monoclonal antibodies (mAbs) against equine IgD for the characterization of IgD+ B-cells in horses.

Methods

A C57BL/6 mouse was immunized with a recombinant IL-4/IgD fusion protein expressed in mammalian ExpiCHO cells. Spleenocytes were fused with murine myeloma cells to generate hybridoma cell lines. ELISA and flow cytometry allowed for the selection of hybridoma clones based on recombinant IgD and native antigen recognition. Two new IgD mAb clones were identified and conjugated with fluorescent dyes. IgD clones were used with an IgM mAb for staining PBMC, lymph node, spleen, and bone marrow derived cells to quantify IgM+IgD+ lymphocytes in assorted tissues.

Results

PBMC (n=2 horses) presented .324% IgM-IgD+, 5.62% IgM+IgD+, 11.57% IgM-IgD+, and 82.5% IgM-IgD-. Lymph node derived cells (n=2 horses) showed 0.056% IgM-IgD+, 3.95% IgM+IgD+, 21.5% IgM+IgD-, and 75% IgM-IgD-. Spleen derived cells (n=4 horses) exhibited 0.39% IgM-IgD+, 3.13% IgM+IgD+, 5.6% IgM+IgD- and 90.9% IgM-IgD-. Lastly, bone marrow derived cells (n=8 horses) displayed 0.13% IgM-IgD+, 1.93% IgM+IgD+, 12.20% IgM+IgD- and 85.7% IgM-IgD-.

Conclusions

We developed two equine mAbs and confirmed their ability to detect equine IgD+ equine B-cells. Our findings support that 2-4% of IgM+IgD+ B-cells are detectable in non-stimulated lymphoid tissues. These mAbs detect native IgD+ B-cells via flow cytometry, enhancing adaptive immune development and B-cell differentiation research during physiological conditions or diseases in horses.

Financial Support
USDA-NIFA

Notes:

102 - Mast cell histamine mediates the intestinal immune response to early weaning in piglets via histamine 2 receptor (H2R) in a sex-dependent manner

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Session: Immunology - 2, Dec. 6, 9:45 - 10:00 AM

Objective

Mast cells are major mediators of stress-related disorders, especially that of the gastrointestinal (GI) tract. Previous studies investigating the impact of early weaning (EW) stress in piglets showed that histamine, a major mediator in mast cell granules, is released shortly after weaning and is followed by increased gene expression of histamine receptor 2 (H2R) in ileal, jejunal, and colonic mucosa. The precise contribution of histamine and histamine receptor subtypes to weaning stress-induced GI immune responses is unknown. Here we tested the hypothesis that EW induced intestinal immune activation is mediated by H2R.

Methods

Fifteen-day-old Yorkshire female and male castrate piglets were administered either saline vehicle or the H2R antagonist, famotidine (10 mg/kg; intramuscular), 30 minutes prior to early weaning. At weaning, piglets were weaned from their dams and housed in nursery pens with ad libitum access to water. At 24 hours post-weaning, mid-jejunum was collected for qPCR gene expression and IHC localization of H2R. Markers of immune activation including myeloperoxidase (MPO), IL1β, TLR4, and β-integrin were measured by Western blot or ELISA. Unweaned control piglets remained with the sow and were collected at the same time as weaned piglets.

Results

Weaning increased gene expression for H2R at 3, 8, and 24 hours post-weaning. Compared with saline-treated controls, piglets administered famotidine had reduced jejunal MPO and IL1-β. Expression of β-integrin in the mesenteric lymph nodes was also reduced in famotidine-treated piglets. Further, these responses were found to be sex dependent with famotidine having a greater effect in male castrates. Immunohistochemical analysis of H2R revealed increased expression and localization in lamina propria cells and in the intestinal epithelium.

Conclusions

Together, these data demonstrate that histamine via H2R plays an important role in early weaning stress-induced intestinal immune responses. This provides a potential target for mediation of the stress response to early weaning practices; however biological sex may need to be considered when targeting H2R.

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

Notes:

103 - Isoprostanes increase endothelial cell barrier integrity independent of altered inflammatory gene expression

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Session: Immunology - 3, Dec. 6, 10:30 - 10:45 AM

Objective

Dysregulated inflammation and oxidative stress contribute to the pathophysiology of several economically important diseases of dairy cattle. A hallmark of these processes is damage to fatty acids in cell membranes, leading to the generation of lipid mediators that regulate many aspects of inflammation. Endothelial cells are crucial for appropriate inflammatory responses because they are responsible for maintaining an effective vascular barrier and forming specialized lipid mediators known as isoprostanes (IsoP). Previous work has indicated that certain IsoP can modulate certain inflammatory outcomes. As the biological action of IsoP are poorly defined, the objective of this study was to investigate the ability of IsoP derived from different fatty acid substrates to impact barrier integrity and inflammatory gene expression during acute bovine inflammation.

Methods

Bovine aortic endothelial cells (n=4) were treated with 15 ng/mL lipopolysaccharide (LPS), 10 nM omega-6- (15-F_{2t}-IsoP) or omega-3-derived (15-F_{3t}-IsoP) IsoP, or LPS cocultured with IsoP. Barrier integrity was assessed with electric cell-substrate impedance sensing for 24 hr. Relative mRNA expression of thromboxane receptor, nuclear factor kappa B, and inducible nitric oxide synthase was assessed with reverse transcriptase qPCR after 1, 4, 8, and 12 hr treatment incubations. Statistics were run with the PROC MIXED procedure in SAS 9.4 ($P<0.05$).

Results

Cells treated with LPS and 15-F_{2t}-IsoP had relatively increased barrier resistance compared to LPS alone after 8 hr. However, mRNA expression of genes associated with inflammation was not different between treatment groups at 1, 4, 8, or 12 hr, suggesting that the increase in barrier integrity is likely not due to alterations in inflammatory gene transcription.

Conclusions

This study benefits animal health by describing how IsoP may influence inflammation in bovine endothelial cells. Future studies should be directed towards further comparing effects of omega-6- and omega-3-derived IsoP, which may implicate dietary modifications capable of mitigating inflammation in dairy cattle.

Financial Support
USDA-NIFA

Notes:

104 - Development of bovine intestinal organoid models to study immune modulation by gut microbes.

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Session: Immunology - 3, Dec. 6, 10:45 - 11:00 AM

Objective

Cattle are born immunologically naïve and due to their epitheliochorial placenta type only receive maternal antibodies during the short-period of colostrum ingestion. Following the waning of maternal antibodies, the animal must develop a self-sustained, robust immunological defense. Consistent with other mammals, our studies have indicated that the voracity of a ruminant's immunological defenses are enhanced by microbial stimulation of the lymphatic tissue located in the ileo-cecal region of the gut (GALT). Microbial stimulation begins following parturition when neonatal ruminants acquire their microbiota from maternal and environmental sources. Studying the microbial effects on the GALT *in-vitro* can be challenging since the physiology and morphology of the gut cannot be fully replicated through primary cell lines. We therefore set out to develop bovine ileal and cecal organoids for use as a model to examine the effects of select microbial species that have a correlative relationship with immune function on bovine immune and barrier function response.

Methods

Tissue from the ileum and cecum of five different bovine genetic lines were collected, stem cells harvested, and organoids developed for both sections of the GIT. Laminin rich Matrigel and WRN (Wnt3a, R-spondin, Noggin) factors were used to mimic the extracellular matrix and provide optimal conditions for specification and passaging respectively. Following development, the size and morphology of the organoid tissue were validated using an inverted compound microscope.

Results

Five bovine ileal and five bovine cecal organoid lines were successfully developed that demonstrate consistent growth and range in size from 200um to 1mm. Villus – like projections are observable providing morphological accuracies to the *in – situ* gut mucosa and providing a valuable model system for subsequent studies.

Conclusions

Organoid models have been developed to provide physiological and morphological accuracy and will be deployed for *in – vitro* studies of the modulatory effects of gut microbiota on the animal's immune response and barrier preservation.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture; Bair Ranch Foundation Montana

Notes:

105 - AEA enhanced bovine vascular endothelial cell barrier integrity during LPS challenge mediated by CB1

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Session: Immunology - 3, Dec. 6, 11:00 - 11:15 AM

Objective

Dysfunctional inflammation associated with coliform mastitis is a major contributor to the severity and potential lethality of acute infections. Breakdown of the vascular endothelium is part of the pathogenesis of coliform mastitis. Ability of the endocannabinoid (EC) system to modulate inflammation was shown in several non-bovine species. The EC system is comprised of the cannabinoid receptor 1 (CB1) and 2 (CB2) and their ligands, fatty acid ethanol amides and glycerols. The EC arachidonoylethanolamide (AEA) improves network formation and proliferation in human and rodent endothelial cell models via activation of several receptors, including CB1. Increased plasma AEA during systemic coliform mastitis *in vivo* and elevated CB1 and 2 expression in cultured bovine aortic endothelial cells (BAEC) challenged with endotoxin (LPS) support the bovine EC system involvement in inflammation. Prior experiments using the electric cell-substrate impedance sensing (ECIS) technology showed a dose dependent increase in barrier resistance of BAEC challenged with LPS.

Methods

Rimonabant, a CB1 inverse agonist, was used to elucidate the involvement of CB1 in the increased barrier resistance observed in BAEC treated with AEA during LPS challenge. Primary BAEC cell lines were cultured in ECIS 96-well arrays and treated with 25 ng/mL of LPS for 6- 8 hours before addition of AEA/rimonabant.

Results

Rimonabant dose of 1 μM decreased barrier resistance within the first 2 hours of treatment, compared to AEA alone. Timing and effect of rimonabant treatment indicate that AEA related increase in barrier resistance is CB1 mediated.

Conclusions

AEA and rimonabant activate non-CB receptors involved in network formation and proliferation and further *in vitro* modeling is necessary to fully elucidate the mechanism of increased barrier resistance of BAEC. Identification of regulatory points of AEA enhanced endothelial cell barrier resistance, may lead to new therapeutic targets to optimize inflammatory responses during acute disease events and reduce incidence and severity of systemic infections, thus reducing dairy cow mortality.

Notes:

106 - Concentration of antibodies against *T. foetus* surface antigen TF1.17 from synthetic mRNA-transfected cells

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Session: Immunology - 3, Dec. 6, 11:15 - 11:30 AM

Objective

Antibodies against the TF1.17 surface antigen of *Tritrichomonas foetus* (TF) inhibit attachment to host cells and decrease parasite viability. Synthetic mRNA encoding these antibodies could be applied to the urogenital epithelium of bulls to prevent or treat trichomoniasis. Antibodies against TF1.17 have been produced by mRNA transfected preputial keratinocytes, but in concentrations too low to demonstrate biologically relevant effects. The objective was to develop an assay to concentrate functional expressed antibody against TF1.17.

Methods

Synthetic mRNAs for membrane anchored, bovine IgG antibodies against 2 epitopes of the TF1.17 antigen (TF1.15 and 1.17) were used to transfect A549 cells (Messenger Max) for 24 hours. Phospholipase C (1 U/mL) was used to cleave the antibodies from the cells and the supernatant was concentrated via centrifugal filtration. The concentration of bovine IgG was determined via ELISA (Abcam) and TF were treated with 0.1 µg/mL of TF1.15 or 1.17, and assayed for cytotoxicity and cellular metabolism via commercially available kits (Promega CellTox Green and CellTiter-Glo 2.0).

Results

Bovine IgG concentration was determined to be 10.0 µg/mL for TF1.15 and 5.5 µg/mL for TF1.17 confirming that cells were expressing antibody and that transfection efficiency was almost doubled for the TF1.15 construct. Cytotoxicity evaluated via a fluorescent marker for membrane permeability revealed a 4-fold and 12-fold increase in relative fluorescent units from TF treated with TF1.15 and 1.17, respectively, as compared to untreated controls. TF metabolism (ATP production) was evaluated via a luminescence assay, and, while there was no effect on TF treated with TF1.15, there was a 23% reduction in ATP production by TF treated with TF1.17, compared to untreated controls.

Conclusions

This method is feasible to concentrate expressed functional antibody for ongoing research to determine the efficacy of mRNA therapy to treat or prevent TF infection in bulls.

Financial Support
U.S. Department of Agriculture, Animal Health Formula Funds; U.S. Department of Agriculture, National Institute for Food and Agriculture

Notes:

107 - Characterization of chicken complement receptor 1-like (CR1L)/GPI-anchored C4 binding protein (C4BPG)

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Session: Immunology - 3, Dec. 6, 11:30 - 11:45 AM

Objective

The complement system consists of multiple soluble and membrane factors that are activated in a cascade manner with the purpose of recognizing foreign pathogens, targeting them for cytolysis and clearance. Since pathogens have been exposed to complement components for millions of years, some viruses have developed mechanisms to usurp the system for their own purposes. Our objective here is to identify and characterize the chicken CRs and develop tools to address our specific hypotheses.

Methods

First, we performed in silico analysis of the chicken genome to identify currently annotated CR genes. Following this analysis, we designed RT-PCR primers to clone the chicken homologs of human and mouse CR1 and CR2, termed CR1-like (CR1L) and CR2-like (CR2L). Following cloning of chicken CR1L (chCR1L), recently renamed GPI-anchored C4 binding protein (C4BPG), plasmids were produced for expression in both eukaryotic and bacterial expression systems. Monoclonal antibodies (mAbs) were generated and characterized using immunofluorescence (IFA) and western blotting (WB). The specificity and isotype of each mAb were determined and chCR1L/C4BPG expression in different chicken cell lines and primary cells and tissues was performed using IFA, WB, and flow cytometry.

Results

RT-PCR analysis using cDNA collected from the chicken B cell and macrophage cell lines, DT40 and HD11, respectively, suggested the predicted chCR2L is not expressed in these cells. However, chCR1L was abundantly expressed in HD11 cells, while DT40 cells had minimal expression. Therefore, chCR1L cDNA was cloned from HD11 cells, sequenced, and confirmed to be the putative chCR1L/C4BPG cDNA. The antigenic portion of chCR1L/C4BPG was expressed in bacteria, purified, and used to generate mAbs in mice. Twelve hybridoma cells lines were further characterized and shown to specifically recognize chCR1L using IFA and WB. Expression of chCR1L was evaluated in various chicken cell lines and primary cells and tissues.

Conclusions

We have successfully cloned and characterized chCR1L that will be of value for further studies including immunological studies.

Notes:

108 - Purinergic receptor expression during natural Marek's disease virus infection in the lungs

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Session: Immunology - 3, Dec. 6, 11:45 - 12:00 PM

Objective

Marek's disease caused by Marek's disease herpesvirus (MDV) and is a major economic concern for the poultry industry. Despite the characteristic manifestations, the pathophysiology of MDV is poorly understood and requires consistent research for understanding disease progression. Purinergic receptors (PRs) have been shown to play important roles during viral-induced pathogenesis, but to date no research has been published with respect to PRs during MDV infection. The main objective of our study is to divulge the importance of purinergic signaling cascade in the MDV replication and disease progression.

Methods

Naïve contact chickens (n=5/breed/time point) from MD-resistant (White Leghorns: WL) and -susceptible chicken (Pure Columbian: PC) lines were housed with experimentally MDV infected PC chickens for 6 or 24 h and euthanized soon after exposure. Suspension cells were obtained from the lungs by flushing with 5% PBS using whole lung lavage. RNA was extracted from the obtained cells and used in RT-qPCR assays to measure specific PR responses.

Results

The gene expression response of each chicken line following exposure was compared to the two time points (6 and 24 h) and expressed as a fold-change to uninfected controls. Overall, WL chickens showed significantly higher expression of PA1A1R, P1A2BR, P1A3R, P2X3R, P2X5R, P2Y1R, and P2Y5R at 6 h ($P< 0.05$), while for the same genes, there were no significant change in PC chickens compared to uninfected control ($P< 0.05$). However, P2X2R at 6 h was significantly higher in PC birds compared to all other groups. No significant differences were observed for P1A1R, P1A2AR, P2X1R, and P2X7R, P2X4R, P2Y2, P2Y6, and P2Y13 ($P> 0.05$).

Conclusions

The purinergic signaling response during virus infection is complex. However, we have identified several PRs that may play a role during natural MDV infection in the lungs that may help us understand different responses to MDV infection in different chicken lines. The data warrant more detailed studies on the PR response during MDV infection.

Financial Support
U.S. Department of Agriculture, Animal Health Formula Funds

Notes:

109 - Brucellosis and reproductive disease: A current update

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Session: AAVI - Immunology Featured Speaker, Dec. 6

Brucellosis, caused by Gram negative intracellular bacteria spp. of the genus Brucella, remains one of the most commonly reported zoonotic diseases worldwide. An intriguing aspect of the bacterium is its ability to evade the host immune response leading to lifelong pathogen persistence while inducing significant reproductive failure. In domestic animals including cattle, sheep, goats and dogs, reproductive disease is typically manifested as abortions, stillbirths and failure to conceive, while in males the disease is characterized by infertility and epididymitis. Recent advances in our understanding of host-pathogen interactions leading to clinical brucellosis have focused on intracellular survival and persistence of the pathogen in the mononuclear phagocyte system with very little known about the mechanism behind reproductive disease both in males and females. Here, we describe the use of different pregnant animal models of infection (guinea pigs and mice) to better understand the mechanism behind reproductive disease as well as a tool to better evaluate vaccine candidates.

Notes:

110 - Understanding host-pathogen interactions to inform on vaccine design to control chlamydial abortion in sheep

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Session: AAVI - Immunology Featured Speaker, Dec. 6

Objective

Chlamydia abortus is an obligate intracellular bacterial pathogen that causes ovine enzootic abortion of ewes (OEA), resulting in major production losses in most sheep-rearing countries worldwide. *C. abortus* establishes a complex relationship with the ovine host. Primary infection of non-pregnant ewes resulting in persistent, subclinical infection leading to OEA in the subsequent pregnancy. The development of effective control methods is dependent on an understanding the host-pathogen interactions that result in disease or protection.

Methods

We developed novel immunological tools to characterise the activation and regulation of the ovine immune system during pregnancy and host immune control of *C. abortus*. We have reproduced OEA by experimental infection of pregnant ewes and used this model to evaluate the safety and efficacy of prototype subunit vaccines to protect against OEA.

Results

Host immune control of *C. abortus* is dependent on Th-1-type cellular immunity, mediated though production and actions of IFN-γ. Humoral immunity to *C. abortus* does not appear to play a major role in protection against disease but has been exploited for the development of diagnostic tests. We have exploited this knowledge to design a prototype adjuvanted subunit vaccine that can be delivered as a single-shot injection prior to pregnancy. This vaccine elicits both humoral and cellular Th-1-type immunity and confers protection against experimental OEA.

Conclusions

By gaining a detailed understanding of host immune responses to *C. abortion* infection, we have developed a prototype safe and effective subunit vaccine for controlling OEA. This subunit approach provides new opportunities for further development of combined management strategies based on discrimination between infected and vaccinated animals (DIVA) approaches to OEA.

Financial Support
Biotechnology and Biological Sciences Research Council; EU Framework Programme for Research and Innovation; Scottish Government Rural and Environment Science and Analytical Services (RESAS) division

Notes:

110 - A molecular perspective on the development of fetal immunotolerance to BVDV

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Session: AAVI - Immunology Featured Speakers, Dec. 6, 3:00 - 3:30 PM

Bovine viral diarrhea virus (BVDV) has afflicted the cattle industry since its discovery in 1946, persevering despite control efforts, and currently costing the cattle industry between $1.5 and $2.5 billion annually. Through vertical transmission, maternal infection with BVDV during early gestation produces persistently infected (PI) fetuses, which, once born will shed the virus asymptomatically throughout their postnatal lives. Congenital malformations such as immune dysfunction, bone, neural, and heart abnormalities, as well as intrauterine growth restriction of fetuses are often observed in postnatal PI calves. It was hypothesized that vertical transmission of BVDV during early gestation contributes to fetal congenital malformations through permanent perturbations of the fetal immune system, specifically through chronic upregulation of the innate immune system and attenuation of the adaptive immune system. BVDV naïve pregnant heifers were infected with BVDV, or sham media, on day 75 of gestation. Fetuses were collected via cesarian section at days 82, 89, 97, 190, and 245 of gestation. Fetal tissues including thymuses, spleens, and placentas were subjected to mRNA analysis. As expected, RT-qPCR revealed the innate immune response in PI fetal tissues was upregulated by day 97 of gestation when compared to controls. However, the PI fetal innate and adaptive immune systems were attenuated by day 190. DNA methylation sequencing was performed to understand the potential permanent postnatal consequences of PI, which revealed hypermethylated pathways associated with bone formation, providing an explanation for several clinical pathologies of PI animals and immune dysregulation. This domestic livestock and agriculturally relevant study offers a unique model and explanation for not only BVDV pathologies, but also transplacental viral infections in humans, many of which exhibit similar pathologies and contributions to the developmental origin of adult health and disease. This work is supported by USDA 2008-35204-04652, 2019-67011-29539, and 2019-67015-29866.

Notes:

111 - Host responses following third trimester maternal PRRSV2 infection

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Session: AAVI - Immunology Featured Speakers, Dec. 6, 4:15 - 4:45 PM

Objective

Late gestation maternal infection by porcine reproductive and respiratory syndrome virus (PRRSV) results in varied fetal outcomes including death, birth of congenitally infected weak pigs, and less frequently, fetuses that escape infection. Historically, fetal compromise and death were thought to associated with umbilical lesions and apoptosis of implantation sites inferring involvement of hypoxia and/or placental separation. Although TNF-alpha and IFN-gamma expression increases in infected fetuses, the infrequency of fetal PRRSV lesions was used to propose that fetal demise was mainly associated with events at the maternal-fetal interface (MFI). PRRSV replicates in CD163 positive macrophages in the MFI with subsequent transplacental transmission proposed by migrating maternal macrophages or free virus translocating through or between the epithelial layers.

The PRRSV2 Pregnant Gilt Models (PGM) were undertaken at the University of Saskatchewan to investigate mechanisms of fetal demise and resilience, and to identify biomarkers that could be used to select more resilient replacement gilts. The core animal model used late gestation maternal infection with a virulent PRRSV2 stain followed by phenotypic assessment of the dam, MFI and fetuses. Dams and fetuses were genotyped using 60K or 660K SNP panels enabling genome wide association studies (GWAS) of host traits associated with resilience.

What is fetal PRRS resilience?

While complete resistance is a lofty goal to attain through natural selection, selecting solely for fetal survival ignores the detrimental effects of congenital infection on the individual and population. We have developed a more sophisticated definition of fetal resilience: ***the ability of a fetus to sustain (near) normal growth and development following maternal exposure to PRRSV2 in 3rd trimester of gestation.*** My presentation will highlight important host responses that provide insight on the mechanisms of fetal demise and resilience following late-gestation PRRSV2 infection.

This research was sponsored by the Genome Canada, Prairie and Alberta.

Notes:

112 - Intrauterine immunization during breeding

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Session: AAVI - Immunology Featured Speakers, Dec. 6

Objective

Productivity and profitability of swine herds are highly dependent on the reproductive performance of gilts and sows as well as piglet survival and growth rates. Group housing increases the difficulty for personnel to safely administer needle-based vaccines so alternative immunization mechanism are being sought. During breeding, pigs undergo a lordosis response (i.e. standing estrus) wherein they become temporarily immobilized, making them safe to inseminate and vaccinate. The majority of commercial pigs are bred by artificial insemination (AI), meaning that the uterus is easily accessible during each reproductive cycle.

Methods

We assessed whether vaccines administered to the uterus during breeding can lead to sufficient colostral antibodies to protect suckling piglets against Porcine Endemic Diarrhea Virus (PEDV). An antigen from *Lawsonia intracellularis*, a bacterial disease that impacts weanling intestinal health, was also included because we have extensive knowledge on the pig immune response to this antigen. Gilts were mock-bred at 2nd estrus with killed sperm that included an intrauterine (i.u.) vaccine comprised of recombinant (r) PEDV Spike protein (rPEDVS1) and *L. intracellularis* flagellin (rFliC) formulated with poly I:C, host defense peptide, and polyphosphazene (TriAdj). Gilts returned to estrus within 3 weeks and they were inseminated with killed sperm (3rd estrus) or live sperm (4th estrus) with rPEDVS1-TriAdj vaccine. They also received an i.m. injection of rFliC-TriAdj at 3rd and 4th estrus to establish whether i.u. vaccination primes systemic immunity without inducing mucosal tolerance. Control gilts were administered semen alone at 2nd estrus which allowed us to compare litter weights and sizes to industry standards. Colostrum from gilts challenged with an intramuscular PEDV vaccine formulated with alum was used as positive reference samples for neutralizing antibodies and passive protection.

Results

Three months after the last vaccine dose, the i.u.-vaccinated gilts showed significant PEDVS1-specific serum, colostral, and uterine antibody titers and colostral PEDVS1-neutralizing antibodies, but poor cell-mediated immunity, which we determined was likely due to lack of robust T cell epitopes in the PEDV antigen. Piglets born to i.u. vaccinated gilts received partial passive protection from PEDV infection 3 days after birth but eventually succumbed to the disease.

Conclusions

Intrauterine immunization at breeding triggers systemic and mucosal immunity in gilts that leads to passive transfer of neutralizing antibodies to suckling piglets. By coupling breeding with vaccination, we save on person-hours and we vaccinate when it is safe to do so, which are significant benefits to producers and allows for passive protection of newborn piglets. Further optimization is needed to promote robust passive protection against neonatal diseases.

Notes:

114 - Effects of two-dose ceftiofur on quantities of third-generation cephalosporin, fluoroquinolone, and macrolide resistance genes in dairy cows

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Session: Antimicrobial Resistance/Use - 2, Dec. 6, 2:00 - 2:15 PM

Objective

The overall objective of this study was to determine the effects of two-dose ceftiofur crystalline-free acid (CCFA) on quantities of third generation cephalosporin (3GC), fluoroquinolone (FQ), and macrolide resistance genes, and determine differences in bacterial community composition in Holstein-Friesian dairy cows in the south-western United States.

Methods

A total of 124 matched pairs of cows were enrolled in a longitudinal study on three dairy farms in the U.S. High-Plains. Cows diagnosed with postpartum metritis received the product twice at the labeled dose of 6.6 mg/kg subcutaneously at the base of alternating ears. Untreated cows–absent clinical metritis–were matched on lactation number and calving date. Feces were collected per rectum on days 0 (baseline), 6, and 16. Samples were pooled (n=192), for community DNA analysis by day and treatment group and 4 cows were matched in each pool. Further characterization of community DNA included quantitative PCR (qPCR) to quantify the antibiotic resistance genes *bla*$_{\text{CMY-2}}$, *bla*$_{\text{CTX-M}}$, *mph(A)*, *qnr(B)*. All genes were standardized with 16S rRNA gene copies/gram of feces. Additionally, metagenomics analyses were used to determine differences in bacterial community composition.

Results

The overall *bla*$_{\text{CMY-2}}$ gene copies/gram feces varied significantly between treated and control groups on days 6 and 16 compared to day 0 ($p<0.05$). The blaCMY-2 quantities also varied significantly between farms. The *bla*$_{\text{CTX-M}}$ and *qnrB19*. quantities did not vary significantly between treatment, days, and farms. Shannon diversity was significantly decreased in the control group compared to the treated group ($p<0.05$). There was also a significant reduction in alpha diversity for days 6 and 16 ($p<0.05$).

Conclusions

Ceftiofur treatment had an effect on *bla*$_{\text{CMY-2}}$ gene quantities in treated cows suggesting a selection pressure. However, a similar effect was not observed for *bla*$_{\text{CTX-M}}$ and *qnrB19*. Additional analysis is needed to determine the effect of ceftiofur treatment on selection of antimicrobial resistant bacterial populations and the overall bacterial composition.

Financial Support
USDA Agriculture and Food Research Initiative (AFRI)

Notes:

115 - Influence of antimicrobial exposures and diet on the fecal microbiome and resistome of pre-weaned dairy calves

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Session: Antimicrobial Resistance/Use - 2, Dec. 6, 2:15 - 2:30 PM

Objective

The use of antimicrobial drugs (AMDs) in food-producing animals has received increased scrutiny because of concerns regarding antimicrobial resistance (AMR). AMDs are commonly administered in dairy calves via milk supplementation or injection. However, how different routes of administration impact the microbiome and resistome remain poorly characterized. The purpose of this study was to evaluate the effect of two different routes of antimicrobial exposure on the microbiome and resistome in young dairy calves.

Methods

Fecal samples were collected from pre-weaned calves (n=220) at participating dairy farms and selected for inclusion using a 2x3 factorial design. The exposure factors were milk source (milk replacer or waste milk) and AMD treatment (no exposure, milk supplementation, or injection). After DNA isolation, 16S rRNA gene sequencing and AMR target-enriched sequencing were performed and data were analyzed using QIIME2, AMR++, and phyloseq.

Results

Calves fed waste milk generally had richer and more diverse microbial communities than those fed milk replacer. Yet, resistome richness and diversity was similar in all groups except calves fed waste milk supplemented with AMDs, which had a significantly richer and more diverse resistome. The resistome composition in those calves was also significantly different from all other groups. This was largely the result of significantly lower abundances of genes conferring resistance to tetracyclines and a significantly higher abundance of genes conferring resistance to β-lactams. The microbiome of calves fed waste milk supplemented with AMDs had significantly lower abundances of Actinobacteria and significantly higher abundances of Bacteroidetes.

Conclusions

Diet had a stronger impact on the microbiome than it did on the resistome. However, waste milk supplemented with AMDs significantly changed the richness, diversity and structure of the resistome. Further work investigating absolute abundances of tetracycline and β-lactam resistance genes would help elucidate the importance of these findings.

Financial Support
U.S. Department of Agriculture, Animal and Plant Health Inspection Services

Notes:

116 - Impact of dry cow therapy on the antimicrobial resistance profile of mastitis pathogens

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Session: Antimicrobial Resistance/Use - 2, Dec. 6, 2:30 - 2:45 PM

Objective

Intramammary antimicrobial drug (AMD) infusion at dry off, also known as dry cow therapy (DCT), is a key component of mastitis control programs on many dairy farms. However, use of AMD in food animal agriculture is a major public concern due to the risk of selection for and spread of antimicrobial resistance (AMR) in both animal and human populations. The aim of this study was to assess the impact of DCT on the AMR profile of mastitis pathogens during the following lactation period.

Methods

A total of 566 bacterial isolates from milk samples of 382 cows were selected for this study. The cows were enrolled at dry off and followed to 150 days in milk (DIM). Milk samples were collected from the study cows at dry off (S1), post-calving (S2) and cultured for bacterial isolation. The AMR profiles of the isolates were determined using broth microdilution.

Results

More than 90% of the coagulase negative *Staphylococcus* (CNS) spp. isolates (n=421) were susceptible to tetracycline, ceftiofur, penicillin/novobiocin and erythromycin. All *Streptococcus* spp. (n=37) were susceptible to penicillin, penicillin/novobiocin, ampicillin and ceftiofur, while non-susceptibility was observed against sulphadimethoxine (17%) and tetracycline (80.55%). All the coliforms (n=21) were susceptible to ceftiofur, while non-susceptibility was recorded for sulphadimethoxine (71%), cephalothin (57%) and tetracycline (43%). Increased AMR was observed in CNS isolates from intramammary AMD-treated cows post-calving, with the highest increases recorded for penicillin (14.95%). Models for cows with CNS isolated at both S1 and S2 showed an increase in resistance against penicillin, ampicillin, oxacillin, cephalothin and ceftiofur in cows that received DCT from the same drug class, or a class with a shared resistance mechanism.

Conclusions

In summary, our results showed low resistance of mastitis pathogens to AMD commonly used for treatment and control of mastitis in dairy cows. However, the study also provided evidence that DCT was associated with antimicrobial resistance during the subsequent lactation.

Financial Support
California Department of Food and Agriculture

Notes:

117 - Farm-level study on the trends and quantitates of antimicrobial use in commercial dairy in Pakistan

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Session: Antimicrobial Resistance/Use - 2, Dec. 6, 2:45 - 3:00 PM

Objective

There is less information about the trends of antimicrobial use (AMU) in large ruminants in Pakistan. The current study was conducted to quantify AMU in corporate dairy farms in Pakistan with the aim to investigate seasonal variations and the use of critically important antimicrobials (CIA).

Methods

The study scheme was cross-sectional to quantify AMU from 10 corporate dairy farms in Pakistan in the summer and winter season 2020-2021. These dairy farms were eligible if they had >100 lactating animals with corporate commercial farming operations. AMU was calculated using antimicrobial treatment incidence (ATI), with measurement unit being the number of defined daily dose animals (DDDA) used per 1,000 animals per day.

Results

It was found that a total of 29 (15.23 kg) antimicrobial drugs were consumed in target dairy farms. The combined ATI for all the respondent farms in this survey was 26.34 DDDA/1,000 animal days. Enrofloxacin (5.59 DDDA/1,000 animal-days), gentamicin (3.38 DDDA/1,000 animal-days) and tylosin (2.61 DDDA/1,000 animal-days) were commonly used for treatment purposes in summer. During winter, the widely used drugs were enrofloxacin (6.27 DDDA/1,000 animal-days), penicillin G (3.61 DDDA/1,000 animal-days) and gentamicin (2.04 DDDA/1,000 animal-days). The combined ATI for all antimicrobial agents for calves was 36.71 DDDA/1,000 animal days. Seasonal trends revealed ATI decreased by 23.9% during winter. Overall, 58% of the total usage in adult dairy cows was of CIA for human medicine as characterized by the WHO.

Conclusions

Our findings call for urgent actions to minimize CIA use in dairy farming operations to minimize human health risk. This baseline data is critical for the design of a robust and reliable AMU monitoring system in Pakistan.

Notes:

118 - Educational interventions to address misperceptions about antibiotic residues in milk

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Session: Antimicrobial Resistance/Use - 2, Dec. 6, 3:00 - 3:15 PM

Objective

The objectives of this study were to assess consumers' perceptions about the quality and production of dairy products in the US, and determine whether educational materials on processes that limit the occurrence of antibiotic residues in milk can change consumers' perceptions of dairy products and purchasing behaviors.

Methods

We surveyed 804 consumers and assigned them to one of three interventions: 1) a control arm (reading the content of the Dairy page of the USDA's myplate.gov website); 2) an educational brochure on the processes that prevent antibiotic residues in milk; and 3) a video on the same processes. Before and after undergoing the intervention, participants were asked questions about their perceptions of dairy products in the United States, emotional perceptions of various dairy labels, and purchasing habits. Changes in levels of reported concerns about antibiotic residues in the milk and the overall quality of milk following the interventions were compared among the three groups.

Results

A majority (86.1%) of participants believe the quality of dairy products in the US is high, though many had concerns about the treatment of dairy animals and chemicals (pesticides, antibiotics, hormones) in dairy products. Compared to the control intervention, the brochure was associated with a significant decrease in the level of concern consumers had about chemicals in their milk (-0.20 points on a Likert scale, $p=0.001$) and a significantly increased comfort in purchasing conventional dairy products (OR 2.43, $p<0.001$). The video was associated with even stronger effects: a 0.29-unit decrease in the level of concern about chemicals in milk ($p<0.001$) and 2.94 times greater odds of purchasing conventional dairy products ($p<0.001$).

Conclusions

While consumer food-decision making is complex and driven by multiple factors, it appears that education about the processes that promote food safety can reassure consumers about their concerns and potentially affect purchasing habits.

Financial Support
Pennsylvania Department of Agriculture

Notes:

119 - Prevalence and profiles of fluoroquinolone and macrolide resistant extended-spectrum beta-lactamase *Escherichia coli* isolated from dairy calf feces

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Session: Antimicrobial Resistance/Use - 2, Dec. 6, 3:15 - 3:30 PM

Objective

Use of cephalosporins, fluoroquinolones, and macrolides in dairy calves allows for the potential selection of multidrug-resistant extended-spectrum beta-lactamase (ESBL) *Escherichia coli*. Identifying emerging resistance patterns to these antibiotics is critical due to their designation as highly important to human health by the WHO. This study investigated potential selection sources and patterns of plasmid-mediated fluoroquinolone (PMQR) and macrolide resistance in ESBL *E. coli* isolated from dairy calf feces.

Methods

Fresh fecal samples (n=150) were collected from a dairy farm in the southern United States for yearling (n=50), post-weaned (n=49), and pre-weaned (n=50) calves and enriched for ESBL *E. coli*; one sample was missing from the post-weaned group. PMQR (*qnrB*), macrolide (*mph*(A)), and beta-lactam ($bla_{\text{CTX-M}}$ groups 1 and 9) resistance genes were identified by PCR in *E. coli* isolates from ESBL positive samples (n=232). Then, beta-lactamase variants and other resistance genes were characterized using whole-genome sequencing (n=8). Also, total and antibiotic-resistant *E. coli* growth was quantified for ten samples from each age group. ANOVA or univariate regressions evaluated associations among age, antibiotic use, and ESBL *E. coli*.

Results

In total, 73 (49.0%) samples were positive for ESBL *E. coli.* ESBL prevalence was significantly higher in the youngest calves, but antibiotic use and respiratory health events had no association with increased prevalence. Of the isolates with $bla_{\text{CTX-M}}$, 165 (71.1%) were identified as group 1 and 67 (28.9%) as group 9. Resistance gene *mph*(A) was more commonly associated with $bla_{\text{CTX-M}}$ group 1, while *qnrB* was more commonly associated with group 9. Additionally, significantly higher quantities of antibiotic resistant *E. coli* were observed in the pre-weaned calves.

Conclusions

The prevalence of ESBL *E. coli* in dairy calves varies significantly by age. Results also indicate $bla_{\text{CTX-M}}$ group has an association with fluoroquinolone or macrolide resistance. Further work is needed to examine antibiotic use in the selection of multidrug-resistant ESBL *E. coli*.

Notes:

120 - Association between antimicrobial class for bovine respiratory disease (BRD) treatment and performance outcomes

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Session: Bovine Respiratory Disease, Dec. 6, 4:15 - 4:30 PM

Objective

Treatment and control of bovine respiratory disease (BRD) is predicated on the use of two categories of antimicrobials, namely bacteriostatic drugs that inhibit bacterial growth and replication (STATIC), and bactericidal drugs that kill bacteria in in-vitro culture systems (CIDAL). The present study was conducted to test the hypothesis that calves administered CIDAL-CIDAL or STATIC-STATIC antimicrobials for first and second BRD treatment would have improved health and performance outcomes compared to calves that received STATIC-CIDAL or CIDAL-STATIC first and second treatments.

Methods

The association between antimicrobial treatments and health, performance and carcass quality outcomes were determined by a retrospective analysis of 4,252 BRD treatment records from a commercial feedlot operation collected from 2001 to 2005. Data were compared using generalized linear mixed statistical models (GLMM) that included gender, season and arrival weight as covariates.

Results

The mean (± SE) percent probability of BRD cases identified as requiring 4 or more treatments compared to 3 treatments was greater in calves that received STATIC-CIDAL (73.58 ± 2.38%) or STATIC-STATIC (71.32 ± 2.52%) first and second antimicrobial treatments compared to calves receiving CIDAL-CIDAL (50.35 ± 3.46%) first and second treatments ($P < 0.001$). Calves receiving CIDAL-CIDAL first and second treatments also had an increased average daily gain (ADG) (1.11 ± 0.03 kg/d) compared to calves receiving STATIC-CIDAL (0.95 ± 0.03 kg/d) and STATIC-STATIC (0.84 ± 0.02 kg/d) treatments ($P < 0.001$). Furthermore, CIDAL-CIDAL treated calves had a higher probability of a choice quality grade at slaughter (36.44 ± 4.80%) compared to STATIC-CIDAL calves (28.09 ± 3.88%) ($P = 0.037$).

Conclusions

These observations suggest that consideration should be given to antimicrobial pharmacodynamics when selecting drugs for retreatment of BRD. These findings have implications for developing BRD treatment protocols that address both post-treatment production and antimicrobial stewardship concerns.

Notes:

121 - Disease outcome and metabolic responses of preweaned dairy calves supplemented with *Saccharomyces cerevisiae* fermentation products to BRSV and *Pasteurella multocida* coinfection

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Session: Bovine Respiratory Disease, Dec. 6, 4:30 - 4:45 PM

Objective

Bovine respiratory disease is a leading cause of mortality in preweaned dairy calves and weaned dairy heifers. *Saccharomyces cerevisiae* fermentation products (SCFP) are known to have positive impacts on health and immune function in calves. The objective of this study was to determine the effect of SCFP supplementation on disease outcome and immunologic, metabolic, and endocrine responses to a co-infection with bovine respiratory syncytial virus (BRSV) and *Pasteurella multocida* (*PM*).

Methods

Twenty-eight, 1-2 day old Holstein-Angus cross calves with adequate passive transfer were enrolled in this 32-day study. Calves were assigned to two treatment groups (14/group): 1) control, base milk replacer and calf starter; or 2) SCFP treated, milk replacer with 1 g/d SmartCare and calf starter top-dressed with 5 g/d NutriTek. One calf in each group was euthanized during the feeding period, so 13 calves/group were included in the challenge period. Calves were infected with ~10^4 (Median Tissue Culture Infectious Dose, $TCID_{50}$) BRSV on day 21, followed 6 days later by intratracheal inoculation with ~10^{10} colony-forming units of *PM* (*PM*, strain P1062). Calves were euthanized on day 10 post-viral infection.

Results

Calves treated with SCFP had a tendency towards lower thoracic ultrasonography scores ($P = 0.089$) and lower lung pathology scores at necropsy ($P = 0.064$). No differences observed in lung viral loads, although BRSV was detected in fewer SCFP fed calves compared to control fed calves (5/12 control vs. 3/13 SCFP). No differences in bacterial lung loads were detected between treatment groups. Calves treated with SCFP tended to have higher serum concentrations of IL-6 ($P = 0.074$), but lower concentrations of β-Hydroxybutyrate ($P = 0.082$) and non-esterified fatty acids ($P = 0.031$) in the days following viral-bacterial coinfection.

Conclusions

Results from this study suggest that supplementing with SCFP may impact metabolic and immunologic responses to disease, as well as improve the outcome of a respiratory viral-bacterial coinfection in preweaned calves.

Financial Support
Diamond V Mills, Inc.

Notes:

122 - Digital mRNA profiling of high-risk beef cattle at arrival substantiates markers of bovine respiratory disease

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Session: Bovine Respiratory Disease, Dec. 6, 4:45 - 5:00 PM

Objective

Previous transcriptome research identified at-arrival differentially expressed genes (DEGs) and mechanisms associated with bovine respiratory disease (BRD) development and severity. However, most transcriptome studies are performed with a limited number of cattle and may not reflect heterogeneity across broader populations. Our objective was to substantiate expressional patterns of select mRNA across independent populations.

Methods

At-arrival blood samples from seven populations were randomly selected from cattle retrospectively never demonstrating clinical BRD (n=115) and treated for BRD within 28 days of arrival (n=119); BRD samples were stratified into treatment frequency cohorts (n=89, treated_1; n=30, treated_2+/died). RNA was isolated via Tempus Spin RNA Isolation kits and hybridized to target-specific reporter/capture probes for 56 genes plus four controls, according to manufacturer's protocol. 30-35µL of hybridized mRNA was profiled with NanoString nCounter SPRINT system at a field of view sensitivity of 555. DEGs were identified through nSolver Advanced Analysis (MAN-C0011-04; $p<0.05$). Overrepresentation analysis for functional enrichment was performed within WebGestalt (FDR<0.05). Statistical analyses were performed within R.

Results

17 DEGs were identified between Healthy and BRD; 30 unique DEGs were identified between the three cohorts. Healthy cattle possessed increased specialized proresolving mediator and defense response expression. BRD cattle possessed increased alternative complement and cell-cell adhesion expression. Type I interferon-related expression was increased in treated_2+ cattle. Average daily weight gain at time of sale significantly decreased with increased treatment frequency ($p<0.0001$).

Conclusions

Data from multiple populations of cattle corroborate findings from previous transcriptome experiments. Collectively, this study establishes key molecules and demonstrates nCounter profiling viability in at-arrival BRD predictions.

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

Notes:

123 - Detecting strain-level shifts in *Mannheimia haemolytica* phylogeny and function via target-enriched metagenomics

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Session: Bovine Respiratory Disease, Dec. 6, 5:00 - 5:15 PM

Objective

Mannheimia haemolytica (Mh) is a commensal member of the microbial community within the upper respiratory tract of cattle. However, it is also a predominant bacterial agent associated with bovine respiratory disease, which ultimately costs the cattle industry in the United States over a billion dollars annually. To circumvent the limitations of culture-dependent techniques, we developed a culture-independent method that characterizes strain-level shifts in Mh phylogeny and function.

Methods

Target-enriched metagenomics was done using a custom bait set that included 42,124 probes for non-redundant Mh genes, 2,131 probes for integrating and conjugative element (ICE) genes, and 1,361 loci previously determined to allow for intra-species differentiation. Following metagenomic sequencing, reads were processed using a custom bioinformatic pipeline that included steps for sequence quality control, removal of host DNA, removal of sequences originating from microorganisms other than Mh, and alignment to a custom-made database of Mh functional genes.

Results

Results demonstrated that the custom bait set is extremely successful in enriching for DNA sequences originating from Mh, with over 99.5% of all classified sequences being discriminant of its lineage. Further, approximately 1% of sequences belonged to ICE genes, which have been proven important to multi-drug resistance within Mh and bovine respiratory disease. A custom database built from the 191 Mh genomes available in NCBI's RefSeq yielded 27, 602 non-redundant coding sequences that were used to functionally profile Mh populations associated with healthy and diseased states.

Conclusions

This research will allow researchers to effectively enrich Mh without being limited by the restrictions of culture-dependent methodologies. Results strongly suggest that the creation of a user-friendly bioinformatic pipeline and custom Mh database in combination with target-enriched metagenomic sequencing will characterize strain variability within individuals and groups of animals, as well as the transmission dynamics of Mh at a level not previously possible.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture; Texas A&M University

Notes:

124 - Characterization of the *Mannheimia haemolytica* biofilm and poly-microbial interaction with other *Pasteurellaceae*

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Session: Bovine Respiratory Disease, Dec. 6, 5:15 - 5:30 PM

Objective

Mannheimia haemolytica is one agent responsible for bovine respiratory disease (BRD). Bacterial biofilms form during chronic infections, and confer the bacteria with resistance to antimicrobial agents. *M. haemolytica* produces a biofilm, but the nature of the biofilm matrix and the interaction with other BRD pathogens is unknown. Here we have characterized factors affecting *M. haemolytica* biofilm formation, biofilm composition, and the interaction between *M. haemolytica, Histophilus somni,* and *Pasteurella multocida*.

Methods

A capsule-deficient mutant of *M. haemolytica* (E09) was obtained following mutagenesis with ethyl methanesulfonate, and the mutation confirmed by sequencing. Encapsulation was measured by transmission electron microscopy (TEM) and Maneval's staining. Biofilm production was assayed by crystal violet staining and confocal laser scanning microscopy with COMSTAT analysis. Biofilm matrix composition was determined by Anthrone and BCA assays, enzyme hydrolysis, and gas chromatography-mass spectrometry.

Results

M. haemolytica made a poorly adherent and low biomass biofilm. Mutant E09, confirmed to be capsule-deficient, produced a biofilm with significantly more biomass but less roughness than the parent. The biofilm matrix of E09 contained predominately protein, significantly more eDNA, but not a distinct exopolysaccharide. Sequencing indicated a point mutation occurred in the capsule biosynthesis gene *wecB*, resulting in a premature stop codon. Treatment with DNase I significantly reduced biofilm content of both the parent and E09. FISH microscopy showed that *M. haemolytica* formed a poly-microbial biofilm with *H. somni* and *P. multocida*.

Conclusions

Mutant E09 contained less capsule than the parent, resulting in enhanced biofilm formation, and an increased amount of eDNA. The reduction in capsule production may have exposed adhesins that enhanced surface adherence. A poly-microbial biofilm was readily formed between *M. haemolytica, H. somni*, and *P. multocida*, suggesting a mutualistic or synergistic interaction that may be beneficial for bacterial colonization in the lungs.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture; Long Island University

Notes:

125 - Do modified-live virus respiratory vaccines influence the respiratory microbiome of cattle?

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Session: Bovine Respiratory Disease, Dec. 6, 5:30 - 5:45 PM

Objective

Modified-live virus respiratory vaccines (MLV) are commonly administered to beef cattle upon arrival in feedlots to prevent bovine respiratory disease (BRD). Yet, the impact of these vaccines on respiratory microbial communities is unknown. The objective of this study was to evaluate the impact of intranasal and parenteral MLV vaccination on the respiratory microbiome of feedlot cattle with a high-risk of BRD.

Methods

Cross-bred beef calves were blocked by truckload and stratified by arrival body weight, sex, and ranch tag status. The cattle were randomly assigned to 1 of 3 treatment groups: no vaccination, intranasal MLV, or parenteral MLV. Nasopharyngeal swabs were collected on D0 and D28, and collected from cattle treated for BRD. A subset of these samples (n=600) was selected from an equal number of healthy and morbid cattle among vaccine treatment groups. Additionally, all chronically ill cattle and mortalities were selected for sequencing. After DNA extraction, 16S rRNA gene sequencing was used to characterize the microbiome of the upper respiratory tract.

Results

The overall composition of respiratory tract microbial communities in all treatment groups was similar to previously described respiratory communities. Members of the families Pasteurellaceae and Mycoplasmataceae, which include common respiratory pathogens (*Mannheimia*, *Pasteurella*, *Histophilus*, *Mycoplasma*), were prevalent in all communities. However, the proportions of these important taxa differed between cattle that developed BRD and cattle that remained healthy. Cattle that received an intranasal vaccine had a significantly higher abundance of Proteobacteria and significantly lower abundance of Tenericutes compared to both control and parenteral cattle.

Conclusions

Results indicate that intranasal vaccination had a significant impact on respiratory microbial communities, while parenteral vaccination did not. These results add to our understanding of the impacts of MLV vaccination on the health and productivity of feedlot cattle.

Notes:

126 - Antigenic profiling of *Flavobacterium columnare* genetic groups affecting cultured fish in the United States

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Session: Aquaculture, Dec. 6, 2:00 - 2:15 PM

Objective

Columnaris disease, caused by the gram-negative bacterium *Flavobacterium columnare,* is a pervasive disease in a wide range of wild and cultured freshwater fish worldwide. Most commercially valuable species cultured in the United States are affected by this disease, including all salmonids, as well as catfish (*Ictalurus* spp.), and tilapia (*Oreochromis* spp.). There are currently no effective preventative methods available to control this pathogen, and consequently, it causes significant economic losses in the aquaculture industry. The genetic diversity of *F. columnare* has recently been elucidated, and four distinct phyletic groups have been established. We hypothesized that these genetic groups share conserved, immunogenic proteins that could be used to generate a polyvalent, cross-protective vaccine.

Methods

One representative strain from each genetic group (n=4) was used to immunize tilapia (*O. niloticus,* n=10), channel catfish (*I. punctatus*, n=10), and rainbow trout (*Oncorhynchus mykiss*, n=10) yearlings (>100g) by intracoelomic injection, with a booster immunization 30 d post-injection. Fish were bled at ~720 and 1000 degree-days and α-*F. columnare* IgM quantified by ELISA. Shared immunogenic proteins were determined by SDS-PAGE and western blot analyses, then identified by liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS). Concurrent *in silico* analysis was used to identify putative conserved antigens in the whole genomes of the representative isolates and publicly available reference genomes.

Results

Each fish species generated an antibody response against all four strains. Titers were generally similar between timepoints, and antisera from each treatment was pooled for immunoproteomics. Both *in vitro* and *in silico* approaches identified shared immunogenic proteins, including candidates that have been used to generate vaccines against other bacterial pathogens.

Conclusions

The four *F. columnare* genetic groups share antigenic proteins that can be further developed and tested as recombinant vaccines to protect against columnaris disease in economically relevant fish species.

Financial Support
USDA-NIFA Special Research Grants Program Aquaculture Research

Notes:

127 - Development of live attenuated *Aeromonas hydrophila* vaccine candidates

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Session: Aquaculture, Dec. 6, 2:15 - 2:30 PM

Objective

Aeromonas hydrophila is a Gram-negative facultatively-anaerobe rod causing motile *Aeromonas* septicemia (MAS) in fish. MAS has been an important problem in the channel catfish industry, causing rapid and high mortalities. Approved antibiotics are added to fish feed for treatment of MAS, but moribund fish become anorexic, limiting the effectiveness of medicated feed. Also, antibiotic use adds to the production cost and can result in antimicrobial-resistant strains. Thus, vaccine-based prevention of MAS is a good alternative.

Methods

In this work, we utilized in-frame deletion technique to modify 24 *A. hydrophila* genes involved in different bacterial functions, including the type IV secretion system, Sec pathway, twin-arginine translocation system, ATP binding, flagellar system, and lipopolysaccharide assembly. Then, these mutants were characterized in catfish fingerlings by intraperitoneal injection.

Results

Of the 24 mutants, three were attenuated in catfish, and after vaccination, one of the attenuated strains provided 55.5% relative percent survival.

Conclusions

The attenuated strain could be used as a potential live attenuated vaccine against MAS in catfish.

Notes:

128 - Evaluation of inactive vaccines against *Lactococcus garvieae* infection in rainbow trout (*Oncorhynchus mykiss*)

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Session: Aquaculture, Dec. 6, 2:30 - 2:45 PM

Objective

Lactococcus garvieae is an important emergent pathogen of farmed fish in the USA. The purpose of this study was to evaluate protection conferred to rainbow trout (*Oncorhynchus mykiss*) against *L. garvieae* by a formalin-killed vaccines in immersion and injectable forms, as well as enhanced protection afforded by booster vaccination.

Methods

In the first experiment, fish were immunized via immersion (IM) or intracoelomic injection (ICi) routes. Approximately 418-degree days (dd) IM, or 622 dd ICi post-vaccination, fish were challenged via ICi with wild-type *L. garvieae.* Additionally, we investigated the immune response of trout to immunization supplemented with the ICi and IM adjuvants using transcriptome analysis. In the second experiment, initial IM vaccination was followed by booster vaccination via IM or ICi routes 273 dd post-immunization along with appropriate PBS controls. The various vaccination protocol efficacies were evaluated by challenging fish with *L. garvieae* by cohabitation with diseased fish 399 dd post-booster administration.

Results

In the first study, a relative percent survival (RPS) of 28% and 89.5% was recorded in the IM and ICi treatments, respectively. Protection correlated with increased IL-10 gene expression in gills, and increase gene expression of IL-6 and TNF-α in spleen of IM immunized fish prior to challenge. On the other hand, only significantly greater TGF-β in gills of IC immunized fish was detected compared to the controls. In the second study, an RPS of 98%, 14%, 3% and -8% was recorded in the IM immunized + ICi boosted, IM immunized + mock ICi boosted, IM immunized + IM boosted and IM immunized + mock IM boosted treatments, respectively.

Conclusions

Both IM and ICi vaccines induce an immune response in gills and spleen of immunized fish. Immune response in vaccinated fish is stronger than in adjuvant and control treatments, and although both IM and ICi vaccines appear to be safe to fish, ICi immunized fish develop a significantly stronger protective response.

Financial Support
U.S. Department of Agriculture, Animal Health Formula Funds; California Department of Fish and Wildlife Statewide Fish Disease Research Program

Notes:

129 - Developing a dual live attenuated vaccine to prevent motile aeromonas septicemia and enteric septicemia of catfish.

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Session: Aquaculture, Dec. 6, 2:45 - 3:00 PM

Objective

Sales from catfish aquaculture totaled $386 million in 2020, and Mississippi produced 54% of total farm-gate sales. *Aeromonas hydrophila* is a pathogen that affects farm-raised catfish causing motile aeromonas septicemia (MAS). Since 2009, outbreaks caused by a genetic clonal group of *A. hydrophila* (virulent *A. hydrophila* or vAh) caused significant losses of market-size catfish in Alabama and Mississippi. There are limitations in the current therapeutic and preventative strategies against vAh. Our preliminary data revealed that recombinant vAh surface proteins (Fim, FimMrfG, ATPase, Tdr, and OmpA1) are effective in protecting catfish against MAS. Furthermore, live attenuated *Edwardsiella ictaluri* vaccine strain ESC-NDKL1 is an efficacious vaccine for enteric septicemia of catfish (ESC), and it is an effective vector for expressing vAh antigens. Our hypothesis is that expression of vAh surface proteins in a live attenuated *E. ictaluri* vaccine will provide significant protection against both MAS caused by vAh and ESC.

Methods

Three pMEG-375 suicide plasmids were constructed and used for conjugation and integration of gene combinations encoding vAh antigens into gene deletion sites in the ESC-NDKL1 chromosome. A total of 32 stable recombinant ESC-NDKL1 strains expressing one, two, or three vAh surface antigens were successfully constructed. Specific pathogen free catfish fingerlings were used for vaccination trials with five replicate tanks per treatment. Fish were vaccinated by immersion, and 21 days after vaccination fish were experimentally infected with $1X10^5$ CFU vAh strain ML09-119 by IP injection.

Results

Recombinant ESC-NDKL1 strains expressing two vAh antigens showing significant protection against MAS and improved protection compared to recombinant ESC-NDKL1 strains expressing one vAh antigen. Additional testing with double and triple antigen combinations inserted in the ESC-NDKL1 chromosome is being conducted.

Conclusions

We expect to identify promising vaccine candidates that will yield an important tool to control two diseases that impact the catfish industry.

Financial Support
U.S. Department of Agriculture, Animal Health Formula Funds

Notes:

130 - Latent acipenserid herpesvirus 2 results in higher mortality of White Sturgeon after *Streptococcus iniae* challenge

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Session: Aquaculture, Dec. 6, 3:00 - 3:15 PM

Objective

White sturgeon (*Acipenser transmontanus*) is an important cultured fish in the United States' caviar and sturgeon meat industries. Acipenserid herpesvirus 2 (AciHV-2) is a large double stranded DNA virus that can cause mortalities in fry and adult white sturgeon. *Streptococcus iniae* is a gram-positive zoonotic bacterium that can result in up to 50% mortality in subadult white sturgeon. Natural coinfections of AciHV-2 and *S. iniae* have been diagnosed in cultured white sturgeon in California since 2015, but little is known regarding disease pathogenesis and immune response during these coinfections.

Methods

To investigate potential immunomodulatory virulence genes in AciHV-2, whole-genome analysis was performed of four isolates recovered from California. To begin to elucidate the role of AciHV-2 during *S. iniae* coinfections, an *in vivo* challenge was conducted in which white sturgeon fingerlings were exposed to AciHV-2 via immersion. Once the population was stable, survivors were exposed to *S. iniae* intramuscularly and mortalities monitored for 3 weeks.

Results

The four AciHV-2 assemblies cluster in a similarity matrix with the published partial AciHV-2 sequence and separate from other herpesviruses. AciHV-2 strains have 133-188 kb, encoding 108-150 predicted proteins. Homology prediction infers that 16-25 proteins in the AciHV-2 genome are homologous to sterlet sturgeon proteins (closest relative of white sturgeon with genome available). White sturgeon previously exposed to AciHV-2 had a significantly lower survival probability after *S. iniae* infection compared to control white sturgeon ($p < 0.05$).

Conclusions

Genome analysis confirms that the sequences obtained correspond to four strains of AciHV-2 and seem to encode candidate virokines that should be investigated further for host immune interactions. Previous infection with AciHV-2 resulted in higher mortalities after infection with *S. iniae*. Based on this data, a better understanding of the host immune response post infection via gene expression studies is essential for elucidating the role of AciHV-2 in coinfection mortality events.

Financial Support
NIH T-32 OD011147 (EMQC); USDA NIFA (Animal Health Project CACALV-AH-410)

Notes:

131 - Virulence, immunogenicity and vaccine potential of *aroA* and *phoP* mutants of *Edwardsiella piscicida* in zebrafish

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Session: Aquaculture, Dec. 6, 2:45 - 3:00 PM

Objective

Study the role of *aroA* and *phoP* genes in *E. piscicida* and investigate the phenotype, degrees of attenuation, immunogenicity, and ability to confer immune protection in zebrafish.

Methods

Insertion of defined deletion mutations was accomplished by conjugational transfer of suicide vectors to *E. piscicida* J118 using the suicide vector donor strain χ7213. We have analyzed the systemic and mucosal IgM responses by ELISA. We have studied cytokine gene expression by qRT-PCR.

Results

Vaccination remains the most effective approach for prevention and control of infectious diseases in aquaculture. *E. piscicida* is a causative agent of edwardsiellosis leading to mass mortality in a variety of fish species, and to huge economic losses in the aquaculture industry. In this study, we have deleted the *aroA* and *phoP* genes in *E. piscicida* and investigated the phenotype, degrees of attenuation, immunogenicity, and ability to confer immune protection in zebrafish. Our vaccine strain c16028 with genotype D*aroA11* D*phoP12*, showed significantly reduced growth, motility, biofilm formation and intracellular replication compared to the wild-type strain J118. In this regard, c16028 exhibited retarded colonization and attenuation phenotype in zebrafish. Studies showed that c16028 induced TLR4 mediated NF-kB pathway and upregulated cytokine gene expression i.e., *tnf-a*, *il-1β*, *il-6*, *il-8* in zebrafish. Zebrafish immunized by intracoelomic injection (i.c.) with c16028 showed systemic and mucosal IgM responses and protection against the wild-type *E. piscicida* i.c. injection challenge. However, the protection was only 25% in zebrafish following i.c. challenge

Conclusions

We speculate that our vaccine strain might be hyper attenuated; a booster dose may trigger better immune responses and increase the percentage survival to a more significant level.

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

Notes:

132 - Evaluation of three different commercial DNA extraction kits for chicken gut microbiota profiling

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Session: Diagnostic Testing - 2, Dec. 6, 4:15 - 4:30 PM

Objective

Examination of chicken gut microbiota using amplicon sequencing techniques has greatly improved the understanding of the critical role of microbiota in host health and physiology. However, it is still largely unknown if the choice of specific kit for fecal DNA extraction could lead to different microbiota profiling and data interpretation. The objective of this study was to analyze both bacterial and fungal communities in the chicken intestine using three commonly used DNA extraction kits.

Methods

The cecum and ileum samples from 8 healthy chickens were subjected to side by side comparison of the performance of three commercial DNA extraction kits from Qiagen, MP Bio, and Zymo. A one-step 16S rRNA gene amplification targeting V4 region and a two-step ITS PCR workflow targeting ITS1 and ITS2 regions were implemented for library preparations for bacterial and fungal communities, respectively. The pooled libraries were sequenced on the Illumina Miseq platform for paired-end sequencing. Qiime2 workflow dada2 was utilized to obtain representative sequences and Scikit-Learn classifier was used to acquire taxonomic profile. Statistical analysis was performed by Kruskal-Wallis test, PCoA, PERMANOVA, ANOSIM, and LEfSe analysis.

Results

All kits generated good quality DNA with slightly higher salt residues in DNA extracted by MPBio. Alpha diversity analysis suggested that the samples extracted by MPBio and Zymo kits had higher richness and evenness than those extracted by Qiagen kit. Both PERMANOVA and ANOSIM analysis indicated that individual differences had a greater contribution to the variations in microbial communities than the choice of DNA kit. LEfSe analysis illustrated that Qiagen kits failed to detect some minor but meaningful populations notably at genus, species, and OTU levels when compared to the other two kits, especially in ileum samples.

Conclusions

Different DNA extraction kits could lead to partially contradictory results aiming at some minor populations although all the kits provided good quality DNA for general microbiota profiling in the chicken gut.

Notes:

133 - Detection of *Mycoplasma hyopneumoniae* antibody in processing fluids using a commercial serum ELISA

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Session: Diagnostic Testing - 2, Dec. 6, 4:30 - 4:45 PM

Objective

Mycoplasma hyopneumoniae (*MHP*) antibody surveillance in breeding herds is usually based on serum samples. Processing fluids (PF), tissue exudates recovered from testicles and tails collected at the time of piglet processing, contain detectable *MHP* antibody and have been proposed as an option for routine sow herd *MHP* surveillance (Boettcher et al., 2010). Therefore, the purpose of this study was to evaluate the diagnostic performance of PF samples in the detection of *MHP* antibodies using a commercial ELISA.

Methods

Processing fluid samples were collected from one farm considered *MHP*-endemic (n = 246) and two farms considered *MHP*-free (n = 248) and then tested at a 1:10 dilution using a commercial *MHP* ELISA designed to detect anti-P46 antibodies in serum (*M. hyo* Ab test, IDEXX Laboratories, Inc.). Results were analyzed by receiver operating characteristic (ROC) analysis and diagnostic sensitivities and specificities were estimated over a range of ELISA sample-to-positive (S/P) cutoffs.

Results

At a cutoff of S/P $\geq$ 0.40, the ROC analysis estimated diagnostic sensitivity and diagnostic specificity as 97.6% (95% CI: 95.5, 99.2) and 100.0 (95% CI: 100, 100), respectively. That is, all samples (n = 248) from *MHP*-free herds produced S/P values < 0.3 and 3 of 246 samples from the *MHP*-endemic farm had S/P values < 0.4.

Conclusions

The *MHP* antibody ELISA provided excellent discrimination between processing fluid samples from *MHP*-free and *MHP*-endemic sow herds. Thus, this study suggested that processing fluids could serve as an alternative approach for routine *MHP* antibody-based sow herd surveillance.

Notes:

134 - Utility of kanamycin to isolate *Mycoplasma hyopneumoniae* from field samples contaminated with *Mycoplasma hyorhinis*

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Session: Diagnostic Testing - 2, Dec. 6, 4:45 - 5:00 PM

Objective

Isolation of *Mycoplasma hyopneumoniae* (*Mhp*) from swine field samples is challenging and easily contaminated by *Mycoplasma hyorhinis* (*Mhr*). The goal of this study was to enhance the ability to isolate *Mhp* from potentially dual *Mhp*/*Mhr* positive clinical specimens.

Methods

Reference isolates *Mhp*232, *Mhr*SK76, and two *Mhp*/*Mhr* dual positive field samples were inoculated into Friis agar plates with 0, 0.5, 1.0, 2.0, or 4.0 µg/ml of kanamycin and evaluated for 28 days. Duplicated samples were inoculated into Friis broth with 0, 0.25, 0.5, 1.0, or 2.0 µg/ml of kanamycin. Three days post-inoculation (DPI), cell viability in broth samples was analyzed by flow cytometry with SYTO 9 and propidium iodide staining.

Results

Both reference strains and clinical samples grew on the positive control plates (no kanamycin), no growth was observed on 4.0 µg/ml kanamycin and the negative control plates. Only *Mhp* 232 grew on 1.0 and 2.0 µg/ml kanamycin plates. By 3 DPI, all samples grew in broth media without kanamycin, with evident color change and none in 2.0 µg/ml kanamycin tubes. Only *Mhp* 232 grew in 0.25, 0.5 and 1.0 µg/ml kanamycin tubes. Cell viability analysis demonstrated a significant reduction in the proportion of viable cells (>50%) in the broth inoculated with *Mhr* SK76 and dual positive field samples. However, at 4, 7, and 15 DPI, there were color changes on *Mhr* SK76 broth samples containing 0.25, 0.5 and 1.0, and 2.0 µg/ml kanamycin, respectively, but *Mhp* 232 at 2.0 µg/ml kanamycin remained negative.

Conclusions

On solid media, the addition of 1.0 and 2.0 µg/ml kanamycin to Friis plates selectively inhibited the growth of *Mhr* over *Mhp*. For broth media, decreased numbers of viable *Mhr* at 3 DPI suggests *Mhr* growth is inhibited early, but later color change suggests survival despite the presence of kanamycin. Moreover, both field dual positive samples were negative on kanamycin Friis plates. We conclude that although kanamycin is selective on reference strains on solid media, further modifications are required for this approach to selectively isolate *Mhp* from *Mhp*/*Mhr* clinical samples.

Notes:

135 - Application of loop mediated isothermal amplification (LAMP) for detection of influenza A virus in swine

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Session: Diagnostic Testing - 2, Dec. 6, 5:00 - 5:15 PM

Objective

The COVID-19 pandemic brought attention to the need for rapid, point-of-care diagnostic testing and the challenges presented by limited reagent ability and assay turnaround time. Many laboratories use RT-qPCR for screening of viral respiratory diseases. Here we describe the application of reverse transcription loop-mediated isothermal amplification (LAMP) to detect the matrix gene of influenza A virus in swine (IAV-S) for future application as a point of care diagnostic assay on-farm.

Methods

Six LAMP primers were designed using M genes isolated from IAV-S between 2017 and 2020 using LAMP Designer software (OptiGene). The WarmStart fluorescent LAMP kit (NEB) was used with a modified protocol for all LAMP reactions. The LAMP assay was placed in a qPCR thermocycler at 65˚C for 30 minutes. The fluorescent signal was read every 20 seconds. A matrix gene standard was used to analyze the sensitivity of the LAMP primers. Ten-fold dilutions of the standard were spiked into two commercial viral nucleic acid extraction kits to compare the limit of detection (LOD) of the recovered nucleic acid. Lab-grown virus was then serially diluted for comparison of recovery from the two commercial kits.

Results

The assay's limit of detection (LOD) was 20 molecules for direct LAMP of the standard matrix gene, and a LOD of 100 molecules from both spiked extraction kits was observed. 161 assays were performed on cell culture virus. Recovery of the extracted virus was less robust, with detection of about a 10^3-fold dilution of the $TCID_{50}$ tested, with an estimated equivalence of 1000 molecules per reaction.

Conclusions

Point-of-care diagnostic technologies are becoming more widely available for production species. The influenza matrix gene LAMP assay is able to detect the presence of IAV under lab conditions. With the appropriate fluorescent reader and heat block, the assay could be quickly validated as a low-cost, rapid, IAV-S screening tool on the farm or in the clinic.

Notes:

136 - Retrospective analysis of *Mycoplasma hyorhinis* infection and correlation of qPCR Ct values and direct detection by RNAscope®

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Session: Diagnostic Testing - 2, Dec. 6, 5:15 - 5:30 PM

Objective

Mycoplasma hyorhinis (*Mhr*) disease is characterized by polyserositis and arthritis in neonatal and nursery pigs. However, it has been suggested that in the absence of other pathogens, *Mhr* can produce similar lesions to those observed during *M. hyopneumoniae* (*Mhp*) infections, suggesting that it could be a primary pneumonic agent. The diagnosis of *Mhr* disease is determined by detecting lesions consistent with polyserositis and *Mhr* detection from fibrin or joint fluid by PCR or bacteriological culture. There is scarce information about the use of different diagnostic tools that correlate the role of *Mhr* with different lesions. Therefore, this study aims to 1) determine the coinfection rate of *Mhr* with different bacterial pathogens in clinical samples using retrospective diagnostic data 2) correlate *in situ* detection and quantification of *Mhr* by RNAscope® with the amount of *Mhr* nucleic acid detected by qPCR in different tissue samples in naturally infected pigs.

Methods

A retrospective assessment was performed on *Mhr* cases evaluated by qPCR on different sample matrices submitted to ISU-VDL between January 2016 and March 2021. The proportion of detection on lung and fibrin and the proportion of bacterial coinfections was evaluated. A subset of random respiratory (n=19) and systemic (n=28) cases were selected for further *Mhr* RNAscope® *in situ* detection, quantification, and correlation with *Mhr* qPCR Ct values.

Results

The detection rate of *Mhr* in lung was 9.8% and fibrin 62.9%, in addition to a high prevalence of *Mhr* coinfection with systemic and respiratory pathogens. No correlation between the amount of *Mhr* detected *in situ* by RNAscope® and qPCR Ct values was observed in systemic cases but there was a strong correlation in pulmonary cases.

Conclusions

The detection of *Mhr* without concomitant pathogens supports its role as a primary agent in systemic disease. Polymicrobial infections are common in the field, leading to the conclusion that pathogen interaction may favor *Mhr* infection, colonization, and replication. The high proportion of respiratory cases and the presence of BALT hyperplasia as a common feature in *Mhr*-positive respiratory cases support the potential role of *Mhr* as a pulmonary pathogen. The use of RNAscope® in conjunction with digital analysis showed a high correlation with qPCR detection in lungs. Thus, this technique can add diagnostic value to *Mhr*-respiratory cases and could be an essential tool for investigating the *Mhr* pathogenesis.

Notes:

137 - Use of an endogenous reference control in a PRRSV RT-qPCR

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Session: Diagnostic Testing - 2, Dec. 6, 5:30 - 5:45 PM

Objective

Performance of qPCR can be affected by testing procedures, i.e., extraction and/or amplification steps, but detection may also be affected by sample handling. Was the sample chilled after collection to protect the analyte? Did it go through multiple freeze/thaw cycles? Ideally, a diagnostic qPCR procedure would include an indicator of sample quality. In fact, endogenous reference controls (ERC) are used for this purpose in basic research. Since ERCs are subjected to the same conditions as the target, detection of the ERC indicates both that the test procedure was conducted correctly and that the sample was of good quality. Herein, we report the ERC response in serum and oral fluids collected from pigs vaccinated against PRRSV under experimental conditions.

Methods

12 pigs were vaccinated with a PRRSV MLV (Ingelvac®) under experimental conditions. Serum (n = 132) and oral fluids (n = 130) were collected from -7 to 42 days post vaccination (DPV) and then tested with a PRRSV RT-qPCR that detects both PRRSV RNA and a pig-specific ERC (IDEXX Laboratories, Inc.). The resulting Cq values of the ERC in serum and oral fluids were evaluated within pigs through DPV's.

Results

The ERC was detected in all serum and oral fluid samples (n = 262). Based on analysis using R 4.1.0 (R core team, 2020), data for ERC Cq in oral fluids was normally distributed (Shapiro-Wilk normality test, $p = 0.584$) and the 95% reference interval was 23.1 to 30.1 (90% CI). For serum, the normality assumption was rejected ($p = 0.031$). Hence, a 95% reference interval with non-parametric percentile method was performed. Upper and lower limits obtained for serum were 23.9 to 29.4 (90% CI).

Conclusions

In samples collected from healthy pigs under experimental conditions, the detection of the ERC was uniform over time and was not affected by PRRSV vaccination. While still work in progress in testing the ERC under different conditions, establishing the utility of an ERC in diagnostic qPCR to control for proper sample handling and testing procedures would be a significant improvement in swine diagnostics.

Notes:

138 - Identification of *Lawsonia intracellularis* proteins that stimulate cell-mediated immune response for subunit vaccine development

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Session: Vaccinology - 2, Dec. 6, 2:00 - 2:15 PM

Objective

To use functional analysis to identify the *Lawsonia intracellularis* antigens that trigger a robust T-cell response (as measured by interferon gamma (IFN-γ) expression) for a subunit vaccine development.

Methods

L. intracellularis proteins extracted from a commercial live-attenuated *L. intracellularis* strain were separated by molecular size ranges using sonication, sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE), gel excision, gel grinding, elution, dialysis, protein renaturation, purification, and quantification. Ten µg/mL of the purified *L. intracellularis* proteins of each molecular size ranges were used to stimulate 10^6 peripheral blood mononuclear cells (PBMCs) isolated from piglets vaccinated with a commercial killed *L. intracellularis* vaccine. IFN-γ enzyme-linked immunosorbent assays (ELISA) were performed to measure the concentration of IFN-γ secreted from the PBMCs in response to the purified *L. intracellularis* proteins or Concanavalin A (Con A) as our positive control. The *L. intracellularis* protein(s) that triggered the highest production of IFN-γ will be subjected to mass spectrometry. Bioinformatics was used to identify the proteins predicted to have T-cell epitopes likely responsible for this IFN-γ response. *L. intracellularis* protein(s) of interest were cloned, expressed, and purified and tested for immunogenicity using PBMC stimulation and IFN-γ ELISA.

Results

IFN-γ production of vaccinated pig's PBMCs and *L. intracellularis*-challenged pig's PBMCs to Con A was 1300~1600 pg/mL and 400~700 pg/mL, respectively, whereas IFN-γ production to all the purified *L. intracellularis* proteins was approximately 200 pg/ml.

Conclusions

The PBMCs of the piglets vaccinated with the killed *L. intracellularis* vaccine responsive to purified *L. intracellularis* proteins extracted from *L. intracellularis* strain suggesting that they triggered a low-level T-cell response. Mass spectrometry will be performed to identify the proteins responsible for initiating this T-cell response.
Funding for this project was provided by Dechra Incorporated.

Financial Support
Saskatchewan Agriculture Development Fund; Dechra Incorporated

Notes:

139 - Immune responses to differing routes of immunization for a *Lawsonia intracellularis* subunit vaccine

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Session: Vaccinology - 2, Dec. 6, 2:15 - 2:30 PM

Objective

Lawsonia intracellularis (*L. intracellularis*) is an economically important bacterium that is the causative agent of ileitis in pigs. Current vaccines for *L. intracellularis* do not differentiate between infected and vaccinated animals, which is imperative for disease surveillance purposes. Previously our lab identified candidate proteins and have since narrowed down to five or six putative surface proteins. As part of our subunit vaccine development, we aimed to evaluate different routes of administration for the vaccine.

Methods

The aim of this study is to evaluate intramuscular, oral, intradermal and intranasal routes of delivery. For the first trial, three groups of pigs (n=6 each) were obtained, and the vaccines were formulated with Emulsigen®, poly I:C, and CpG as adjuvants. Group 1 pigs were given proteins G and F intramuscularly and proteins C and Y via the oral route. Group 2 pigs were given proteins C and Y intramuscularly and proteins G and F via the oral route. For both intramuscular vaccines, protein T – a viral protein – was added to assess the DIVA immune response. Group 3 pigs were mock vaccinated with saline and served as the control group. Tissues and samples were collected after primary vaccination, secondary booster (2 weeks later) and after euthanization, 40 days later.

Results

Jejunal and ileal samples were used to evaluate the mucosal antibody mediated immune response for each protein. The oral and intramuscular vaccine with F antigen triggered significant ileal IgA antibodies. The other antigens failed to trigger significant antigen-specific immunity, regardless of route or antigen. Due to the adjuvant formulation, we anticipate a strong antigen-specific cell-mediated immune response to at least some of the antigens.

Conclusions

Both routes triggered significant antigen-specific immunity for one of the proteins. This work will assist us in design of a subunit *Lawsonia intracellularis* vaccine. Future trials will compare intranasal and intradermal routes of immunization using the same format as this trial.

Financial Support
Saskatchewan Agriculture Development Fund; Dechra Incorporated

Notes:

140 - Epigraph hemagglutinin vaccine induces broad cross-reactive immunity against swine H3 influenza virus

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Session: Vaccinology - 2, Dec. 6, 2:30 - 2:45 PM

Objective

Influenza A virus infection in swine impacts the agricultural industry in addition to its zoonotic potential. Here, we utilize epigraph, a computational algorithm, to design a universal swine H3 influenza vaccine.

Methods

The epigraph hemagglutinin proteins are delivered using an Adenovirus type 5 vector and are compared to a wild type hemagglutinin and the commercial inactivated vaccine, FluSure. Mice and swine were vaccinated, immune correlates were evaluated and challenge studies with mouse-adapted SwH3 influenza studies were performed.

Results

In mice, epigraph vaccination leads to significant cross-reactive antibody and T-cell responses against a diverse panel of swH3 isolates. Epigraph vaccination also reduces weight loss and lung viral titers in mice after challenge with three divergent swH3 viruses. Vaccination studies in swine, the target species for this vaccine, show stronger levels of cross-reactive antibodies and T-cell responses after immunization with the epigraph vaccine compared to the wild type and FluSure vaccines. In Swine, most importantly, the epigraph vaccine resulted in cross-reactive antibodies (≥40) to 11 of the 13 (85%) North American strains and both 2010 human-like strains after a single immunization. In contrast, TX98 only resulted in strong antibody titers (≥40) to the matched Texas/1998 strain and FluSure vaccination did not result in significant titers to any of the swH3 after a single immunization.

Conclusions

In both murine and swine models, epigraph vaccination shows superior cross-reactive immunity and is being investigated as a universal swH3 vaccine in Swine challenge studies.

Financial Support
University of Nebraska

Notes:

141 - Transcriptome profiling of porcine dendritic cells activated by a novel nanoparticle/poly(I:C) combination adjuvant

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Session: Vaccinology - 2, Dec. 6, 2:45 - 3:00 PM

Objective

Adjuvants are required for inactivated and subunit vaccines to induce optimum immune responses. Diverse vaccine adjuvants that can be employed for alternate delivery routes and to elicit specific immune responses are needed. In this study, the transcriptional response induced by a novel phytoglycogen-based nanoparticle (Nano-11) combined with the TLR3 agonist poly(I:C) in porcine monocyte-derived dendritic cells (Mo-DCs) was investigated.

Methods

Peripheral blood mononuclear cells were isolated from the blood of domestic pigs and differentiated into monocyte-derived dendritic cells (Mo-DCs). The porcine Mo-DCs were subsequently treated with Nano-11 or Nano-11/poly(I:C). RNA was extracted and sequenced to examine the adjuvant effects on the transcriptome of Mo-DCs.

Results

The Nano-11/poly(I:C) combination adjuvant induced upregulation of antiviral interferon-stimulated genes (ISGs), including the family of IFIT proteins (interferon-induced proteins with tetratricopeptide repeats), *IFNA* and *IFNB1*. Combining poly(I:C) with Nano-11 increased the gene expression of costimulatory molecules *CD40*, *CD80*, *CD83*, *OX40L*, *ICAM1*, and *ICOSLG*. Increased expression of CD80 by Nano-11 alone and the combination adjuvant was verified by flow cytometry. The combination adjuvant increased the expression of *TNF* and *IL1B* mRNA. Significant secretion of TNF and IL-1β was measured in the cell culture supernatant. Nano-11 induced mRNA expression of the chemokines *CCL3L1*, *CCL4*, *CCL8*, *CCL20*, *CCL22*, *CXCL2* and *CXCL14*, and this was further enhanced by the addition of poly(I:C). An increase in *MAPKAPK2* mRNA was identified using differential gene expression analysis. Immunoblotting showed greater phosphorylation of MAPKAPK-2 in Mo-DCs treated with Nano-11 and Nano-11/poly(I:C).

Conclusions

Adsorbing poly(I:C) on Nano-11 improved the immunostimulatory effect on porcine Mo-DCs while producing minimal cytotoxicity. These findings support the development of Nano-11 and Nano-11/poly(I:C) as safe and effective vaccine adjuvants.

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

Notes:

142 - Cryptic promoter activity in cDNA sequence corresponding to *Arterivirus* 5' untranslated region

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Session: Vaccinology - 2, Dec. 6, 3:00 - 3:15 PM

Objective

As a part of our research to develop a maker vaccine for PRRSV, we generated an infectious cDNA clone, designated pCMV-NCV1-Nluc, that carries a luciferase gene under the control of the TRS6. Cells transfected with pCVM-NCV1-Nluc DNA plasmid produced infectious virus and high levels of luciferase activity. Interestingly, cells transfected with mutant pCVM-NCV1-Nluc constructs carrying deletions in nsp7 or nsp9 regions also exhibited luciferase activity although no infectious virus was produced. This observation suggested that DNA sequence corresponding to TRS6 might possess promoter activity. This study was designed to demonstrate the presence of promoter activity in the DNA sequence corresponding to 5' UTR and TRS of PRRSV and related Arteriviruses.

Methods

cDNA sequences corresponding to PRRSV 5'UTR and TRS were cloned into a reporter plasmid upstream of *Nluc*. The resulting plasmids were transfected into HEK-293T cells and luciferase activity measured and compared to empty vector carrying *Nluc* without a promoter. Subsequently, serial deletion of PRRSV 5'UTR cDNA sequence was performed to identify the core sequence responsible for its promotor activity. Finally, cDNA sequences corresponding to the 5'UTR of related *Arteriviruses* including EAV and LDV were cloned into the reporter plasmid to evaluate for promoter activity.

Results

Cells transfected with PRRSV 5'UTR-*Nluc* plasmid exhibited 8-fold greater luciferase activity than those transfected empty plasmid, demonstrating the presence of a cryptic promoter in the 5'UTR cDNA sequence. Serial deletion revealed that nucleotide sequence from position 61 to 90 of the 5'UTR was important for its promoter activity. Luciferase signal was also detected from cells transfected with reporter plasmids carrying cDNA sequence corresponding to six PRRSV TRS sequences as well as plasmids carrying 5'UTR sequence EAV and LDV.

Conclusions

cDNA sequence of *Arterivirus* 5'UTR as well as PRRSV TRS possess cryptic promoter activity in eukaryotic cells. This study provides adition insights into the mechanisms of replication and transcription or Arteriviruses.

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

Notes:

143 - Establishment of outbred pre-exposed pigs as an animal model for *Chlamydia trachomatis* vaccine development

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Session: Vaccinology - 2, Dec. 6, 3:15 - 3:30 PM

Objective

Chlamydia trachomatis (*Ct*) is the most frequent sexually transmitted bacterial infection worldwide. Infections in women can lead to infertility, chronic pelvic pain and ectopic pregnancy. Nevertheless, a vaccine is currently not available partly due to the lack of appropriate animal models. The pig has been proven to be a valuable animal model for vaccine development: i) It is receptive to *Ct*; ii) It is the natural host to a close relative of *Ct* – *Chlamydia suis* (*Cs*); and iii) It accurately resembles *Ct* infection and immunity in humans. We established *Cs* pre-exposed pigs as a model for *Ct* vaccine development: this model has the unique ability to mimic the main population used during phase III clinical trials for *Ct* vaccine development – genetically diverse, and mostly *Ct* pre-exposed humans.

Methods

In Trial 1, pigs were infected with either *Cs* or *Ct* and followed the infection and immune response for three weeks. We studied infection and the resulting immune response of *Cs* and *Ct* infections in pigs via anti *Cs*/*Ct* antibody ELISA and multi-color flow cytometry. In Trial 2, *Cs* pre-exposed pigs were prime/boost-vaccinated with either MOCK or a TriAdj-adjuvanted UV-inactivated *Cs* vaccine. Two weeks post boost, we trans-cervically challenged the pigs with *Cs* and studied the genital *Cs* load and the induced immune response.

Results

Most importantly, Trial 1 demonstrated that *Cs* infection induced an immune response that is cross-reactive with *Ct*. Trial 2 verified that *Cs* vaccination induced CD4 T-cell differentiation into IFN-γ^+ tissue-homing effector memory T cells; on top, it significantly reduced the genital *Cs* burden.

Conclusions

Trials 1+2 demonstrated that this *Cs* pre-exposed pig model not only mimics genetically divers and *Ct*-pre-exposed humans, but that it can also be used to assess both, vaccine immunogenicity and efficacy of a chlamydia vaccine. Therefore, future studies will be able to use this biologically highly relevant animal model for *Ct* vaccine development.

Notes:

144 - Predicting lameness and its impact on dairy herds: The welfare sub-module in the Ruminant Farm Systems model.

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Session: Modeling - 2, Dec. 6, 4:15 - 4:30 PM

Objective

1) Find risk factors associated with lameness in dairy cows, 2) develop a model to predict cow lameness, 3) estimate impacts of lameness on production and reproduction, and 4) incorporate the model into the Ruminant Farm Systems (RuFaS) model.

Methods

A literature review was carried out with a focus on the risk factors related to lameness and on how it affects productive and reproductive performance on dairy herds. Risk factors were separated into farm- and animal-level factors and used for model parameterization. Quantitative information related to the odds ratio (OR) was collected from variables presented in the papers. After collecting and verifying the most relevant factors, a logistic model was developed with those factors to predict the risk of each cow in the herd presenting lameness during lactation. The review was also focused on lameness impacts on dry matter intake, milk yield, average daily gain, and reproductive indicators.

Results

The logistic model developed to predict lameness for each cow at herd level comprised housing type [free-stall (OR=1.38) or tie-stall (OR=reference)], stall surface [concrete or rubber mattress (OR=reference), geotextile mattress (OR=0.77), sand (OR=0.53), and water bed (OR=0.55)], footbath [present (OR=0.71) or not present (OR=reference)], and season [summer (OR=1.84) vs others (OR=reference)]. At animal level, the predictive variables were body condition score [≤2.75 (OR=reference), ≤3.25 (OR=0.73), and >3.0 (OR=0.55)], parity [≤2 (OR=reference), 3 (OR=1.63), and ≥4 (OR=2.46)], and lameness previously [yes (OR=2.31) and no (reference)]. Days in milk were used to set up when lameness events should happen, according to a gamma distribution (α=2, β=60). For a case of lameness, the average reduction in milk yield was 420 kg/lactation. The reduction in dry matter intake and weight gain were 4% and 0.5 kg/day, respectively, for 35 days. The average increase in days from calving to conception was 29.6.

Conclusions

The proposed model is suitable to be incorporated into the RuFaS, allowing to measure systemically the effects of lameness on dairy herds.

Notes:

145 - A simulation model to study the effects of dog density on illness and release rates in animal shelters

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Session: Modeling - 2, Dec. 6, 4:30 - 4:45 PM

Objective

The animal sheltering system in the United States is complex, with many moving parts that affect an organization's ability to care for a dog and provide a successful outcome. Decisions made regarding the size of the population in the care of the shelter, whether to euthanize animals, disease control strategies, and how quickly animals get released may ultimately affect the health of the animals in the shelter and the rate at which animals get adopted or transferred. The objective of this study was to develop a systemic dynamics model to simulate how decisions made regarding animal intake, population management, and release affect animal health and adoption rates. This goal of this tool is to help educate and inform policy for veterinarians, animal shelter employees, and that can be extrapolated to other animal populations.

Methods

A system dynamics model of the dog sheltering system was developed using Vensim DSS modeling software and was informed with data from an in-person survey that was conducted of 348 shelters across 5 states (Mississippi, Pennsylvania, Michigan, Colorado, Oklahoma).

Results

Parameters that can be modified as part of a "flight simulator" interface are intake rate, length of stay, death rate, duration of illness, euthanasia rate, intake rate, initial dog population, and the illness prevalence. The model simulates increases and decreases in sick animals based on dog density and contact with infected individuals.

Conclusions

This model demonstrates that increases in dog population within a shelter lead to increased disease, increased length of stay, and, long-term, decreased adoption rates.

Notes:

146 - Swine enteroids reproduce gene expression changes observed in *Lawsonia intracellularis*-infected pig intestine

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Session: Modeling - 2, Dec. 6, 4:45 - 5:00 PM

Objective

In a previous RNAseq study of *Lawsonia intracellularis* (Li) infected pig ileal crypts, *ADAMTS1*, *CD63*, *UNC93B1* and *SLAMF7* expression changed, suggesting a role for the metalloproteinases, adhesion and immune response molecules coded by these genes in Li pathogenesis. This study aimed to validate if swine enteroids infected with Li reproduce the changes observed in tissues from infected pigs.

Methods

RNA from archive tissues (n=2 per time) from infected and non-infected (control; CTL) pigs after 3, 5, 8, 15, 19 and 24 days post infection (dpi) was extracted and evaluated for gene expression by qPCR. Organoids from swine ileum (enteroids) were cultured in transwells as monolayer and infected with Li isolate PHE/MN1-00. Enteroids were collected 5, 7, 10, 14 and 21 dpi. Non-infected enteroids cultured under the same conditions were CTL. Gene expression changes were determined by qPCR in four independent experiments. Data were analyzed by one-way ANOVA followed by Kruskal-Wallis test.

Results

In tissues, *ADAMTS1* expression was numerically highest on day 5 and reduced on day 8 (P=0.03 vs CTL and 0.01 vs 5 dpi). In enteroids, the expression was greatest at 5 dpi (P=0.08 vs CTL) followed by a reduction on 14 dpi (P=0.07). *CD63* expression was decreased in 8 dpi tissues compared with 5 dpi (P=0.01) while a trend on expression reduction was observed in enteroids between 7 and 14 dpi (P=0.06). *UNC93B1* expression was less on 24 dpi compared with 5 and 15 dpi (P=0.02 and P=0.03, respectively) but no differences or trends were identified in infected enteroids. *SLAMF7* in tissues was also less on 24 dpi compared with 5 and 15 dpi (P=0.02), while a different profile was observed in enteroids, showing increased expression at 5 dpi (P=0.06 vs CTL and P=0.03 vs 7 dpi).

Conclusions

Although all the genes of interest were modulated by Li infection in tissues and enteroids, *SLAMF7* expression in enteroids did not reproduce the profile observed in tissues. Overall, swine enteroids infected with Li reproduced changes observed in infected pigs, further supporting their use as *in vitro* models to study Li pathogenesis.

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

Notes:

147 - Revealing invisible links and modes of between-farm PRRSV transmission using genetic-based network analysis

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Session: Modeling - 2, Dec. 6, 5:00 - 5:15 PM

Objective

Porcine reproductive and respiratory syndrome virus (PRRSV) is considered endemic and is the costliest pathogen affecting the United States (US) swine population. Control efforts could be improved if tailored to the most pervasive mechanisms of transmission or targeted towards super-spreading farms. However, the relative importance of different transmission mechanisms (e.g., animal transport, semen, localized spread via wind/fomites) remains unclear, nor do we understand what drives variability in effective reproduction numbers (Re) quantified at the farm-level.

Methods

We investigated the potential modes of transmission within a swine-dense region of the U.S. by integrating sequence-based transmission tree inference and network analyses. We inferred transmission trees for three distinct clades of genetically closely related ORF5 sequences (open reading frame5, n =206) collected from 125 farms between 2014-2017. Farm-to-farm transmission networks were extracted from trees by utilizing observed pig movement data to set a threshold for the maximum length of pig-to-pig transmission chains (inferred from genetic relationships) that would be compatible with a direct farm-to-farm transmission event. Exponential random graph models (ERGMs) were then applied to the networks to investigate determinants of farm-to-farm transmission links and variability in farm-level Res, including farm production type, herd size, regional farm density, movement network connectivity, season of outbreak, and spatial proximity.

Results

Overall, the median farm-level Re was 1 and the maximum was 5 (IQR = 1-2). Preliminary results of the ERGMs suggests that the odds of between-farm transmission links in the network were increased by spatial proximity (coefficient 1.63 ± 0.31 SD, $p < 0.0001$) and the occurrence of animal movement between farms (coefficient 1.92 ± 0.36 SD, $p < 0.0001$).

Conclusions

This study not only informs swine producers of the region-specific risk factors, but also introduces novel integrative approaches that may allow for deeper investigation of outbreaks in humans and animals.

Financial Support
USDA-NIFA Food Animal Residue Avoidance Databank

Notes:

148 - Integrating animal movements with phylogeography to model the spread of PRRS virus in the U.S.

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Session: Modeling - 2, Dec. 6, 5:15 - 5:30 PM

Objective

For highly mobile hosts frequently involved in long-distance movements (like humans and livestock) inclusion of movement data and other population connectivity attributes in phylodynamic analysis enables us to quantify the relative importance of specific types of population connectivity, like age-stratified animal movements, in pathogen spread. To understand spatial diffusion and evolution of a rapidly spreading sub-lineage (denoted L1A) of porcine reproductive and respiratory syndrome virus (PRRSV) type 2 in the U.S. between 2014-17, we incorporated empirical pig movement, environmental and spatial data in a Bayesian phylodynamic model. PRRSV is the costliest endemic pathogen affecting pigs in the U.S.; this particular virulent sub-lineage emerged in 2014 and is the dominant lineage in the U.S. swine industry to date.

Methods

Data included 984 ORF5 PRRSV-L1A sequences from a swine dense production region (~ 85,000 mi2) in the U.S. from 2014-17. We divided the study area into sectors over which we summarized model covariates and between-sector animal movements by age (wean: 3-4 weeks; feeder: 8-25 weeks; breeding: ≥21 weeks). We implemented a discrete-space phylogeographic generalized linear model using Bayesian evolutionary analysis by sampling trees (BEAST) to infer factors associated with variability in between-sector diffusion rates of PRRSV L1A.

Results

We found that between-sector spread was enhanced by the movement of feeder pigs, spatial adjacency of sectors, and farm density in the destination sector. After introduction in the study area in early 2013, L1A genetic diversity and effective population size peaked in 2015 and fluctuated seasonally in subsequent years (summer peaks). This study highlights the importance of animal movements, and for the foremost demonstrates, that feeder-pig movements (8-25 weeks old) shaped spatial spread of PRRSV more than wean/breeding movements.

Conclusions

The inclusion of type-stratified movement data in phylodynamic models may enhance detection of vital routes for pathogen spread and intervention opportunities to manage human and animal infections.

Financial Support
Swine Health Information Center; USDA National Institute of Food and Agriculture, and by the joint NIFA-NSF-NIH-BBSRC Ecology and Evolution of Infectious Disease award 2019-67015-29918 and BB/T004401/1

Notes:

149 - Domestic pigs represent a novel translational animal model for eosinophilic esophagitis

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Session: Modeling - 2, Dec. 6, 5:30 - 5:45 PM

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

Food allergy was induced by intraperitoneal sensitization with hen egg white protein (HEWP) with cholera toxin as adjuvant followed by seven daily oral HEWP challenges. Systemic IgG, IgE and CD4 T-cell responses were monitored weekly. At necropsy, esophageal tissue was assessed for pathology and eosinophil infiltration and RNA was isolated for qPCR and RNAseq analysis.

Results

Sensitized and challenged pigs showed clinical signs of food allergy, increased serum IgG and IgE levels, and a strong systemic CD4 T-cell response; their esophagi also revealed RNA and pathological changes seen in human EoE patients; moreover, these pigs developed the hallmark of EoE – esophageal eosinophilia.

Conclusions

This study establishes the pig as a relevant animal model for EoE: this model has the potential to highly improve the development of new diagnosis and treatment strategies for EoE.

Financial Support
U.S. National Institutes of Health; University of North Carolina; UNC Center for Gastrointestinal Biology and Disease

Notes:

150 - An analysis of companion animal tick encounters as revealed by photograph-based crowdsourced data

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Session: Preventive Medicine, Dec. 6, 2:00 - 2:15 PM

Objective

Community science is increasingly used to track important vectors of companion animal disease, providing a scalable, cost-effective strategy for identifying new foci, changing phenology, and disease prevalence across wide geographies. We examined photographs of ticks found attached to predominately dogs and cats reported to a photograph-based tick surveillance program to identify potential areas for improvements in tick prevention education and risk intervention.

Methods

We compared estimated days of tick attachment using a Kruskal-Wallis one-way analysis of variance, and a Pearson's chi-square analysis of variance on the number of submissions by host type submitted for each season.

Results

The blacklegged tick (*Ixodes scapularis*) was the most common species reported (39.8%). Tick photographs submitted were almost entirely adults (89.5%), and ticks found on companion animals exhibited an estimated median engorgement time of 2.5 days. *Ixodes scapularis* displayed the highest median engorgement of the top tick species found feeding on companion animals ($\chi 2 = 98.96$, $p < 0.001$). Ticks were spotted year-round; during spring and summer, ticks collected from pets represented 15.4% and 12.8% of all submissions, but increased to 28.5% and 35.2% during autumn and winter, respectively.

Conclusions

Crowdsourced data reveal that mostly adult ticks are detected on pets, and they are found at a point in the blood-feeding process that puts pets at heightened risk for disease transmission. The increase in proportion of ticks found on pets during colder months may reveal a critical knowledge gap amongst pet owners regarding seasonal activity of *I. scapularis*, a vector of Lyme disease, providing an opportunity for prevention education.

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

Notes:

151 - Towards novel acaricide development against cattle fever tick: GPCR target validation by RNAi and chemical leads

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Session: Preventive Medicine, Dec. 6, 2:15 - 2:30 PM

Objective

The project advances the discovery of novel chemistries to control the tick *R. microplus,* a vector of babesiosis. Innovation is in the validation of G protein-coupled receptors (GPCRs) as acaricide targets, and in advancing tick neurobiology and endocrinology using a multidisciplinary approach. Aims: 1. Define pharmacological profiles of tick GPCRs expressed in CHO-K1 cells using peptide ligands and small-molecule chemical libraries. 2. Validate GPCRs as targets for tick control by RNAi silencing. 3. Perform chemical validation with tick bioassays of discovered compounds. The tick kinin signaling system that is hypothesized to regulate water balance, metamorphosis, and feeding. We silenced the pyrokinin (PK) and periviscerokinin receptors and verified PK activity on the cognate receptor.

Methods

1. To determine the identity and function of the tick kinin and pyrokinin neuropeptides, their cDNA was cloned from *R. microplus* synganglia and predicted the endogenous peptides; we also sequenced the kinin gene. Predicted kinins and pyrokinins and designed pyrokinin analogs were tested on the receptors expressed in CHO-K1 cells. 2. RNAi in females was performed using pyrokinin or periviscerokinin receptor dsRNA constructs validated in a luciferase cell assay system. 3. High-throughput screening (HTS) identified antagonists of the kinin receptor (KR).

Results

The kinin gene encodes 17 kinins. Fourteen kinins tested on the kinin receptor were highly active (nM level). Thirty-six kinin receptor antagonists were identified by HTS; the most potent had an IC_{50} = 600 nM. Structure-activity relationships of the potent antagonists identified a pharmacophore needed for antagonism; a potent kinin antagonist has paralytic action on tick reproductive tissue and an international patent application was filed in 2021. PK and analog can elicit muscle contractions in ticks. Both KR- and PKR-RNAi caused delays in oviposition and hatching (P <0.05).

Conclusions

Tick neuropeptide GPCRs can be interfered with dsRNA and small molecules. Peptidomimetics and small molecules aid to elucidate functions of tick neuropeptides.

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

Notes:

152 - Effect of monensin supplementation in stocker calves on coccidia shedding and weight gain

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Session: Preventive Medicine, Dec. 6, 2:30 - 2:45 PM

Objective

To compare coccidia shedding and weight gain in stocker calves grazing on California rangeland supplemented with 50, 125, or 200 mg monensin daily to a control.

Methods

The study followed two groups of 148 male stocker calves from December to May 2019/20 and 2020/21. Stockers were stratified by starting weight and randomly assigned to 4 treatment groups consisting of 0 (control), 50, 125, and 200 mg monensin supplementation per head per day based on recommended label dosage.Treatment groups were rotated over 4 pastures approximately every 40 days. Monensin was supplied in mineral mixes and was provided free choice of a known quantity. Mineral consumption was estimated by weighing the residual mineral after rotation. Cattle were held off feed and water overnight and shrunk weights recorded before every rotation. Fecal samples were collected in the second year at the first four weight recordings. A modified McMaster technique was used to obtain oocysts per gram (OPG) and to identify *Eimeria* species. Mixed model repeated measure analysis was used to assess differences in oocyst counts on a log scale and weights between study groups.

Results

On day 42 and 82 of the study, total oocyst and pathogenic oocyst counts from 3 treatment groups were significantly lower than the control group ($P < 0.001$) but did not significantly differ among treatment groups ($P > 0.05$). At study start, weight was similar between groups ($P = 0.95$). Weight gain was significantly different between groups over time ($P < 0.001$). The least square means of weights (SE) for control, 50mg ,125 mg, and 200 mg groups at 150 days were 333.3 (±1.5), 337.9 (±1.5), 343.9 (±1.5), and 337.4 (±1.5) kg respectively. At 150 days, weights in every treatment group were significantly higher than control ($P < 0.001$) and among the monensin supplemented groups, calves with 125 mg supplementation weighed more than those with 50 and 200 mg supplementation ($P < 0.05$).

Conclusions

The results suggest daily monensin supplementation for stockers on California rangeland has advantages to significantly reduce coccidia shedding and increase weight gain.

Financial Support
USDA-NIFA

Notes:

153 - Understanding health-associated issues in dairy farms by exploring integrated data

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Session: Preventive Medicine, Dec. 6, 2:45 - 3:00 PM

Objective

The aim of this study was to distinguish healthy from sick cows using integrated data streams.

Methods

Data from Holstein's lactating dairy cows in a farm during 2020 were used for this analysis. Records from 3 different data streams, management software, Dairy Herd Improvement (DHI) and genetics were integrated. Only complete records were used (n=6,912 records from n=1,079 cows). Selected health issues were clinical mastitis (CM), ketosis (KT) and lameness (LM). Cows were grouped as sick or healthy for each disease, and the average of each genetic trait and DHI variables were calculated for each group. Sick animals were those that had at least one episode of the disease. Statistical differences between healthy and sick groups for each disease were evaluated using a T-test.

Results

Results showed that 305-d mature equivalent milk production was lower for the sick KT and CM groups (595 and 1,797 kg, respectively; P=0.01). Milk fat percentage was lower for the group that had CM (0.26%; P=0.01), and higher for cows with KT (0.66%; P=0.01). As expected, the somatic cell count and linear score were higher for the CM cows (P<0.01). Among the genetic variables, TPI was higher in the healthy groups of KT and CM (P<0.001), however it tended to be higher in the sick LM group (P=0.07). For the LM cows, the milk trait was higher than for the healthy cows (P=0.01), whereas, it was the opposite for the livability trait. The ketosis and metritis traits were lower for the sick CM and KT groups (P<0.01). The traits for milk fever and displaced abomasum were lower for the sick KT group (P=0.01).

Conclusions

This exploratory analysis showed that there are differences in genetic traits and phenotypic production characteristics between healthy and sick cows that could be used to detect anomalies and likely anticipate health issues when a constant flow of aggregated data are available. We are exploring the same analysis in a longer period of time and more herds to better understand the implications of some traits such as livability and health traits in the overall herd management.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture; Food and Agriculture Cyberinformatics and Tools grant

Notes:

154 - Revealing RNA and protein partners of small RNAs CjNC110 and CjNC140 of *Campylobacter jejuni* IA3902

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Session: Preventive Medicine, Dec. 6, 3:00 - 3:15 PM

Objective

Campylobacter jejuni is a prevailing zoonotic pathogen. A hypervirulent strain named 'clone SA,' represented as isolate IA3902, causes most ovine abortions inflicted by *Campylobacter* spp in the U.S. and is associated with human gastrointestinal disease. Recently, small non-coding RNAs (ncRNAs) of *C. jejuni* have been established as transcribed RNA products; however, defining the regulatory networks of these ncRNAs has lagged. Our previous work has demonstrated several vital phenotypes associated with the ncRNAs CjNC110 and CjNC140, however, the binding partners of these regulators remain unknown. To investigate the regulatory networks of CjNC110 and CjNC140 and to determine their binding partners, here we report a unique 'RNA centric approach' using an adaptation of previously described methods of MS2 tagging, which utilizes a bacteriophage-derived tagging system to identify potential ncRNA binding partners.

Methods

MS2-tagged complements IA3902ΔCjNC140c and IA3902ΔCjNC140c, and respective untagged controls were created, and validated using Sanger sequencing and northern blotting. Next, MS2 affinity purification coupled with RNA sequencing (MAPS) was conducted to discover ncRNA-mRNA partners of CjNC140 and CjNC110. Finally, putative ncRNA-protein partners of CjNC140 and CjNC110 were assessed via proteomics using mass spectrometry (LC-MS/MS).

Results

MAPS successfully revealed ncRNA-mRNA targets of CjNC140 and CjNC110, and identified several binding partners previously suggested via RNAseq related to the activated methyl cycle (AMC), iron homeostasis, motility, and flagellar glycosylation. Additionally, proteomics revealed binding of both CjNC140 and CjNC110 to protein Fur, a central transcriptional regulator for iron homeostasis, and other proteins associated with flagella structure, arginine biosynthesis, and the AMC.

Conclusions

These results support the notion that CjNC110 and CjNC140 interact within regulatory networks connected to many important biological processes, and demonstrates that CjNC140 and CjNC110 may serve as key regulators critical for the pathobiology of *C. jejuni.*

Financial Support
Iowa State University; USDA-NIFA AFRI

Notes:

155 - Swine industry stakeholder perception on depopulation using water-based foam

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Session: Preventive Medicine, Dec. 6, 3:15 - 3:30 PM

Objective

Swine populations are susceptible to emerging diseases, which may require emergency responses including large-scale depopulation. Currently, there are a few methods available for swine depopulation, but their applicability in large farms is debatable. Water-based foam (WBF) is an approved method for poultry in the US but is not approved for swine. The objective of this study was to assess pre- and post-application perceptions of WBF depopulation through a survey of swine industry-affiliated stakeholders.

Methods

Invited stakeholders (n=33) completed pre- and post- surveys on perceptions regarding animal behavior, methodology, and likelihood of field success. The WBF depopulation process was applied in cull sows. Animals were loaded into an adapted rendering dump trailer (8.5’ wide x 40’ length x 6’ height) and a 1% foam-water solution was generated using 3 gas-powered water pumps and medium-expansion foam nozzles. Following trailer fill, sows were immersed in the WBF for a period of 5 minutes. Comparisons between the pre- and post- responses were conducted using Wilcoxon Rank Sum Test or Fisher’s Exact Test.

Results

Participants self-identified as animal health officials (n=11), educators/researchers (n=9), and veterinarians (n=8), indicating 0 to 43 years of experience in the swine industry (10.0±23.0 [median ± IQR]). Comparing pre- to post- survey responses, the actual time (in minutes) to fill the trailer with foam (5.0±12.0 vs. 1.0±0.7), stop hearing animal vocalizations (5.0±6.5 vs. 0±1.0), and stop hearing animal movements (7.0±6.0 vs. 2.0±0.25) were all shorter than anticipated ($P<0.001$). Additionally, the majority of participants indicated WBF was a better method than currently approved depopulation methods (e.g. captive bolt, CO_2), which was significantly higher compared to before they observed the process (86.7% vs. 56.3%; $P=0.008$).

Conclusions

Stakeholder perception of WBF as a viable depopulation option increased after respondents had the opportunity to observe the process in situ, a finding supporting efforts to seek conditional approval of WBF as an emergency depopulation option.

Financial Support
National Pork Board

Notes:

156 - *In-feed* vs. *in-water* chlortetracycline on the prevalence of *E. coli* pathotypes involved in swine colibacillosis

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Session: Microbial Ecology, Dec. 6, 4:15 - 4:30 PM

Objective

Colibacillosis in swine, which includes neonatal enteritis, postweaning diarrhea, and edema disease, is one of the major economically important diseases. The pathotypes of *E. coli* involved are enterotoxigenic (ETEC), enteropathogenic (EPEC), enteroaggregative (EAEC), and Shigatoxigenic (STEC) *E. coli.* Our objectives were to determine the effects of in-feed or in-water chlortetracycline (CTC) administration on prevalence of virulence genes and pathotypes implicated in colibacillosis and compare phenotypic and genotypic susceptibilities to CTC.

Methods

A total of 648 weaned piglets (21 days age) were used in a 35-d study. Piglets were allocated to 24 pens (27 per pen) and pens were assigned randomly to no CTC, in-feed CTC, or in-water CTC groups. Fecal samples were collected from 5 piglets from each pen on days 0, 14 and 28. Samples were enriched in *E. coli* broth and subjected to a 11-plex PCR assay to detect major virulence genes targeting the four pathotypes and to a culture method to isolate and identify the pathotypes. Isolates were subjected to the CTC susceptibility testing by micro-broth dilution and major *tet* genes were determined by PCR.

Results

The fecal prevalence of the virulence genes and the pathotypes were not affected by CTC administration ($P < 0.05$). The predominant enterotoxin genes in feces were *estB* (heat stable enterotoxin B; 96.9%), *astA* (enteroaggregative heat stable; 92.7%), and *estA* (heat stable enterotoxin A; 55.5%). Shiga toxin genes were detected only in day-28 fecal samples and the *stx*2 gene was more predominant than *stx*1. The pathotypes of *E. coli* isolated were ETEC, atypical EPEC (positive for intimin and enterotoxin genes), STEC and ETEC and STEC hybrids. Although the samples were positive for enteroaggregative enterotoxin gene (*astA*), EAEC pathotype was not detected. All isolates were resistant to CTC and *tet*A gene (91.5%) was the most predominant.

Conclusions

In-feed or *in-water* CTC administration had no effect on the fecal prevalence of virulence genes and pathotypes implicated in swine colibacillosis and on phenotypic and genotypic CTC susceptibilities.

Notes:

157 - Characterizing the developing microbiome and resistome in young dairy calves without antimicrobial influence

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Session: Microbial Ecology, Dec. 6, 4:30 - 4:45 PM

Objective

Changes in the gut microbiome during early-stage development can be associated with lasting effects on the structure and function of microbial communities, which in turn can affect the health and performance of animals. The purpose of this study was to characterize changes in the fecal microbiome and resistome of calves during early life without exposure to antimicrobial drugs.

Methods

Fecal samples were collected per rectum from 50 Holstein calves that were born and raised at an organic dairy in Texas. Ten calves were enrolled in each of 5 age groups:

Results

Microbial richness significantly increased between the 3 youngest groups, but then remained consistent. Interestingly, resistome richness followed an opposite pattern; decreasing significantly between the three youngest age cohorts. Microbiome composition changed significantly during the first 6-8 weeks of a calf's life but remained stable afterwards. These changes were the result of significantly more Proteobacteria in calves

Conclusions

The microbiome stabilized at 3-4 weeks of age, while the resistome stabilized earlier. Comparisons to calves with gut disease or treated with antimicrobial drugs, in addition to longitudinal studies of calf health and productivity may help elucidate potential impacts of dysbiosis.

Financial Support
U.S. Department of Agriculture; U.S. Department of Agriculture, Center for Epidemiology and Animal Health

Notes:

158 - An Hfq mutant of *Histophilus somni* strain 2336 is attenuated and modified in multiple phenotypic properties

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Session: Microbial Ecology, Dec. 6, 4:45 - 5:00 PM

Objective

Histophilus somni is one of the primary bacterial agents responsible for bovine respiratory disease in cattle. Many *H. somni* virulence factors have been identified, but little is known regarding how these factors are regulated. The post-transcriptional regulatory protein Hfq, a chaperone for small RNAs, may be important in maintaining bacterial virulence. In this study, *hfq* was deleted in *H. somni* strain 2336 (2336Δ*hfq*) to investigate the effect of Hfq loss on virulence and phenotypic expression of several known virulence factors.

Methods

Fusion PCR was used to clone *a* DNA fragment containing the 5' and upstream region of *hfq*, a chloramphenicol resistance cassette (Cm^R), and the 3' and downstream region of *hfq*. The construct was electro-transformed into *H. somni* strain 2336, and chloramphenicol resistant colonies were selected. The *hfq* gene was cloned into shuttle vector pNS3K to complement the mutation. Growth rate, biofilm formation, lipooligosaccharide (LOS) and outer membrane protein (OMP) electrophoretic profiles, serum susceptibility, and virulence for mice was determined.

Results

DNA sequencing confirmed the Cm^R gene replaced most of the *hfq* gene in mutant 2336*Δhfq*. Hfq mRNA or Hfq protein could not be detected by reverse transcriptase PCR or western blotting, respectively. The mutant grew slower than the parent strain, produced less biofilm biomass, and less matrix-associated protein and carbohydrate than the parent, but the differences were not significant. However, the roughness and surface to biovolume ratio of the 2336Δ*hfq* biofilm was significantly greater than the parent biofilm. Following gel electrophoresis, the OMP profile was altered and the LOS was truncated and not sialylated. Complementation of the mutant corrected the LOS truncation. Mutant 2336Δ*hfq* was also more sensitive to the bactericidal action of serum and complement, and more virulent in a mouse model of bacteremia.

Conclusions

Mutant 2336Δ*hfq* will prove valuable in determining which genes that contribute to virulence are regulated by Hfq, and the role of small RNAs in gene regulation and virulence in *H. somni.*

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture; Long Island University

Notes:

159 - Impacts of host density on vector abundance and transmission of bluetongue virus

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Session: Microbial Ecology, Dec. 6, 5:00 - 5:15 PM

Objective

Culicoides biting midges are vectors of bluetongue virus (BTV), an arbovirus affecting both wild and domestic ruminants. Recent outbreaks and expanding range have highlighted the need to better understand factors contributing to transmission. Specifically, it is not well understood how differences in host density impact vector populations and, in turn, transmission. Using vector abundance as a proxy for transmission, we sought to examine whether BTV transmission is density-dependent.

Methods

We conducted trapping of *Culicoides* using CDC-style suction traps baited with carbon dioxide attractant using dry ice. Our sites included four dairies and four feedlots in northern Colorado, ranging in density from 200 to 69,000 cows. Trapping was conducted overnight weekly at each site from mid-July through early September, 2020. Four traps were placed at each site at varying distances from the host aggregation, controlling for surrounding habitat. Generalized additive mixed effect models with a negative binomial distribution were used to assess the effect of host density, distance from hosts, and site type (dairy or feedlot) on midge abundance.

Results

Results suggest a three-way interaction between host density, distance from hosts, and site type, with high host density leading to increased midge abundance. This effect was detected at far distances from hosts, which were less subject to sampling artifacts affecting traps nearest the hosts. Examining the effect of host density on midge abundance seen at far distances revealed evidence for density-dependent midge abundance on dairies but not on feedlots, likely because of the very high host densities on feedlots. This indicates a threshold point after which higher host density no longer leads to increased transmission.

Conclusions

Our work supports the potential for density-dependent BTV transmission up to a threshold point in areas with concentrated ruminant host populations. These finding can help contribute to a greater understanding of vector populations and transmission dynamics for use in predictive modeling efforts.

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

Notes:

160 - Multi-strain dynamics of PRRSV type-2 in U.S. pig populations

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Session: Microbial Ecology, Dec. 6, 5:15 - 5:30 PM

Objective

Ecological interactions between different variants or strains of a pathogen may occur if there is partial cross-immunity between strains, thus creating the potential for immune-mediated competition amongst co-circulating viruses. Porcine reproductive and respiratory syndrome virus (PRRSV) type-2, is characterized by extensive genetic diversity and frequent emergence of variants. This contributes to inadequate disease control, resulting in economic losses for the U.S. swine industry. Despite this, drivers of multi-strain dynamics of PRRSV remain poorly understood.

Methods

Utilizing a database of >12,000 *orf5* sequences obtained from the University of Minnesota Veterinary Diagnostic Laboratory (UMN VDL), our objective was to phylogenetically classify co-circulating PRRSV variants in the U.S., quantify evolutionary dynamics of variant emergence, and describe potential antigenic differences among variants.

Results

We subdivided the most prevalent strain (called Lineage 1, responsible for ~60% of sequences) into eight sub-lineages. Using a Bayesian coalescent SkyGrid models to estimate each sub-lineage's effective population size over time, we identified a pattern of sequential dominance of sub-lineages, with a new sub-lineage replacing its predecessor approximately every three years. New sub-lineages emerged every 1 to 4 years and the time between emergence and peak population size was 4.5 years on average (range: 2–8 years). Each sub-lineage's consensus amino acid sequences differed in key GP5 sites related to immunity response, suggesting sub-lineage turnover may be linked to immune-mediated competition. This has important implications for understanding drivers of genetic diversity and emergence of new PRRSV variants in the U.S. Given the rich data available in livestock host-pathogen systems, our project provides new insight into multi-lineage ecological theory.

Conclusions

Our results suggest that PRRSV may be an interesting model to study the evolution of diversity and sequential dominance of viral variants in a system where immune-mediated strain selection may occur.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture; USDA National Institute of Food and Agriculture, and by the joint NIFA-NSF-NIH-BBSRC Ecology and Evolution of Infectious Disease award 2019-67015-29918 and BB/T004401/1

Notes:

162 - Risk management in an ever more complex world

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Session: AVEPM - Schwabe Symposium, Dec. 7, 8:30 - 9:15 AM

The world has gone through incredible changes in the last 100 years, and a major impact has been how it has connected us with each other more and more, until we reached a stage where we are able to reach virtually every location on the planet within a few days. The main driving force has been a desire for economic prosperity, and it has led to major positive but also negative changes to eco-social systems at a global scale. From a veterinary perspective, the development of highly complex global food systems and associated risk environments is of particular significance. The latter has resulted in the global emergence of infectious diseases with major societal impact, such as avian influenza, African swine fever and COVID-19. Prevention, detection and response to such events requires an integrated approach to both science and policy development that goes beyond effective linkage between the veterinary and medical knowledge dimensions by also embracing knowledge generated by the environmental and social sciences. This now widely recognised One Health approach needs to be seen as part of a global effort towards a sustainable future for the planetary ecosystem, since infectious disease emergence is also a symptom of much more significant and complex threats to global ecosystems, such as climate change. When implementing a One Health approach, a significant challenge for researchers from different scientific disciplines is the necessity to become much more effective at true co-production of knowledge to be used for the purpose of the development and implementation of risk management interventions. In that context, another challenge have been the recent changes towards a multipolar global political landscape. In addition, it needs to be taken into consideration that visions of what 'living a good life' means are now changing very rapidly between generations and along different trajectories between geographical regions and political systems. Both these issues have major implications for the ability to translate risk management policies into effective and sustainable action.

Notes:

163 - "The cost and the lessons of the COVID pandemic"

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Session: AVEPM - Schwabe Symposium, Dec. 7, 9:15 - 10:00 AM

Covid-19 has caused more than 5 million death globally, and the magnitude of its impact was magnified by inadequate policies and lack of cooperation. The Covid-19 pandemic has cost thus far about $5 trillion in terms of lost lives and another 5% of 2020 GDP in terms of reduced economic activity which is $6.75 trillion for a global GDP of $125 trillion. Much of the cost of the Covid-19 pandemic was the result of a lack of monitoring and preparedness. We would have benefited from a global research program, monitoring emergence of zoonotic diseases, preparing strategies to control them and increasing investment in preventing future infections are likely to reduce these costs drastically. The political system in many countries failed to respond to the pandemic in a timely manner, reflecting underinvestment in public health and politicization of the public health system. The key for damage control was a speedy reaction and effective monitoring and containment of the spread of the pandemic. The burden of the pandemic wasn't shared equally, and some low-income groups were highly exposed and suffered devastating economic loss.

At the same time, there was some encouraging news. First, the fast development of vaccines has been a major achievement, demonstrating the importance of integrating modern science with industry. The safety net legislations providing support to the unemployed and other victims of the pandemic reduced its social cost and improved social cohesion. The agri-food system has proven to be highly resilient. It modified its supply chain to overcome the constraints of social distancing, and to a large extent, avoiding a nutritional crisis.
The pandemic has several lessons for the ongoing global challenge of climate change. First, it is crucial to supporting research to understand climate change processes and how to address them. Second, political leadership must be capable of taking bold and decisive actions based on science. Third, one of the challenges in addressing climate change is building trust in government and having a culture where experts initiate improved policies and actions. Fourth, investments in mitigation, prevention, and preparedness are very valuable. Fifth, effective climate policy should establish safety nets to share mitigation and adaptation burden and assure social acceptance of policy actions. Finally, addressing climate change will require worldwide collaboration and knowledge exchange.

Notes:

164 - Post normal One health: Baselines and boundaries gone feral

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Session: AVEPM - Schwabe Symposium, Dec. 7, 10:30 - 11:15 AM

Much of what we think we know about the world is based on assumptions of stability and predictability. In its simplest form, this idea of stability has been reflected in how we practiced science, health, and public policy. We created health and agricultural programs that assumed a level cultural, economic and ecological playing field that usually—no surprise here—looked just like what we had created in North America and Europe. If there were problems, they could be solved by technical experts. We knew of course that the world changed, but changes were assumed to follow predictable patterns; some argued that human society has been steadily improving, others have seen collapse and destruction everywhere. Many business analysts, political theorists and ecologists have taken the view that all systems pass through phases of exploitation, conservation, release, and reorganization, a model economists call creative destruction. If understood and managed, could we use such models to promote a more resilient, sustainable society? In a stable world, perhaps, sometimes. In a politically, economically, ecologically and climatically unstable and tightly inter-connected world, however, our biggest problems are all embedded in a complex web of "wickedness," where striving to reach one goal (control a pandemic) can undermine our ability to address other pressing problems (climate change, pollution, water shortages, energy sustainability, economic and social equity, racism, indigenous land claims, food production, animal welfare). In this situation, laboratory-based, normal sciences, which require a tight focus on small pieces of protein or nucleic acid, are useful, but insufficient. Models that require quantitative precision are helpful guides, but vulnerable to over- and under- interpretation. Post Normal Science (PNS) was first proposed in 1993 as an enrichment of science for situations where facts are uncertain, values are in dispute, stakes are high and decisions are urgent. PNS requires an understanding that this is not a battle we are trying to win, but a massive orchestral performance, composed on the fly by millions of players (not all of them human), where we are each other's peripheral vision, and where the quality of our performance depends on improving our collective decision-making. It is, in perhaps all the best senses of that word, a practice.

Notes:

165 - Better bridging science and animal health policy

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Session: AVEPM - Schwabe Symposium, Dec. 7, 11:15 - 12:00 PM

Millions of people rely on animals not only for food but also for livelihoods and core needs such as clothing, transport and power. While livestock systems support the livelihoods of millions of people and contribute to healthy diets and resilience, the rapid growth in production and trade has increased risks as well as opportunities. Among others, major concerns are threats to human and animal health, animal welfare and the environment. Strong policies are needed in the livestock sector including animal health to optimise its contribution to achieving many sustainable development objectives.

It is clear that evidence use does matter as it advocates a more rational, rigorous and systematic approach. Policy including one for animal health aspect should be informed by a wide breadth of evidence which include the quality, credibility, relevance and cost of the policy in all the different components of policy processes.

It is needed to recognize that animal health policy-making is a complex process and is not based on scientific evidence alone. Gaps between science and policy-maker generally recognized include: researchers often fail to see the policy relevance of their own knowledge and experience; researchers do not make extra effort to communicate their study results in non-technical language to policy-makers, or to shape messages specifically for policy-oriented audiences; policy-makers lack appreciation for the use of evidence, either because they do not see any value in its use or because they consider such information irrelevant to their planning and decision-making. Moreover, animal health policy and decision-making is influenced by political will, existing governance structures, public opinion, and other exogenous factors.

To better bridging science and policy, researchers need to engage with all of these facets in a holistic way though it is challenging in the context of traditional research environments. It is critical to highlight the need for a commitment to: integrate policy relevance to the research focus from the outset; engage with policy-makers and other stakeholders throughout; use platforms to facilitate science-policy dialogue; and disseminate simplified research findings in form of policy brief and recommendations. It is also to note that the concept and strategies of bridging the gap between science and policy are not static, but varying in space and evolving over time.

Notes:

166 - Acute anaplasmosis reduces breeding soundness in experimentally-infected beef bulls

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Session: Pathogen-Host Interaction - 2, Dec. 7, 8:30 - 8:45 AM

Objective

The causative agent of bovine anaplasmosis, *Anaplasma marginale*, costs the U.S. cattle industry an estimated $300 million per year. Natural service breeding by bulls is common in U.S. beef cow-calf operations. Anemia and fever associated with clinical anaplasmosis may reduce bull breeding soundness. The study objective was to evaluate breeding soundness outcomes and clinical changes in beef bulls over the course of clinical anaplasmosis and after recovery.

Methods

Six healthy, *Anaplasma*-negative, mature, Angus bulls of satisfactory breeding status were included. Fresh blood from an infected donor cow was used to challenge three bulls, the other three remaining as unchallenged controls. All bulls were observed for disease progression and soundness of breeding. Fever, anemia via packed cell volume (PCV), pallor, and icterus were monitored weekly. Progression of anaplasmosis was evaluated via quantitative PCR and percent parasitized erythrocytes (PPE). Seroconversion was monitored by cELISA. Injectable oxytetracycline was given to bulls with a PCV <15% or a temperature >105°F. Weekly breeding soundness examinations using electroejaculation were performed on all bulls for 16 weeks. Breeding soundness parameters included sperm morphology and motility, external and internal genitalia exam, and physical exam.

Results

All *A. marginale*-challenged bulls were PCR-positive, seropositive, and showed clinical signs by 3-, 17-, and 24-days post-challenge, respectively. Common clinical signs of acute anaplasmosis included weight loss, pallor, icterus and fever (>104.3°F). Acute anemia was observed in all challenged bulls with PCV nadirs ≤18% and peak PPEs ≥50%. Reduced breeding soundness outcomes were observed only days after onset of clinical signs and continued for weeks beyond resolution of clinical anaplasmosis. Bulls in the control group remained negative for *A. marginale* by PCR and cELISA, and never developed dramatic reductions in breeding soundness outcomes.

Conclusions

Findings from this study suggest acute anaplasmosis is a driver of reduced breeding soundness in beef bulls.

Financial Support
Kansas State University Global Food Systems Seed Grant

Notes:

167 - Integrative interactomics approach applied to bovine fescue toxicosis

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Session: Pathogen-Host Interaction - 2, Dec. 7, 8:45 - 9:00 AM

Objective

Bovine fescue toxicosis (FT) is caused by grazing ergot alkaloid-producing endophyte (*Epichloë coenophiala*)-infected tall fescue. FT has complex pathophysiology and, in grazing beef, economic losses due to decreased weight gains are substantial. In our recent investigations we reported on toxic endophyte's effects on the animal's microbiota and metabolism, but its effects *in planta*, on the plant-animal interactions and on their association with key signs of FT have not been considered.

Methods

Therefore, we examined multi-compartment (plant, rumen, plasma, urine and fecal samples) microbiota-metabolome perturbations using multi-'omics (16S and ITS2 rRNA sequencing and untargeted metabolomics) in Angus steers grazing non-toxic (Max-Q) or toxic (E+) tall fescue for 28 days and in E+ plants. Complex bioinformatic analyses where then performed to determine the structure of the FT integrome and key integrome components that are associated with signs of FT. We aslo established new experimental pastures with E+, Max-Q, and endophyte-free (E-) tall fescue in the fall of 2020; these pastures will be ready for experimentation in the fall of 2021.

Results

E+ altered the plant/animal microbiota; it decreased most ruminal fungi and had mixed effects on rumen bacteria and fecal microbiota. Metabolic perturbations occurred in all matrices, with some plant-animal overlap (e.g., Vitamin B6 metabolism). Importantly, integrative interactomics revealed unique E+ network constituents. Only E+ had ruminal solids operational taxonomic units (OTUs) within the network and fecal fungal OTUs in E+ had unique taxa (e.g., *Anaeromyces*). Three E+-unique urinary metabolites that could be potential biomarkers of FT and targeted therapeutically were identified and their biomarker utility will be followed up upon in the upcoming studies.

Conclusions

Overall, this work is the first integrative description of the plant-endophyte-animal interaction and it points to the value of such approaches in the quest for better understanding of FT pathogenesis and its therapeutic management.

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

Notes:

168 - Characterization of immortalized feline respiratory epithelial cells

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Session: Pathogen-Host Interaction - 2, Dec. 7, 9:00 - 9:15 AM

Objective

Feline herpesvirus 1 (FHV-1) is the leading cause of respiratory and ocular disease in cats and is particularly severe in 6–9-week-old kittens. Primary Feline Respiratory Epithelial Cell (FREC) cultures have been used to study FHV-1 immunomodulation and growth *in vitro*; however, cell yields limit experimental design and paradigms that can be performed. To further reduce the number of cats needed for experimental research, we immortalized FRECs to generate a sustainable cell line that will allow the study of more elaborate assays and experimental repeats. Our hypothesis was to demonstrate that immortalized Feline Respiratory Epithelial Cells (iFRECs) retain morphological and immunological characteristics of the natural airway and primary cells and can be used to study FHV-1 and other respiratory pathogens in cats.

Methods

FRECS were purified following isolation by positive selection with a cytokeratin-specific antibody before commercial immortalization with HPV+SV40T. iFRECs were grown and compared to primary FRECs morphologically and immunologically using microscopy and conventional PCR. In addition, FRECs and iFRECs were inoculated with FHV-1 and viral growth kinetics were determined by virus titration and real-time quantitative PCR (qPCR). Finally, cytokine expression in response to viral inoculation was compared by qPCR

Results

Morphologically and immunologically, there were no differences between iFRECS and FRECs, but there was less variability in the expression of immunological receptors for iFRECs. Infection of iFRECs was readily achieved and viral growth kinetics were comparable between iFRECs and FRECs, although intracellular endpoint titers were slightly higher iFRECs. In addition, IFNα, IL-10, IL-1β, GM-CSF, TLR9, and TNFα mRNA expression was increased in iFRECS at 72 hpi with FHV-1, which was similar to what has been observed following inoculation of FRECs.

Conclusions

Our results highlight the potential of using iFRECs for studying FHV-1-host interactions while reducing the number of animals needed for future studies

Financial Support
Sheila McMonagle Fund for Feline Health at the College of Veterinary Medicine at Michigan State University

Notes:

169 - Dissecting the cellular landscape and transcriptome network in viral myocarditis by single-cell RNA sequencing

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Session: Pathogen-Host Interaction - 2, Dec. 7, 9:15 - 9:30 AM

Objective

Viral myocarditis is a predominant cause of heart failure in children and young adolescents that can lead to dilated cardiomyopathy (DCM). Experimentally, Coxsackievirus B3 (CVB3) is commonly employed to study the pathogenesis of myocarditis. Post-infectiously, affected mice can develop DCM, and viral RNA may be present with or without inflammation raising a question as to the events culminating in the development of DCM. To dissect this complexity, we sought to characterize heart infiltrates from CVB3-infected mice by single-cell RNA seq and compared the profiles with healthy mice.

Methods

Six to eight-week-old male A/J mice were infected with CVB3-Nancy strain, and 21 days later hearts from infected and age-matched healthy controls were collected, perfused, and enzymatically digested to obtain a single-cell suspension. The viable, metabolically active cells were sorted by flow cytometry and were processed using 10X genomics Single-cell 3' RNA-seq to prepare unique barcoded libraries. Raw data obtained from sequencing were then processed downstream using the SEURAT R package to analyze and visualize the data.

Results

Our analysis revealed 26 subtypes of cells, with myocarditic mice having significantly higher proportions of myeloid cells, CD4 and CD8 T cells, and fibroblasts, whereas NK cells, ILCs, and B cells were low. By investigating the transcriptome signatures of T cells in myocarditic hearts, transcripts critical for cytotoxic functions were noted. Additionally, T cells in myocarditic mice revealed uniquely upregulated transcription factors such as *Elf1, Ets1, Irf7,* and *Stat1* that may modulate cardiac remodeling functions.

Conclusions

Our data suggest that T cells, but not B cells appear to play a role in the pathogenesis of viral myocarditis. We have identified novel transcription factors, importantly *Elf1*, and *Ets1* that have roles in anti-viral response, fibrosis, and expression of cytokines, which could play a role in disease pathogenesis. Detailed investigation of these may offer new therapeutic targets of clinical relevance for DCM and heart failure.

Notes:

170 - Ribosomal protein sequence 17 (RPS17) insertion increases avian hepatitis E virus (aHEV) replication

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Session: Pathogen-Host Interaction - 2, Dec. 7, 9:30 - 9:45 AM

Objective

Avian HEV (*Orthohepevirus* B) is the causative agent of hepatitis-splenomegaly syndrome in chickens, turkeys, and wild birds and the recently reported hepatic rupture hemorrhage in chickens. aHEV infection in chickens causes sudden mortality and drastic reduction in egg production. Lack of robust tissue culture hinders significant molecular research on aHEV. *Orthohepevirus* A genotype (gt)3 HEV (Kernow C-1 P6) identified in a chronically infected patient allowed for efficient cell culture growth due to the natural insertion of a 171-nucleotide sequence of human ribosomal protein S17 (RPS17) within the hypervariable region (HVR) of HEV ORF1. Whether RPS17 can contribute to enhanced replication in *Orthohepevirus* B strains remains unknown. We investigated RPS17 in avian species, specifically chickens, and whether RPS17-mediated replication enhancement can be transferred to aHEV allowing enhanced replication in LMH (leghorn male hepatoma) liver cells, providing a suitable cell culture model for aHEV.

Methods

RPS17 amino acid (aa) sequence alignments were created and compared between mammalian, avian, and other species using CLUSTAL Omega. aHEV infectious clone containing BspEI/SexAI- restriction sites flanking the HVR was created using overlap extension PCR. Insertion of 171 aa from RPS17 in the pT7-aHEV backbone was done by PCR amplifying RPS17 from the Kernow C1-P6 infectious clone using oligonucleotides containing BspEI and SexAI restriction sites. HEV infection (aHEV wild type, aHEV S17, aHEV empty, human HEV gt3) was initiated in LMH liver and huh7 human hepatoma cell lines via transfection with *in vitro* transcribed capped HEV RNA and replication was assessed. Immunofluorescence assay (IFA) to detect ORF2 protein using flow cytometry was performed after harvesting transfected cells at 5 days post transfection.

Results

RPS17 is highly conserved across different species. RPS17 insertion into the aHEV genome resulted in higher ORF2 positive LMH cells compared to wild type aHEV.

Conclusions

RPS17 insertion enhanced aHEV replication in LMH cells and allowed for replication in human liver cell lines.

Notes:

171 - *Salmonella* Kentucky ST152 and ST198 genetic lineages show lineage-specific metabolic phenotypes

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Session: Pathogen-Host Interaction - 2, Dec. 7, 9:45 - 10:00 AM

Objective

Salmonella Kentucky is an emerging foodborne pathogen comprised of two major genetic lineages, sequence types (ST)198 and ST152. ST152 is prevalent in US poultry and sporadically associated with human disease while ST198 is prevalent in international poultry and commonly associated with human disease. We recently reported that these lineages can be clearly distinguished by lineage-specific mutations. Here, we hypothesized that mutations differentiating these lineages underlie lineage-specific metabolic phenotypes that contribute to the metabolic adaptation of ST198 to a human host. The objective of this study was to identify specific metabolic differences between ST152 and ST198 which may explain differences in nutritional virulence and/or host adaptation.

Methods

A total of 8 *S.* Kentucky strains (ST198 n=3; ST152 n=5) isolated from US poultry and human sources were tested using Phenotype Microarray (PM) to compare their respiratory activity (RA) in the presence of 192 carbon compounds as sole energy sources. The two lineages were considered metabolically distinct when a mean RA difference threshold of >50 with $P \leq 0.01$ (Student's t-test) was considered significant.

Results

The RA of ST198 strains was significantly higher in the presence of 18 (9.4%) out of 192 energy sources. These energy sources included 1,2-propanediol and m-inositol, which have previously been associated with virulence in other *Salmonella* serotypes. The differential RA activity in 1,2-propanediol and m-inositol correlated with the lineage-specific mutations in genes involved in their respective metabolic pathways.

Conclusions

The predominant lineages of *S.* Kentucky, ST198 and ST152, are differentiated by unique genetic mutations which can be linked to observed phenotypic differences. The ability of ST198 strains to utilize 1,2-propanediol and m-inositol as energy sources may contribute to increased virulence or host adaptation. These metabolic phenotypes may serve as a tool for differential detection of ST198 and ST152 lineages, and potentially as targets for the development of therapeutics or prophylactics.

Financial Support
Washington State University; USDA-ARS

Notes:

172 - Porcine gut innate lymphoid and T cells differ from circulating populations – novel single-cell RNAseq findings

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Session: Immunology - 4, Dec. 7, 10:30 - 10:45 AM

Objective

Understanding porcine immunity is crucial for improving animal well-being to sustain pork as a global food source. As gut health influences overall health, a thorough understanding of ileal immune cells, such as T cells and innate lymphoid cells (ILCs), is important, yet most knowledge of T cells and ILCs in pigs is derived from studying cells in periphery. Hence, we sought to characterize and compare ileal and peripheral T cells and ILCs.

Methods

Ileal T cells and ILCs were identified and profiled by single-cell RNA sequencing (scRNA-seq), resulting in 14,742 cells annotated into 16 cell types. Ileal cells were projected onto a multidimensional space of reference porcine PBMC scRNA-seq data based on conserved gene expression to calculate mapping scores for each cell. Higher mapping scores indicated greater similarity between ileal and peripheral cells.

Results

Ileal naïve alpha/beta (ab) T cells and CD2- gamma/delta (gd) T cells were most similar to comparable peripheral cells (mapping mean >0.94), while non-naïve T-cells and ILCs had lower mapping scores to peripheral cells. A previously undescribed subset of ileal SELLhi gd T cells mapped poorly to peripheral cells (mapping mean 0.47), suggesting a unique subset of gd T cells found in ileum but not blood. Cytotoxic gd T, CD8 ab T, and group 1 ILCs had mapping scores lower than other remaining non-naïve populations of the same lineages (<0.75), indicating ileal cytotoxic cells were more different from peripheral cells than other non-naïve subsets. Other ileal cell types with low mean mapping scores to peripheral cells included follicular CD4 T cells (0.73) and group 3 ILCs (0.63).

Conclusions

Comparing scRNA-seq datasets of ileal and circulating T cells and ILCs led to identifying commonalities and differences in cell populations. Non-naïve ileal lymphocytes were poorly represented in the periphery, suggesting programs of cell activation/differentiation in the ileum but not blood under steady state. Findings bring into question applicability of dogmas established through study of porcine peripheral T cells and ILCs to intestinal counterparts.

Financial Support
U.S. Department of Agriculture, Agriculture and Research Services; Oakridge Institute for Science and Education; USDA-NIFA

Notes:

173 - A single cell analysis of porcine thymopoiesis and thymic iNKT cells

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Session: Immunology - 4, Dec. 7, 10:45 - 11:00 AM

Objective

Many aspects of the porcine immune system remain poorly characterized which poses a barrier to improving swine health and utilizing pigs as preclinical models.

Methods

We employed single-cell RNA sequencing (scRNA-seq) to create a cell atlas of the postnatal pig thymus and to compare its composition to the human thymus. We also used this approach to interrogate rare invariant natural killer T (iNKT) cells purified using CD1d tetramers from the thymi of the same pigs.

Results

The overall kinetics of conventional ab T cells development mirrored T cell differentiation in humans. We identified several unconventional T cell types, including a $CD8^+$ T cells subpopulation that expressed a NK-like transcriptional profile and a $CD8aa^+$ population primed for transcription factor ZNF683. In addition, the transcriptional landscape of porcine iNKT cell revealed that the subsets were transcriptionally more homogeneous than mouse iNKT cell subsets, and shared expression of some genes with murine iNKT2 cells, but lacked clearly distinguishable iNKT1 and iNKT17 cells, which make up key iNKT effector subsets in mice. Instead, pig iNKT cells contained a rare population of $TBX21^+$ cells that expressed a NK-like transcriptional profile but lacked detectable levels of some prototypal iNKT1 genes.

Conclusions

Our data provides new insight into porcine thymopoiesis and pig-specific cell types and gene expression signatures, which is needed to fully understand mechanisms of T cell immunity and T cell disorders in pigs.

Financial Support
U.S. Department of Agriculture; U.S. National Institutes of Health

Notes:

174 - Combination of antiviral therapies in the control of influenza infection in pigs

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Session: Immunology - 4, Dec. 7, 11:00 - 11:15 AM

Objective

Influenza A virus (IAV) infections are a leading cause of morbidity is swine, poultry, and humans. Antiviral therapies targeting the virus are the most common strategy for treating infections in humans. However, there is growing interest in controlling influenza infections though immune-based therapies, which inhibit virus replication through multiple host immune pathways without inducing viral resistance. We used the swine influenza model to compare the efficacy of two antiviral therapies; oseltamivir (OS), a neuraminidase inhibitor targeting the virus, and α-galactosylceramide (α-GC), a synthetic glycolipid that controls IAV infections in mice by stimulating natural killer T (NKT) cells to produce cytokines and trans activate NK cells. We also tested the efficacy of combining both drugs since several studies have shown that combination therapies are more effective than monotherapies for inhibiting virus replication.

Methods

Forty-two weaner pigs were divided into 5 treatment groups: Group 1 was sham infected, while groups 2-5 were intratracheally infected with pandemic H1N1 California/07/2009. Group 3 received α-GC intranasally at the time of infection, group 4 received OS twice a day for 5 days, and group 5 received both α-GC and OS. Clinical signs and virus shedding were assessed daily. Respiratory tissues were collected at day 5 post infection to quantify viral titers, lung lesions, and innate and adaptive immune responses.

Results

OS treatment reduced virus shedding and lung pathology but did not reduce virus replication in lavage fluid. α-GC had no effect on virus titers or influenza induced disease. Combination did not reduce virus shedding or improve disease outcome compared to oseltamivir monotherapy, despite the induction of cytotoxic T cells in this treatment group.

Conclusions

α-GC therapy did not alter the course of an influenza infections in pigs, which have much lower concentrations of NKT cells than mice. This result raises questions about current interest in harnessing NKT cells to treat virus infections in humans, which also have much lower concentrations of NKT cells.

Financial Support
U.S. National Institutes of Health; U.S. Department of Agriculture

Notes:

175 - Modified-live virus vaccination induces heterologous immunity against different type-2 PRRSV strains.

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Session: Immunology - 4, Dec. 7, 11:15 - 11:30 AM

Objective

Objective: Cross-protection against heterologous strains is a major hurdle of vaccines against PRRSV – the porcine reproductive and respiratory syndrome virus. Heterologous vaccine efficacy relies on the induction of both humoral and cellular immunity that reacts against various PRRSV strains. Thus, this study investigated vaccine efficacy and immunogenicity of the Prevacent modified live virus (MLV) vaccine against four type-2 PRRSV strains.

Methods

Methods: Sixty weaners were divided into five MOCK- and five MLV-vaccinated groups. After four weeks, each of these groups were challenged for two weeks with MOCK or one of four PRRSV-2 strains – NC174, NADC20, NADC30, or VR2332. Heterologous vaccine efficacy was assessed by lung pathology and viremia. Heterologous vaccine immunogenicity was determined via nasal swab IgA, serum IgG and neutralizing antibody levels, and a detailed T-cell response analysis – proliferation, IFN-γ production, and differentiation of CD4, CD8, and TCR-γδ T cells.

Results

Results: Vaccination showed heterologous efficacy against VR2332 (reduced viremia), and NADC20 and NADC30 (reduced lung pathology and viremia). Vaccination also induced a strong systemic IgG response and increased the number of animals with neutralizing antibody titers against VR2332, NADC20, and NADC30. Vaccination also improved the heterologous T-cell response: Vaccinated animals not only had a higher frequency of memory/effector CD4 T cells but also an improved heterologous CD4 and CD8 IFN-γ response against NC174, NADC20, and NADC30.

Conclusions

Conclusions: Overall, the MLV vaccine Prevacent elicited various degrees of both vaccine efficacy and immunogenicity against different heterologous PRRSV-2 strains.

Financial Support
Elanco

Notes:

176 - Characterization of porcine γδ T cells based on their expression of cell surface markers and Toll-like receptors

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Session: Immunology - 4, Dec. 7, 11:30 - 11:45 AM

Objective

γδ T cells represent a prominent lymphocyte subset in pigs. Their role and function, however, remains largely unknown. Direct antimicrobial functions have been described in humans, with certain subsets resembling the characteristics of innate and adaptive immune cells. A thorough phenotypic characterization and reliable purification methods are required to assess the role of this immune cell subset. In this project, we determined the influence of age on γδ T cell subsets, evaluated purification methods and compared expression levels of Toll-like receptors (TLRs).

Methods

Flow Cytometry was used to analyze the expression of cell surface markers involved in the identification of γδ T cell subsets (CD2, CD8α) and potential markers for differentiation/activation (CD27, MHCII, CD16). To determine TLR expression, γδ T cells were purified by magnetic activated cell sorting. After purity was confirmed to be over 98%, quantitative PCR was used to determine TLR levels and sorted cells were cultured with varying TLR ligands and cytokines.

Results

We found that γδ T cell percentages not only decline with age within all peripheral mononuclear cells (PBMCs) but also within T cells. The major γδ T cell phenotype significantly shifts from $CD2^{-}CD8\alpha^{-/dim}CD27^{+}$, $MHCII^{-}$ and $CD16^{-}$ in young pigs to $CD2^{+}CD8\alpha^{+}CD27^{-}$, $MHCII^{+}$ and partly $CD16^{+}$, in sows, indicating a terminal differentiation stage for the latter phenotype. Additionally, TLR 2, 3, 4, 7, 8 and 9 expression in γδ T cells was confirmed. Especially within PBMCs, γδ T cells reacted to TLR 7/8, but not to TLR 3 stimulation by upregulating CD25.

Conclusions

This research highlights the influence of age and possible antigen contact on the frequency and activation status of γδ T cell subsets. Expression of TLRs, in particular TLR7, indicates that these cells have the ability to directly respond to exposure with viral pathogens. Future research will focus on the functional relevance of the different γδ T cell phenotypes and TLR expression in response to the Porcine Reproductive and Respiratory Syndrome Virus.

Financial Support
Natural Sciences and Engineering Research Council of Canada; Saskatchewan Agriculture Development Fund

Natural Sciences and Engineering Research Council of Canada | Conseil de recherches en sciences naturelles et en génie du Canada

Notes:

177 - Butyrate modulates lipopolysaccharide-induced innate responses of porcine monocytes

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Session: Immunology - 4, Dec. 7, 11:45 - 12:00 PM

Objective

The short-chain fatty acid butyrate is a major metabolite produced by microbes in the lower gastrointestinal tract. Known to enhance mucosal immunity and intestinal barrier function, butyrate has demonstrated anti-inflammatory properties. Monocytes from the blood can migrate into the intestine and respond to environmental signals. Pro-inflammatory responses to bacterial agonists, such as lipopolysaccharide (LPS), include evaluation of IL-1b, TNF, IL-6 and IL-18; whereas IL-10 is often assessed as an anti-inflammatory marker.

Methods

To investigate the impact of butyrate on monocyte responses to LPS, as a model for monocyte responses in the intestine, porcine blood-derived monocytes were cultured with LPS +/- butyrate for 4 or 24 hours (h). RNA was isolated for gene expression at 4 h and supernatants were collected for protein at 24 h. Gene expression data is presented as log2 fold change.

Results

LPS stimulation alone increased *IL1B, TNF, IL6, IL18* and *IL10* average gene expression relative to mock-stimulated cells. Co-culture of LPS with butyrate (0.25 mM) caused a 3- to 4.5-fold decrease in the average gene expression of *IL1B*, *TNF* and *IL6* compared to LPS-only exposure. Though LPS alone had little effect on expression of *TLR4*, the co-culture with LPS and 0.25 or 1 mM butyrate slightly decreased *TLR4* gene expression when compared to LPS exposure alone. The addition of 0.25 mM butyrate with LPS for 4 h slightly increased expression of anti-inflammatory cytokine *IL10*. However, after 24 h, LPS with 1 mM of butyrate increased IL-10 protein production, but 0.25 mM did not. Thus, while *IL10* gene expression was increased at 4 h with 0.25 mM butyrate and LPS, it did not translate into increased IL-10 protein levels.

Conclusions

In conclusion, butyrate had differing effects on LPS-induced pro- and anti-inflammatory gene expression, and increased production of anti-inflammatory cytokine IL-10 when given at a higher dose. Further exploration is warranted to understand the mechanism of butyrate modulation of porcine monocyte responses to LPS, which may be used to improve intestinal health.

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

Notes:

178 - The novel ORFV protein ORFV113 activates LPA-p38 signaling

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Session: Virology - 2, Dec. 7, 8:30 - 8:45 AM

Objective

To determine the molecular mechanism(s) by which novel orf virus (ORFV) immunomodulatory protein ORFV113 interferes with immune signaling pathway and the function(s) of ORFV113 in infection and disease in natural host

Methods

A preliminary mass spectrometry experiment using immunoprecipitates from cells transiently expressing ORFV113 identified Lysophosphatidic acid receptor 1 (LPA_1), a p38 kinase activator, as a putative ORFV113 interactor. Reciprocal co-immunoprecipitations and confocal microscopy was performed to confirm the interaction. Effect of ORFV113 on LPA-p38 signaling in primary ovine fetal turbinate cells was assessed using Western blot for p38 phosphorylation, real time PCR for p38-regulated gene expression and functional assays (LPA_1 siRNA, CRISPR, antibody and pathway inhibitors). To assess function of ORFV113 in natural host, parameters of infection and disease was compared between mock infected animals, animals inoculated with ORFV113 revertant virus (OV-IA82RV113) and ORFV113 gene-deletion virus (OV-IA82Δ113) using a sheep model of infection.

Results

We describe a novel ORFV protein, ORFV113, that interacts with the G protein-coupled receptor LPA_1. Consistent with its interaction with LPA_1, ORFV113 enhances p38 kinase phosphorylation in ORFV infected cells *in vitro* and *in vivo*, and in cells transiently expressing ORFV113 or treated with soluble ORFV113. Infection of cells with OV-IA82Δ113 significantly decreased p38 phosphorylation and viral plaque size. Infection of cells with ORFV in the presence of a p38 kinase inhibitor markedly diminished ORFV replication, highlighting importance of p38 signaling during ORFV infection. ORFV113 enhancement of p38 activation was prevented in cells in which LPA_1 expression was knocked down and in cells treated with LPA_1 inhibitor. Infection of sheep with OV-IA82Δ113 led to a strikingly attenuated disease phenotype, indicating that ORFV113 is a major virulence determinant in the natural host.

Conclusions

Notably, ORFV113 represents the first viral protein that modulates p38 signaling via interaction with LPA_1 receptor.

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

Notes:

179 - Mutations in PEDV nsp1 causes increased viral sensitivity to host interferon responses and attenuation in vivo

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Session: Virology - 2, Dec. 7, 8:45 - 9:00 AM

Objective

PEDV was introduced into the United States in 2013 and rapidly spread nationwide. Its infection of suckling piglets can cause up to 100% mortality. Efficacious and safe vaccines are in urgent need to prevent PEDV outbreaks. Recent in vitro studies showed that PEDV nsp1 is the most potent interferon (IFN) suppressor among all viral proteins. In this study, we evaluated whether nsp1 is a virulence determinant in vivo and a good target for the development of live attenuated vaccines (LAVs).

Methods

We generated a recombinant PEDV (rPEDV) N93/95A carrying N93A and N95A mutations in nsp1 protein using the infectious clone of a highly virulent PEDV strain PC22A (icPC22A). Sensitivity to host IFN responses of the N93/95A was examined by infecting Vero cells pretreated with type I IFN (IFNβ) or type III IFN (IFNλ1 and IFNλ3). LLC-PK1 cells infected with rPEDVs were used to evaluate whether N93/95A mutant triggers enhanced IFN responses in vitro. To investigate the pathogenesis and immunogenicity of N93/95A mutant, 5-day-old gnotobiotic pigs were orally inoculated with the N93/95A, the parental virus icPC22A, or mock, and challenged at 22 days post-inoculation (dpi) with icPC22A.

Results

In both Vero and LLC-PK1 cells, N93/95A mutant replicated to 0.5 - 1.0 $\log_{10}$ $TCID_{50}$-lower titers compared with icPC22A. The N93/95A mutant was more sensitive to IFN pretreatment and induced significantly higher mRNA levels of IFNs at 24 hours post-inoculation compared with icPC22A. In the pig study, no mock pigs had diarrhea. icPC22A caused all pigs (100%, 5/5) severe diarrhea and death within 6 dpi. Although all N93/95A-inoculated piglets had severe diarrhea, only one of four pigs died. Post challenge, the piglets in N93/95A group were 100% protected from severe diarrhea and mortality, whereas all pigs in mock-challenge group developed severe diarrhea and one of them died.

Conclusions

Collectively, N93/95A mutant showed decreased replication and increased sensitivity to host IFN responses in vitro. It is attenuated but retained immunogenicity in vivo and can be a potential target for the development of LAVs for PEDV.

Notes:

180 - Modeling porcine hemagglutinating encephalomyelitis virus infection *in vivo* and *ex vivo*

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Session: Virology - 2, Dec. 7, 9:00 - 9:15 AM

Objective

Porcine hemagglutinating encephalomyelitis virus (PHEV) causes vomiting and wasting disease and/or encephalomyelitis in suckling pigs. This study characterized PHEV infection, pathogenesis, and immune response in CDCD 6-day-old pigs.

Methods

Piglets (n=18) were randomly distributed into PHEV (n=12) or control (n=6) groups. Before inoculation and immediately prior to euthanasia, blood was collected to evaluate viremia and humoral response by RT-qPCR and ELISA, respectively. Viral shedding was evaluated daily using nasal and rectal swabs throughout the study. Piglets in each group were euthanized at 5, 10, or 15 days post-inoculation (dpi). A variety of tissue sections were collected for viral RNA detection, histopathological and immunohistochemical evaluation.

Results

Infected animals developed mild respiratory, enteric, and neurological clinical signs between 2 to 13 dpi. PHEV did not produce viremia, but virus shedding was detected in nasal secretions (1-10 dpi) and feces (2-7 dpi) by RT-qPCR. Of all the tested tissue samples, only liver tissue had no viral RNA detected. The detection rate and RT-qPCR Ct values decreased over time. The highest concentration of the virus was detected in turbinate and trachea, followed by tonsils, lungs, tracheobronchial lymph nodes, and stomach from inoculated piglets necropsied at 5 dpi. The most representative microscopic lesions were gastritis lymphoplasmacytic, moderate, multifocal, with perivasculitis, and neuritis with ganglia degeneration. A moderate inflammatory response, characterized by increased levels of IFN-α in plasma (5 dpi) and infiltration of T lymphocytes and macrophages, was also observed. Increased plasma levels of IL-8 were detected at 10 and 15 dpi, coinciding with the progressive resolution of the infection. Moreover, a robust antibody response was detected by 10 dpi. An *ex vivo* air-liquid porcine respiratory cells culture system showed virus replication and cytopathic changes and disruption of ciliated columnar epithelia, thereby confirming the upper respiratory tract as a primary site of infection for PHEV.

Conclusions

This study provides a platform for further multidisciplinary studies of coronavirus infections.

Notes:

181 - Shedding dynamics, genetic variation, and reassortment of heterosubtypic influenza secondary infection in pigs.

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Session: Virology - 2, Dec. 7, 9:15 - 9:30 AM

Objective

Influenza (IAV) infection in pigs causes substantial production losses in the US swine industry and poses a constant threat to public health for its zoonotic potential. Pigs are susceptible to IAVs from multiple hosts, and the co-circulation of different subtypes of IAVs in US farms is common which facilitates IAV reassortment and long-term evolution of the virus. The objective of this study was to evaluate the extent of heterosubtypic IAV secondary infection at the individual pig level and how it impacts virus diversity in pigs following an experimental co-infection model.

Methods

Fourteen naive pigs were inoculated with either an H1N1 or an H3N2 IAV, and distributed in 7 rooms with one pig of each subtype housed together. Nasal swabs were taken daily, and bronchoalveolar lavage fluid (BALF) samples were collected during necropsy seven days post-contact. Samples were tested by matrix and HA subtyping real-time PCR. Positive samples (matrix Ct < 35) were virus quantified (TCID50) and whole genome sequenced by Illumina Nextseq platform. Up to 14 plaques were isolated from each BALF sample to identify virus reassortment in the pigs.

Results

Heterosubtypic IAV secondary infection was detected in 43% of pigs (6/14) through the lungs or nasal cavities during the 7-day observation period. We identified five pigs that apparently cleared their original IAV infection and at necropsy (7 dpc) mainly had the subtype from the other pigs detected in BALF. Compared with the primary infection, the IAV secondary infection exhibited similar variation patterns in the pigs although we found a positive selection on H1 and M1 protein in H1N1 primary infected pigs. The purified selection and antigenic drift are the primary selection forces shaping the IAV genome regardless of infection statuses. About 10% (4/40) of plaques isolated from 3 pigs (BALF samples) were identified as reassortants which resulted in 2 distinct genotypes. Overall, there was limited IAV reassortment in the secondarily infected pigs.

Conclusions

Our study demonstrated that pigs that may become infected consecutively by multiple subtypes of IAVs can have extended IAV shedding patterns over time which may affect virus diversity in the pigs. More research is needed to validate these results under field conditions.

Financial Support
Zoetis

Notes:

182 - Molecular evolution of ORF5 gene of field PRRSV type 2 strains in the United States from 2012 to 2020

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Session: Virology - 2, Dec. 7, 9:30 - 9:45 AM

Objective

Porcine reproductive and respiratory syndrome virus (PRRSV) poses an extensive economic impact on the United States (US) swine industry. PRRSV is characterized by a higher degree of genetic and antigenic variability thus, the virus is continuously evolving challenging the existing vaccination programs. In this study, we aimed to investigate the genetic diversity of PRRSV type 2 (PRRSV-2) isolates circulating in the US and the underlying evolutionary mechanisms among the heterologous isolates on a molecular level that may impact vaccination efficacy.

Methods

We assessed the ORF5 gene sequences of 1931 PRRSV-2 field isolates collected from two porcine production systems in the US from 2012 to 2020. Phylogenetic analysis was performed to estimate the genetic relatedness between field strains and labeled by the global PRRSV classification system. Global and local section pressure and N-glycosylation in the predicted glycoprotein 5 (GP5) were evaluated.

Results

The field PRRSV-2 isolates were classified into either Lineage 5 (L5; 22.7%) or Lineage 1 (L1; 77.3%). The L1 strains belonged to one of three sub-clades, L1A (63.4%), L1B (3.6%), and L1C (10.3%). A total of 10 N-glycosylation sites were detected. Most isolates (93.3%) shared the N-glycosylation at 44th and 51st sites. We found clade-specific N-glycosylation sites at 57th in L1A, 33rd in L1B, 30th and 34th in L1C, and 30th and 33rd in L5. We identified 9 (L5) and 19 sites (L1) under positive selection. The local positive selection was observed uniquely at the 13th, 151st, and 200th positions of GP5 in the L5 strains.

Conclusions

Heterogeneity of N-glycosylation and positive selection sites may play a role in shaping the evolutionary characteristics of the ORF5 gene in US field PRRSV-2 strains. L1A and L5 clades signified an excellency in adaptation to the current swine population in the US, which was ascertained by their extensive positive selection sites with higher site-specific selection pressure. Our findings provide meaningful insights into the evolutionary processes of field PRRSV-2 strains in the US and designing effective vaccines.

Financial Support
U.S. National Science Foundation; University of California at Davis; This study was funded by the NSF BigData: AI award # 1838207, NSF Track-D award # 2134901, and Fellowship of the Graduate Group in Epidemiology, University of California Davis, USA.

Notes:

183 - Bovine rhinitis B virus is highly prevalent in BRD and causes upper respiratory tract infection in calves

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Session: Virology - 2, Dec. 7, 9:45 - 10:00 AM

Objective

Bovine respiratory disease (BRD) is the most significant cause of cattle morbidity and mortality worldwide. The multifactorial disease has complex etiology. Dogma posits a primary viral infection followed by secondary bacterial pneumonia. Bovine rhinitis B virus (BRBV) is an established etiological agent of BRD however little is known on its pathogenesis. Here we evaluated the prevalence of BRBV in BRD diagnostic submissions and characterized the pathogenesis of a contemporary virus isolate.

Methods

The prevalence of BRBV was evaluated by PCR in lung and nasal swabs submitted for BRD diagnostic testing. Isolated virus was used to evaluate pathogenesis in calves.

Results

A BRD PCR panel identified 18/153 (11.8%) lung samples and 20/49 (40.8%) nasal swabs collected from cattle with respiratory signs were positive for BRBV, which was the most prevalent virus in nasal swabs. Primary bovine tracheal epithelial cells were used to isolate BRBV that was phylogenetically related to contemporary sequences from the U.S. and Mexico and genetically divergent from the previous sole BRBV isolate. To investigate virus pathogenesis, one-week-old colostrum-deprived dairy calves were inoculated intranasally with 7.0 $\log_{10}$ $TCID_{50}$ BRBV. Virus was isolated from nasal swabs, nasal turbinates, trachea, and the brain of the challenged animals. Neutralizing antibodies were detected beginning 7 days post inoculation and peaked at day 14. In situ hybridization (ISH) localized BRBV infection in the upper respiratory ciliated epithelial and goblet cells, occasionally associated with small defects of the superficial cilia lining. Sporadically, pinpoint ISH signals were also detected in cells resembling glial cells in the cerebrum in one calf.

Conclusions

These results demonstrate the BRBV infection is highly prevalent in acute BRD samples and while the pathogenicity of BRBV is minimal with infection largely limited to the upper respiratory tract, further research is needed to elucidate a possible initiatory role in BRD.

Financial Support
South Dakota State University Agricultural Experiment Station

Notes:

184 - Novel pseudorabies virus (PRV) vectored bivalent vaccine against classical swine fever and porcine circo viruses

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Session: Vaccinology - 3, Dec. 7, 8:30 - 8:45 AM

Objective

Classical swine fever, porcine circovirus 2b, and pseudorabies viruses (CSFV, PCV2b, and PRV) are important swine viral diseases worldwide. PCV2b, recently renamed PCV2d is prevalent in domestic and feral pigs. PCV2b infected pigs are prone to coinfection with other swine viruses, resulting in the pig industry's fatal outcome and significant economic losses worldwide. Even though CSFV and PRV have been eradicated in commercial swine in the U.S. and many European countries, there is a constant risk for PRV spillover from wild/feral pigs and accidental CSFV introduction. Therefore, this research aims to develop a safe and protective PRV vectored CSFV and PCV2b subunit vaccine against the three diseases.

Methods

We have constructed a triple gene (thymidine kinase, glycoprotein E [gE] and gG)-deleted (PRV-TMV) vaccine vector expressing chimeric PCV2b-capsid, CSFV-E2 and E^{rns}-fused with bovine granulocytic monocyte-colony stimulating factor (E^{rns}-GMCSF), designated as PRV-TMV.PCV-2/CSFV-sub vaccine. Here we compared the immunogenicity and protective efficacy of the vaccine in pigs against wild-type PCV2b challenge in comparison to the Zoetis Fostera Gold PCV commercial vaccine.

Results

We have characterized the PRV-TMV.PCV-2/CSFV vaccine virus for growth kinetics and confirmed chimeric protein expression. PRV-TMV.PCV-2/CSFV-sub is safe and highly attenuated in pigs. Based on PCV2b-specific neutralizing antibody, viremia, virus replication in tissues, nasal- and fecal-virus shedding, pigs immunized with a single dose of PRV-TMV.PCV-2/CSFV prototype subunit vaccine and challenged with PCV2b protected the pigs slightly better than the Fostera Gold PCV- vaccinated pigs. The pigs inoculated with PRV-TMV.PCV-2/CSFV subunit vaccine also generated moderate levels of CSFV-specific serum neutralizing antibody titers.

Conclusions

The PRV-TMV.PCV-2/CSFV subunit vaccine is safe and efficacious against virulent PCV-2 challenge in pigs. Protective efficacy of the vaccine against CSFV, and PCV2b-/CSFV-specific cellular response experiments will be completed in year three of the project.

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

Notes:

185 - Natural killer T cell activation induces influenza vaccine-associated enhanced respiratory disease (VAERD) in pigs

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Session: Vaccinology - 3, Dec. 7, 8:45 - 9:00 AM

Objective

Vaccination is critical for controlling influenza A virus (IAV) infections. Broader cross-protection against circulating strains is needed; however, there are reports of vaccine-associated enhanced respiratory disease (VAERD) when vaccinated pigs are challenged with a mismatched virus strain. Natural killer T (NKT) cells are immunoregulatory innate-like T cells, capable of being activated with synthetic glycolipids that can be used as adjuvant to enhance vaccine response against a range of infectious diseases. The NKT cell agonist alpha-galactosylceramide (αGC) used as an adjuvant improved protection against homologous influenza challenge in pigs immunized with a killed H1N1 IAV vaccine. However, increased lung pathology and no improvement in protection were seen with heterologous IAV challenge. Here, we used a heterologous vaccination-challenge experiment to induce VAERD and evaluate the effect of NKT cell activation.

Methods

Three groups of 6 pigs were vaccinated with UV-inactivated H1N2 MN08, combined with an oil-in-water adjuvant, αGC (100 μg/kg), or with both adjuvants. A fourth group was unvaccinated, and 3 additional pigs served as unvaccinated non-challenged controls. After 21 days, a second dose of vaccine and adjuvant was given, followed 16 days later by challenge with heterologous pandemic H1N1 CA04 virus. Pigs were euthanized at 5 days post-challenge (dpc).

Results

Pigs that received αGC had increased NKT cells in peripheral blood, bronchoalveolar lavage fluid, lung tissue, and tracheobronchial lymph nodes. αGC-treated pigs also had higher concentrations of IFN-γ producing cells from lung tissue after *in vitro* stimulation with both, the vaccine and challenge IAV strains. Pigs in all three vaccinated groups had increased macroscopic lung lesion scores and reduced viral titers at 5 dpc when compared to unvaccinated, pH1N1-infected pigs.

Conclusions

αGC alone or in combination with oil-in-water adjuvant increased lung pathology in a heterologous influenza vaccination-challenge model of VAERD. Caution should be used when considering αGC as an adjuvant for inactivated influenza vaccines.

Financial Support
U.S. National Institute of Child Health and Human Development

Notes:

186 - Assessment of local innate immune responses induced by polydi-(sodium carboxylatoethylphenoxy)-phosphazene in pigs

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Session: Vaccinology - 3, Dec. 7, 9:00 - 9:15 AM

Objective

Adjuvants induce local innate immune responses, which in turn influence the development of antigen-specific immune responses. The objective of this study is to evaluate the capacity of novel adjuvant polydi(sodium carboxylatoethylphenoxy)phosphazene (PCEP) to stimulate potent innate immune responses in piglets and evaluate the differences between intradermal (ID) and intramuscular (IM) routes of immunization.

Methods

Two groups of 3-week-old piglets (n=6/group) will be assessed in this experiment. One group will be immunized ID, the other IM. Each pig will be injected four times on their left and right back (for a total of 8 injections), with either 100 microliters of endotoxin free, sterile PBS on the left back, or with 100 micrograms of PCEP dissolved in 100 microliters of endotoxin free, sterile PBS on the right back. Each injection site will be approximately 3 inches apart. Animals will be humanely euthanized 48 hours after injection. Punch biopsies (8 mm each) will be collected from the injection sites, as well as the prescapular lymph nodes. One of each of the right and left punch biopsy samples will be processed for PCR, histology, ELISA, and kinome analysis. Genes and cytokines to be measured include but are not limited to: CCL2, CCL5, IFN-γ, IL-1β, IL-18, and IL-6. This experiment is slated to be completed October 1, 2021.

Results

We will show that there will be significant recruitment of innate immune cells when comparing PCEP to the control PBS. These immune cells will be detected directly using histology and indirectly using cytokine ELISAs. Furthermore, we expect ID immunization to outperform IM due to the increased presence of dendritic cells, antigen presenting cells and Langerhans cells in the dermis.

Conclusions

This experiment will further inform the mechanisms of action surrounding novel adjuvant PCEP when stimulating innate immune effector mechanisms. From these conclusions we plan to use them as a baseline to inform future studies on how PCEP contributes to innate immune responses and how combination adjuvants work mechanistically.

Financial Support
Natural Sciences and Engineering Research Council of Canada

Natural Sciences and Engineering Research Council of Canada | Conseil de recherches en sciences naturelles et en génie du Canada

Notes:

187 - Immunogenicity and pathogenicity of a cell adapted PEDV oral vaccine candidate belong to non-S INDEL cluster

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Session: Vaccinology - 3, Dec. 7, 9:15 - 9:30 AM

Objective

The antigenic diversity makes porcine epidemic diarrhea (PED) as a challenge for the global pig industry. The emergence of PED virus (PEDV) causes significant economic loss to pig farms. In this study, we investigated the prevalence of PEDV in Vietnam in 2018. More important, we developed a new attenuated vaccine candidate from the emerging PEDV strain belongs to non-S INDEL cluster.

Methods

A phylogenetic tree was constructed based on full *spike* genes of eight positive PEDV samples, which were collected from outbreaks in Vietnam during 2018, and reference strains using BioEdit and MEGA 6.06 programs. PEDV2 strain was isolated from a small intestine sample of an infected piglet in Vietnam, in 2015. PEDV2 continuously passaged until passage level 103 in the VERO-CCL81 cell. PEDV2 strains at four passage levels, PEDV2-p10, PEDV2-p26, PEDV2-p46, and PEDV2-p103, were tested for pathogenicity in piglets. Five-day-old piglets in each group were orally inoculated with 1 ml of one passage level of PEDV2, or placebo. The dose of the viral inoculum was 10^5 $TCID_{50}$ in 1 ml volume per piglet. After inoculation, clinical signs were recorded, fecal swabs were collected daily to evaluate viral shedding.

Results

Eight of the PEDV strains circulating in Vietnam during 2018 belong to three different clusters as Asian non-S INDEL, S INDEL and classical. The cell-adapted PEDV2-p103 strain belonged to the emerging non-S INDEL cluster, resulted in low virus shedding, and did not induce lesions in the small intestines of challenged piglets. PEDV2-p103 reached the peak titer in the VERO-CCL81 cell at 48-hour post-infection.

Conclusions

In this study, we reported the genetic diversity of PEDV circulating in Vietnam in 2018 to highlight the need for new vaccines, which are effective for the prevention of emerging PEDV strains. In preliminary *in vivo* test, the highly cell passaged PEDV2 strain showed no pathogenicity and significant change of seroconversion after oral inoculation. The PEDV2-p103 strain might be evaluated for oral vaccine candidate after further *in vivo* immunogenicity and challenge test.

Financial Support
Korea University

Notes:

188 - Prescription platform vaccines as next-generation approaches for emerging pathogens FAD outbreaks.

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Session: Vaccinology - 3, Dec. 7, 9:30 - 9:45 AM

Objective

Vaccines are critical to support the Animal Agriculture industry. The current USDA regulatory process ensures a pure, safe, potent and efficacious product, but does take years and significant expense to develop and license. The USDA recently created new subcategories for platform and prescription platform vaccines. These new regulatory pathways can now be used to rapidly respond with products that address pathogens with high diversity as well as new and emerging diseases. Although Medgene Labs has a focus on swine and cattle vaccines, we used this approach to develop and deploy efficacious vaccines against an emerging foreign animal disease, Rabbit Hemorrhagic Disease virus (RHDv).

Methods

Vaccines developed under these new guidelines must use a well-established, highly uniform production process, must be non-replicating subunit approaches, and demonstrate safety in the target species of interest. Multiple prescription vaccine products can be generated based off this backbone to respond to targets of commercial interest, as well as FADs. As an example, we have deployed a vaccine formulation showing strong protection in challenge studies directed against an emerging pathogen – RHDv – which can now be used as the basis to address additional viral mutants, as well as form the basis of protective vaccines against other pathogens (e.g. Classical Swine Fever) in other species.

Results

Using this system, we rapidly developed, tested, and deployed a vaccine to protect the United States against a FAD incursion. This vaccine protected rabbits in direct challenge studies, appears safe, and most importantly can now be added to several other licensed formulations for future preparedness.

Conclusions

This approach provides a roadmap for generation of new vaccines for animal health that can be implemented rapidly in response to immediate need. A future goal is to expand the utility of our platforms through development of new subunit vaccine approaches to address animal health issues of commercial relevance or national security.

Notes:

189 - U.S. dairy producers' attitudes on male calf care and its relationship with on-farm care

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Session: Vaccinology - 3, Dec. 7, 9:45 - 10:00 AM

Objective

This study aimed to characterize and identify associations between dairy producer attitudes and the differences in early-life care of surplus male calves and female calves on dairy farms in 5 U.S. states.

Methods

In February 2021, 1,000 dairy producers in 5 states (FL, MI, OH, VT, WI) were selected through stratified random sampling and mailed a survey consisting of 45 questions. Calf care practices were characterized by reported colostrum quality, quantity, and timing of delivery relative to calving, and compared between male and female calves using a non-parametric Wilcoxon rank-sum test. Using a 5-point Likert Scale, each participant responded to 10 statements regarding their attitudes towards the value and care of male calves. Factor analysis revealed 2 composite scores (cost of care, perceived value of male calves), and preference of male calves raised by dairy beef growers as variables of interest. Established factors were used as dependent variables in multiple analysis of variance (MANOVA) and univariate regression analysis, with farm demographics and calf care practices included as independent variables.

Results

By May 2021, 315 usable responses of 953 delivered surveys were collected (33.1%). Overall, 16.1% of respondents reported feeding different types of colostrum to female and male calves ($P=0.28$), while 15.6% delivered colostrum with different methods ($P=0.49$). Less volume ($P=0.02$) and slower time to the first colostrum feeding ($P=0.001$) were identified in male calves in 5.14% and 8.57% of respondents, respectively. Using 3 univariate models, respondents that treated female and male calves differently regarding type, delivery method, or time to colostrum delivery after birth considered costs and benefits in male calf care more than those that treated male and female calves equally ($P<0.05$). No other significant relationships were identified.

Conclusions

Dairy producers with more cost and benefit concerns used different early-life care practices for surplus male calves. This association may be used to design interventions in animal welfare improvement programs.

Notes:

190 - Clinical effects and *Histophilus somni* prevalence in high-risk calves administered intranasal or parenteral vaccine

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Session: Vaccinology - 4, Dec. 7, 10:30 - 10:45 AM

Objective

Previous research indicates mucosal BRSV infection enhances *Histophilus somni* (Hs) clinical disease. We hypothesized that intranasal vaccination with BRSV antigens in high-risk cattle with naturally occurring Hs would result in greater Hs colonization. Our objective was to determine safety, efficiency and BRSV and Hs nasal shedding in intranasal or parenteral vaccinated high-risk beef calves.

Methods

Auction-derived beef calves (n=525; 5 truckload blocks) were stratified by body weight (213±18.4 kg), sex, and presence of a pre-existing ear-tag. Pens were spatially arranged and randomly assigned to treatment with an empty pen between treatment groups. Treatments were applied on d 0: 1) no viral respiratory vaccination (CON), 2) intranasal administration of a trivalent (IBRV, BRSV, PI3V) modified-live virus (MLV) vaccine with a parenteral BVDV type I and II vaccine (INF), and 3) parenteral administration of a pentavalent (IBRV, BRSV, PI3V, BVDV type I and II) MLV vaccine (INJ). Pen was the experimental unit, with a total of 15 pens/treatment and 11 or 12 calves/pen in this 70-d receiving study. Health and performance outcomes, and BRSV and Hs frequency of carriage and cycle time in nasal swabs via rtPCR, BRSV-specific antibody titer, and serum IFN-γ concentration via ELISA were evaluated periodically.

Results

Morbidity (P=0.83), mortality (P=0.68) and average daily gain ($P^3$0.08) did not differ. Serum antibody against BRSV increased with time (P<0.01), and was numerically greatest for INF. On d 14 and 28, INF (21.1 and 57.1%) had more (P<0.01) nasal swabs become Hs positive than CON (3.6 and 25.3%) and INJ (3.4 and 8.4%). Also, INF had reduced (P=0.03) cycle time of Hs positive samples on d 28. No treatment differences (P=0.55) were detected for serum IFN-γ.

Conclusions

These data indicate MLV vaccination of high-risk calves at arrival, either parenterally or intranasally, had little effect on health or growth during the feedlot receiving period. However, INF increased the prevalence of Hs in the naris.

Notes:

191 - Development and testing of *Mycobacterium avium* subsp. *paratuberculosis* DIVA vaccines in ruminants

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Session: Vaccinology - 4, Dec. 7, 10:45 - 11:00 AM

Objective

Johne's Disease (JD), caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP), is a significant problem in animal health. We generated live-attenuated strains that can differentiate vaccinated from infected animals (DIVA) and engender protective T-cell responses. We previously reported that marked deletion mutants in MAP_1152 (DMAP52) and MAP_1156 (DMAP56) are attenuated in bovine macrophages and immunogenic in tissue culture of primary bovine macrophages. The objective of this study is to test apoptotic stimulation of MAP mutants and also to develop unmarked mutants of DMAP52 and DMAP56.

Methods

Wild type and marked deletion mutant strains were assayed for the induction of apoptosis and necrosis using cell cultures of RAW 264.7 macrophages. MAP strains were grown to mid-exponential phase in Middlebrook media, and used to infect (MOI, 10) macrophages for 1, 3, 6,12, 24 or 48 h. RealTime-Glo™ Annexin V Apoptosis and Necrosis Assay (Promega) reagents were added, measuring the output with a multi-mode detection platform. To unmark the deletion mutants, the marked (Hyg^R) strains were transformed with pYUB870 (Kan^R) that carries the *sacB* gene and gamma-delta resolvase.

Results

We observed activation of apoptosis at 3 and 6 h followed by secondary necrosis at 12, 24 and 48 h. The mutant strains DMAP52 and DMAP56 displayed apoptotic and necrotic trends compared to wild type NADC K-10, UNL K-10 and wild type like transposon mutant 4H2. These assays were confirmed by flow cytometry, apoptotic nuclear morphology and Caspase-Glo® 3/7 (Promega). Mutant unmarking proceeded by first selecting on Kan^R to obtain pYUB870 transformants that have lost Hyg^R, followed by counter selection on sucrose to remove the helper plasmid to obtain Kan^S, Hyg^S, Suc^R transformants.

Conclusions

The marked mutants were clearly apoptotic, a landmark of good vaccine candidates. Steps for unmarking were completed. We plan to characterize the attenuated unmarked in-frame deletion mutants, test antigens for DIVA capabilities and assess the immunogenicity and pathogenicity of unmarked mutants in calves.

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

Notes:

192 - A vaccine conferring protection against virulent bovine anaplasmosis is accomplished by targeted mutagenesis

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Session: Vaccinology - 4, Dec. 7, 11:00 - 11:15 AM

Objective

Anaplasma marginale is responsible for causing a major economically important tick-borne disease, bovine anaplasmosis, throughout the world. Vaccines offering protection against the disease are unavailable. Targeted mutagenesis methods are yet to be developed to define genes essential for this and other *Anaplasma* pathogens.

Methods

We developed a targeted mutagenesis method to delete a phage head-to-tail connector protein gene from *A. marginale*, as its homolog is identified as essential for *in vivo* growth and persistence in *Ehrlichia chaffeensis* (another closely related pathogen). We then assessed its growth defect *in vivo* and then determined if it confers protection against virulent infection challenge. We also tested inactivated *A. marginale* whole cell antigens as a vaccine (WCV).

Results

The *A. marginale* mutant displayed a growth defect and abated the clinical disease in cattle. Cattle that received the virulent *A. marginale* St. Maries strain developed severe disease, as evidenced by 52% drop in PCV between days 26-31 post inoculation, with a peak bacteremia reaching to 12% on day 25 (assessed by thin blood smear and quantitative PCR analysis). The infected animals also exhibited anisocytosis from day 30 onwards. Conversely, animals inoculated with live mutant (MLV) exhibited no clinical signs and maintained blood parameters within the normal range. When these animals were challenged with the virulent St. Maries strain four weeks after the MLV infection, clinical signs were also not observed for the 44-day assessment with no evidence of bacteremia or anisocytosis or drop in PCV below the normal range. Contrary to this, WCV vaccinated animals exhibited disease similar to the infected controls, although the bacteremia and drop in PCV was lower at 5% and 40%, respectively.

Conclusions

This is the first study describing targeted mutagenesis and its application in determining virulence and vaccine development for an *Anaplasma* species pathogen.

Financial Support
Center of Excellence for Vector-Borne Diseases foundation fund, KSU-CVM, Manhattan, KS; Russell L. Rustici Rangeland and Cattle Research Endowment fund, College of Agricultural and Environmental Sciences, UC Davis, CA

Notes:

193 - A heterologous prime boost vaccination strategy against Johne's disease

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Session: Vaccinology - 4, Dec. 7, 11:15 - 11:30 AM

Objective

Johne's disease (JD) is a contagious, chronic, and potentially fatal infection caused by *Mycobacterium avium ss. paratuberculosis* (*M. ap).* JD causes significant economic losses in the dairy industry, estimated at $500 million in the USA alone. Currently, there is no approved vaccine against JD in the USA. Previously, we showed that a single dose live attenuated vaccine (LAV) developed after deleting the lipN gene (dubbed pgsN) provided partial protection against JD in cattle. To further enhance the efficacy of the pgsN vaccine, we utilized a prime/boost (PB) vaccination strategy in combination with a novel nanoadjuvant system composed of Quil A and chitosan (QAC).

Methods

In this work, we evaluated the safety, immunogenicity and protective efficacy of pgsN administered either as live or inactivated vaccine in a PB regimen in cattle. Calves were primed at 4 weeks of age and boosted 4 weeks following the initial priming. Calves were challenged 2 months post vaccination with a virulent bovine *M. ap* strain and followed for 6 months.

Results

Significant changes in body weight, temperature, general health conditions were not observed between sham- and pgsN-vaccinated calves suggesting an overall safety of the vaccines. Calves vaccinated with the PB regimen showed significant skin induration (compared to PBS group) in response to Johnin PPD indicating a robust cellular immune response and potentially enhanced protection. Importantly, the same animals did not respond to *M. bovis* antigens, suggesting its utility in bovine tuberculosis enzootic areas. So far, challenged animals showed bacterial shedding in their feces only up to 8 days post challenge. Bacterial, blood and histological analyses of collected samples are currently underway.

Conclusions

The results demonstrated that the pgsN vaccine formulations tested were safe and induced a potent cellular immunity and potential protective immunity in cattle, further paving the way for testing in the field.

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

Notes:

196 - Identification of genetic mechanisms of antimicrobial resistant in aquaculture pathogens

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Session: Antimicrobial Resistance/Use - 3, Dec. 7, 8:30 - 8:45 AM

Objective

Aquaculture represents the upsurging agricultural industry, and pond-raised catfish industry dominates aquaculture in the United States. The ability to treat catfish bacterial pathogens with antimicrobials is important for animal welfare, and it is important economically for producers to prevent losses. Unfortunately, multidrug resistant (MDR) *Edwardsiella ictaluri* MS 17-156, *Edwardsiella piscicida* MS 18-199, and *Plesiomonas shigelloides* MS 17-188 strains have been reported from moribund catfish in east Mississippi. The objective of this study is to explore the genetic mechanisms of antimicrobial resistance in these strains

Methods

To explore resistance mechanisms in these multidrug resistant (MDR) strains, genomic DNA was extracted and subjected to whole genome sequencing using a combination of long (Oxford Nanopore) and short (Illumina) reads

Results

The genome of *E. ictaluri* strain MS-17-156 revealed an IncA/C incompatibility group plasmid (named pEIMS-171561), which carries florfenicol efflux pump(floR), tetracycline efflux pump(tetD), and sulfonamide resistance(sul2). The genome of *E. piscicida* strain MS-18-199 revealed a novel conjugative plasmid named pEIMS-18199 (NCBI accession number is CP035669.1). The plasmid size is 117,448bp in length and it shares a high degree of similarity (99.97% identity and 95% coverage) to a previously identified plasmid from *Edwardsiella anguillarum* ET080813. Among the three plasmids identified in *P. shigelloides*, pPSMS-171883 was found to carry florfenicol and tetracycline resistant genes. Plasmid pPSMS-171883 is 18,970bp in length and is a novel IncP-6 resistance plasmid. Results from mobilization and stability experiments revealed that these three MDR-plasmids are highly stable in the host cell and can transfer to *Escherichia coli* by conjugation

Conclusions

A better understanding of the transmission of these three plasmids is vital to develop management options for reducing the spread of AMR determinants via environmental pathways. In aquaculture, continuous monitoring of MDR strains and sequence are important to determine the genetic basis of resistant

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

Notes:

197 - Antibiotic resistance profile of bacterial isolates from aquaculture production in Ikorodu, Lagos, Nigeria

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Session: Antimicrobial Resistance/Use - 3, Dec. 7, 8:45 - 9:00 AM

Objective

Aquaculture production provide cheap animal protein for Nigerians. Indiscriminate use of antibiotics commonly practiced among fish farmers and for their fish contributes to the global menace of antimicrobial resistance . To evaluate amr risk in aquaculture production, antibiotic profile of enteric bacterial isolates from fish farmers, the fish and aquaculture environmental samples in Ikorodu Fish Farms in Lagos State which is a hub of aquaculture in Nigeria.

Methods

Feacal samples from aquaculture farmers (n= 45) and pooled fish gut (n=100) as well as pond-water (n=100) were collected. Escherichia coli and Salmonella spp. were isolated and tested for susceptibility to 10 antimicrobials using a multi-disc diffusion.

Results

A total of 195 fecal *E.coli* isolates and 64 *Salmonella* isolates were obtained. Sixty five *E.coli* were pan-susceptible, but high proportion of isolates were resistant to tetracycline (78% fish farmer, 81% fish and 75% pond-water isolates), streptomycin (62% fish farmer, 73% fish, and 68% pond-water isolates), sulfonamides , and ampicillin . While E.coli isolates from 37% of fish farmers, 48% fish and 54% pond-water exhibited multidrug resistance. Salmonella isolates were most frequently resistant to streptomycin, tetracycline, and sulfonamides. Both the *E.coli* and *Salmonella* isolates were moderately resistance to cephalosporins, macrolides, and quinolones.Streptomycin-tetracycline-sulfisoxazole-trimethoprim-sulfamethoxazole was most common resistance pattern in fish *E.coli* isolates with highest odds of resistance to tetracycline and ampicillin.

Conclusions

In this study *Escherichia coli* and *Salmonella* isolates from farmers, fish and pond-water were highly resistant to most commonly used antibiotics. This portends One-health challenge of antibiotic resistance and risk to aquaculture production, public health, food safety and environmental health, which necessitate drastic actions to enforce prudent use of antibiotics and reduce the emergence of multidrug resistant bacteria

Notes:

198 - Antimicrobial resistance trends among *Escherichia coli* obtained from canine clinical samples in the Northeastern US

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Session: Antimicrobial Resistance/Use - 3, Dec. 7, 9:00 - 9:15 AM

Objective

We aimed to describe the antimicrobial susceptibility patterns, identify temporal resistance trends, and describe associations between resistance phenotypes among canine clinical *Escherichia coli* isolates in the Northeastern US.

Methods

We collected minimum inhibitory concentrations from 5,695 *E. coli* isolated from canine urinary and non-urinary infections at the Cornell University Animal Health Diagnostic Center between 2007 and 2017. The available clinical data were limited to body site. Isolates were classified as resistant or susceptible to 6 (urinary) and 16 antibiotics (non-urinary) based on CLSI breakpoints. We used the Mann-Kendall test (MKT) to detect the presence of a significant trend in the percent of resistant isolates over the study period. Six multivariable logistic regression (MLR) models were built with ceftiofur (CFT), enrofloxacin (ENR), or trimethoprim-sulfamethoxazole (SXT) resistance as the outcome and either (1) body site and isolation date, or (2) resistance to other antibiotics as predictors.

Results

Overall, 15.8% were resistant to ENR, 14.6% to CFT, and 13.9% to SXT. The MKT revealed a significant decreasing temporal trend for SXT resistance. No significant temporal resistance trends were detected for other antibiotics. MLR showed that non-urinary isolates were significantly more likely than urinary isolates to demonstrate *in vitro* resistance to CFT, ENR, and SXT after controlling for year of isolation. A decrease in resistance rates to CFT, ENR, and SXT was observed after 2010 in the MLR models. We identified a higher level of CFT resistance among ENR resistant isolates from urinary and non-urinary origin. Among non-urinary isolates, ticarcillin resistance was the strongest predictor of resistance to both SXT and ENR.

Conclusions

Although CFT, ENR, and SXT resistance decreased over time, our findings confirmed that dogs are still a reservoir of drug-resistant *E. coli* in the Northeastern US. Interestingly, the decrease in SXT resistance suggests that this older drug could be a good first-line choice for empiric therapy; it is already recommended for canine urinary tract infections.

Notes:

199 - WGS analysis of plasmid-mediated colistin-resistant *Escherichia coli* isolates from the poultry sector in Lebanon

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Session: Antimicrobial Resistance/Use - 3, Dec. 7, 9:15 - 9:30 AM

Objective

We aimed to look for the presence of colistin resistance in chicken fecal samples collected from 32 chicken farms located in three governorates of Lebanon.

Methods

Based on both phenotypic and molecular analyses (including next-generation sequencing), we characterized the population structure of colistin-resistant *Escherichia coli*, the genetic support of *mcr*-dependent or *mcr*-independent colistin-resistance, and the plasmid types carrying the *mcr* genes.

Results

This study reports the prevalence of *mcr-1*-positive *E. coli* in poultry originating from 32 farms across three Lebanese governorates and slaughtered in the same place. We report 27/32 (84.4%) *mcr-1* positive farms, leading to a total of 84 non-duplicate *E. coli* collected, of which 62 presented the *mcr-1* gene. Numerous associated resistance mechanisms were identified, including to extended-spectrum cephalosporins through the presence of bla_{CTX-M} or bla_{CMY} genes. The *mcr-1* gene was mostly carried by IncX4 (n = 36) and IncI2 (n = 24) plasmids, which are both known for their efficient transfer capacities. High genetic diversity was detected, arguing for the lack of contamination during the slaughter process. ST744 and ST1011 were the most widely identified clones, which have been both regularly associated to *mcr-1*-carrying *E. coli* and to the poultry sector.

Conclusions

The wide dissemination of colistin resistance, coupled with resistance to extended-spectrum cephalosporins and numerous other molecules, should urge authorities to implement efficient guidelines for the use of antibiotics in the poultry sector in Lebanon.

Notes:

200 - Exposure to antimicrobials in PRRSV infected nursery pigs and impacts on the resistome of pre-retail pork products

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Session: Antimicrobial Resistance/Use - 3, Dec. 7, 9:45 - 10:00 AM

Objective

In swine, a major driver of antimicrobial use is porcine respiratory and reproductive syndrome (PRRS), which is caused by a virus but predisposes infected animals to severe secondary bacterial infection. The goal of this study was to determine whether different PRRS-relevant antibiotic treatment protocols were associated with differences in the total AMR profile and microbiome of skin and carcass samples obtained from treated pigs and their carcasses at slaughter.

Methods

At weaning, pigs were divided into three treatment groups (N=36 pigs/group): 1) PRRSV negative / minimal antibiotic use, 2) PRRSV challenged / moderate antibiotic use, and 3) PRRSV challenged / intensive antibiotic use. At the plant, skin samples were collected in lairage and on the shackle chain post-stunning; and carcass swabs were collected pre-and post-evisceration and in the cooler. DNA was extracted from each sample and subjected to target-enriched shotgun and 16S rRNA gene sequencing to characterize the resistome and microbiome, respectively. The resistome and microbiome were then compared by sample type, sampling event, and treatment group.

Results

There was a major reduction in relative AMR gene abundance between lairage/shackles and evisceration, and the AMR gene profile obtained from carcass samples was significantly different from that obtained from skin. Previous antibiotic exposures accounted for only 2% of the variation in the AMR profile on carcasses. Similarly, microbiome composition also varied across sampling events, with relatively higher abundance of Firmicutes in the skin samples taken in lairage and shackles, while Proteobacteria were present in higher abundance in pre- and post-evisceration and cooler carcass samples.

Conclusions

Using a sensitive enrichment approach, we were able to profile the very low-abundance AMR genes on carcass swabs. Our results support the efficacy of slaughter-based food safety interventions in reducing overall microbial load and AMR genes, consistent with previous work demonstrating log-fold reductions in aerobic bacteria and specific pathogens during the slaughter process.

Financial Support
National Pork Board

Notes:

202 - Surveillance for antibiotic residues in beef, eggs, and honey sold in East Tennessee farmers' markets, 2020

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Session: Food Safety, Dec. 7, 10:30 - 10:45 AM

Objective

There is a lack of surveillance of antibiotic residues in foods, particularly eggs and honey that are sold at farmers' markets in the USA. Active surveillance was conducted to determine the pattern and concentration of tetracycline, erythromycin, and sulfonamide residues in beef, eggs, and honey sold at East Tennessee farmers' markets in 2020.

Methods

Between July 2020 and September 2020, beef (n=9), eggs (n=18), and honey (n=9) samples were purchased from selected East Tennessee farmers' markets. Using an enzyme-linked immunosorbent assay, tetracycline, erythromycin, and sulfonamide residues were detected. Chi-square or Fisher exact test was done to find out the association between antibiotic residues and all categorical variables.

Results

Over half (55.5%) of beef, nearly two-thirds (66.6%) of honey, and almost half (38.8%) of the egg samples had detectable residues for tetracycline. All beef and honey had detectable residues for erythromycin, and 22.2% beef and 5.5% egg samples had detectable residues for sulfonamides. In beef, the median concentration of tetracycline, erythromycin, and sulfonamides was 3.3 ppb, 12.9 ppb, and 812.5 ppb, respectively. In egg, the median concentration of tetracycline and sulfonamides were 17.2 and 876.4 ppb respectively. In honey, the median concentration of tetracycline and erythromycin were 142.1 ppb and 3.0 ppb respectively. With the exception of honey, one beef, and one egg sample, the rest of these concentrations did not exceed the allowable maximum residue limits (MRL). The erythromycin and sulfonamides test kits were not adaptable for the testing of eggs and honey, respectively. No statistically significant association was found between the county, month of sampling, type of food samples, farmers' markets, and presence of tetracycline and sulfonamides residues in the foods.

Conclusions

Surveillance findings show most of these samples had antibiotic residues that did not exceed the MRLs set in the U.S, except for a few individual samples. However, the presence of the residues in animal products could have a negative health effect among consumers.

Notes:

203 - Methods for estimating withdrawal interval recommendations for fenbendazole administered in feed to ring-necked pheasants

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Session: Food Safety, Dec. 7, 10:45 - 11:00 AM

Objective

Pheasants are considered game birds in the USA. They are categorized by the Food and Drug Administration (FDA) as a "minor" food-animal species. Fenbendazole is a highly effective benzimidazole-class anthelmintic that is not approved for treating game birds in the USA. Since there are 4 FDA-approved medications for treating pheasants in the USA, extra-label drug use is commonly utilized by veterinarians. Consequently, there is a need to establish a conservative withdrawal interval (WDI) following extra-label drug use of fenbendazole in pheasants to protect human health.

Methods

A drug residue tissue depletion study was performed in ring-necked pheasants. Liver, pectoral muscle, and thigh muscle samples were analyzed following a fenbendazole continuous feeding regimen (100 ppm in the feed for 7 days). Time versus concentration data was analyzed using the FDA and EMA tolerance regulatory methods. In addition, log-linear regression analysis and a non-parametric approach were evaluated as methods for establishing WDI recommendations following ELDU since published experimental pharmacokinetic studies have more data variability and are not performed according to FDA and EMA guidelines for drug residue human food safety studies. For all methods, turkey tolerances, maximum residue limit, and analytical limit of detection were applied as limits.

Results

The longest WDIs were estimated using liver, thigh, and pectoral tissue data and applying the FDA method and the LOD as the limit. In addition, the longest WDI (7 days; 153 hr) was calculated using pectoral muscle data. WDIs obtained using the EMA method 99% percentile and FDA tolerance methods were very similar.

Conclusions

This study demonstrated that non-regulatory methods for estimating WDIs following extra-label use of fenbendazole in pheasants resulted in WDIs that were similar to those derived using regulatory methods. In addition, a non-parametric approach might be useful for published scientific time versus concentration data that does not meet the FDA and EMA assumptions but could be helpful for estimating WDI recommendations following ELDU.

Financial Support
University of California at Davis

Notes:

204 - Transcriptome analysis of *Campylobacter jejuni* and *Campylobacter coli* during cold stress

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Session: Food Safety, Dec. 7, 11:00 - 11:15 AM

Objective

Campylobacter spp. are known to cause campylobacteriosis, a bacterial disease that remains a public health threat. *Campylobacter* spp. are prevalent in retail meat and liver products, and the prolonged survival of *Campylobacter* in the low temperatures needed for storage is a challenge for food safety. The main objective of this research was to determine the transcriptomic response of *C. jejuni* and *C. coli* to cold stress.

Methods

In this study, RNA-seq was used for analysis of the *C. coli* HC2-48 and *C. jejuni* OD2-67 transcriptomes at 4°C in a nutrient-rich medium (chicken juice, CJ) and Mueller Hinton Broth (MHB) for 0 h, 30 min, 24 h and 48 h.

Results

Differentially expressed genes (DEGs) involved in flagellar assembly were highly impacted by low temperatures (4°C) in *C. coli* HC2-48, whereas genes related to the ribosome and ribonucleoprotein complex were modulated for *C. jejuni* OD2-67 at 4°C. Most of the DEGs in cells grown at 4°C in the two media formulations were not significantly expressed at different incubation times.

Conclusions

Although more DEGs were observed in CJ as compared to MHB in both *Campylobacter* strains, the absence of common genes expressed at all incubation times indicates that the food matrix environment is not the sole determinant of differential expression in *Campylobacter* spp. at low temperatures.

Notes:

205 - Evaluation of the Hazard Analysis Critical Control Point in the processing of 'suya' meat in Abuja, Nigeria

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Session: Food Safety, Dec. 7, 11:15 - 11:30 AM

Objective

The processing of 'Suya' in Nigeria has generated a lot of problems regarding its safety for human consumption. The recommended systematic approach to managing process hygiene and controlling undetectable hazards in foods is based chiefly on Hazard Analysis Critical Control Points (HACCP) principles. The aim of the work was to determine the critical control points in the processing of 'Suya' in Nigeria.

Methods

The study was carried out in Abuja, Nigeria. Two 'suya' spots were purposively selected. The 6 standard principles of HACCP were conducted in accordance with the National Food Processors Association (1950) hand book. A total of 74 swabbed samples were aseptically collected at 3 different points for analysis. First from the raw meat samples freshly delivered from the abattoir. Secondly from the sliced, sticked, and spiced meat before roasting and finally from processed 'suya' meat. Samples were analyzed for total aerobic plate count (APC), total coliforms and Salmonella/Shigella using poured plate count method.

Results

Washing and heating were identified as effective control measures to eliminate hazards in the processing of 'Suya'. Three critical control points presented in a flow chart were identified as possible points of contamination. In the raw abattoir meat, APC was 4.2 x 10^6, total coliforms was 2.3 x 10^6 and Salmonella/Shigella was 1.1 x 10^4. In the case of sliced, sticked and spiced meat before roasting, APC was 4.8 x 10^6, total coliforms was 2.5 x 10^6 and Salmonella/Shigella was 0.8 x 10^4. For the processed 'suya' meat, APC was 2.9 x 10^6, total coliforms was 0.6 x 10^6 and Salmonella/Shigella was 0.1 x 10^4 .

Conclusions

The result indicated that HACCP is a very strong tool to assess hazards and establish control systems that focus on prevention during food processing. Washing and heating are the major preventive measures to control hazards in suya processing. Education of Suya meat processors on the importance of HACCP, personal hygiene and clean environment is very imperative.

Notes:

206 - Association of litter and cecal microbiomes with *Campylobacter* colonization in broilers under commercial settings

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Session: Food Safety, Dec. 7, 11:30 - 11:45 AM

Objective

Campylobacteriosis is a serious foodborne disease in humans and is the most common cause of bacterial gastroenteritis globally. The reduction of campylobacteriosis attributed to poultry demands a multiple stepwise program of pre- and post-harvest interventions, including *Campylobacter* load reduction on the farm. However, the potential to manipulate cecal and litter microbiomes to reduce *Campylobacter* colonization remains underexplored. The objective of this study was to determine the association of litter and cecal microbiomes with *Campylobacter* within-flock prevalence and concentration in ceca from broilers in commercial settings.

Methods

Broiler flocks were followed weekly throughout the production cycle until six (small broilers, n=10 flocks) or nine (big broilers, n=16 flocks) weeks of age, respectively. Weekly sample collections included two litter samples (from front and back sections of each house) and ceca from five broilers per flock. DNA was extracted from the samples and used for *Campylobacter*-specific qPCR and 16S rRNA gene V4 region sequencing. Inverse probability weighted (IPW) linear and logistic regression models were built to assess the effect of bacterial genera from litter and ceca at two, three, and four weeks of flock age on *Campylobacter* concentration in ceca and within-flock prevalence at six weeks in both small and big broilers. Propensity scores were estimated using random forest.

Results

Bifidobacterium in litter and *Negativibacillus*, DTU089, GCA-900066575, and *Oscillibacter* in ceca were associated with a statistically significant reduction of *Campylobacter* colonization in small and big broilers at six weeks of flock age. Interestingly, *Negativibacillus* and *Oscillibacter* high abundance in ceca at three and four weeks of flock age, respectively, was translated into a public health-relevant decrease in *Campylobacter* colonization in small and big broilers (>2-$\log_{10}$ reduction of *Campylobacter* loads).

Conclusions

A targeted manipulation of the litter and cecal microbiomes could become an important component of pre-harvest *Campylobacter* control in broilers in the future.

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

Notes:

207 - Molecular enrichment of whole bacterial genomes for metagenomic profiling of foodborne pathogens

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Session: Food Safety, Dec. 7, 11:45 - 12:00 PM

Objective

Food safety threats of antimicrobial resistance (AMR) and virulence are mediated by horizontal transfer of antimicrobial resistance genes (ARGs) and virulence factors (VFs) via mobile genetic elements (MGEs). Public health risks of such microbiome-level dynamics are difficult to predict or control via surveillance due to limitations of current culture-based and culture-independent techniques to reconstruct and contextualize ARGs with VFs, and MGEs in complex metagenomic samples. We demonstrate the use of a novel metagenomic sequencing approach– *Target-enriched long-read sequencing* (TELS) – to achieve accurate resolution of microbial resistomes, virulomes, mobilomes, and pathogen profiles from surveillance samples. TELS sequencing involves *in situ* enrichment using custom probes covering >130,000 ARGs, VFs, and MGEs, as well as whole genomes of priority serovars of: *Salmonella*, *Escherichia coli*, *Campylobacter*, and *Enterococci*. Here we report on the first phase of this study: Molecular probe design for enrichment of pathogens from metagenomes.

Methods

Whole-genome-sequenced isolates (n=1000) for 2018–2021 were systematically abstracted from the NCBI pathogen detection system representing recent SNP-clusters of priority serovars. Following parameterization, these genomes served as the basis for probe design using the algorithm—*CATCH*— which was implemented in parallel to a new custom probe design algorithm. The performance of both algorithms to generate molecular probes was assessed.

Results

CATCH design coverage rate decays in a power fashion over time before reaching equilibrium rate. For *Salmonella* serovars, representing the largest proportion of genomes, polynomial probe synthesis converges on a theoretical completion of coverage in 3.4 months of *CATCH* runtime. In all cases *CATCH* failed to converge on a probe design covering all genomes. This is contrasted with the custom algorithm producing 230,000 120-mer probes covering all intended genomes in < 50 minutes of runtime.

Conclusions

We extend bioinformatic techniques for purposes of metagenomic surveillance of foodborne pathogen threats.

Financial Support
U.S. Department of Agriculture, Food Safety Inspection Services; U.S. National Institute of Allergy and Infectious Diseases

Notes:

P001 - Evaluation of enrofloxacin and oxytetracycline to eliminate persistent *Anaplasma marginale* infection in cattle

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Session: Antimicrobial use/resistance/stewardship, Dec. 6, 6:00 - 8:00 PM

Objective

Bovine anaplasmosis is a bacterial disease of cattle caused by the rickettsial pathogen *Anaplasma marginale*. Common clinical symptoms include anemia, lethargy, pyrexia, and death. Antimicrobial treatment of anaplasmosis has historically relied on oxytetracyline products; however, Baytril® 100-CA1 (enrofloxacin) was recently granted conditional approval for the treatment of clinical anaplasmosis. In most cases, anaplasmosis challenged animals survive infection with or without treatment intervention and remain carriers of *A. marginale*, serving as disease reservoirs. This study was designed to investigate the efficacy of enrofloxacin and oxytetracyline to eliminate *Anaplasma marginale* infection in persistently infected steers.

Methods

Fourteen Holstein steers previously inoculated with a Virginia or KS2 *A. marginale* strain and confirmed positive using a PCR test targeting the *A. marginale* Major Surface Protein 5 gene (*msp5*) were allocated to treatment groups receiving injections of enrofloxacin (Baytril® 100-CA1) or oxytetracycline (Bio-Mycin® 200). The recommended dosage for each treatment (Bio-Mycin® 200 at 4.5mL/100, 10 mL/injection site: Baytril® 100-CA1 at 5.7 mL/100 lb, 20mL/injection site) was administered subcutaneously for five consecutive days (off-label experimental dosing regimen). Blood samples were collected daily during treatment and twice weekly following the last treatment to monitor *A. marginale* infection status and bacterial level using the quantitative *msp5* PCR assay.

Results

All seven steers receiving oxytetracyline and five of the seven steers receiving enrofloxacin remained positive for *A. marginale* infection. Injection site reactions were observed for some steers treated with oxytetracyline.

Conclusions

Identification of effective antimicrobial-based protocols to clear anaplasmosis infection would provide producers options to reduce anaplasmosis transmission potential in their herd and retain valuable stock.

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

Notes:

P002 - Behavior-change interventions to improve antimicrobial stewardship in the human and animal sectors

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Session: Antimicrobial use/resistance/stewardship, Dec. 6, 6:00 - 8:00 PM

Objective

Antimicrobial resistance (AMR) is a rapidly emerging public health threat that causes an estimated 700,000 deaths annually. The overuse and misuse of antimicrobials in the human and animal health sectors are major drivers of AMR and render many available antimicrobials ineffective. There is an urgent need to identify effective interventions that reduce inappropriate antimicrobial use (AMU) and improve antimicrobial stewardship (AMS) in both sectors in a variety of settings including low- and high-resource contexts. We conducted a systematic search of MEDLINE, Cochrane, and Web of Science for peer-reviewed articles that described knowledge or behavior change interventions aimed at optimizing AMU and improving AMS in the human and animal health sectors.

Methods

Papers published in any language and written on interventions conducted in any country were included. Non-relevant papers; those that did not clearly describe intervention aims, outcomes, or results; or entries for which the full text was unavailable were excluded. For entries that met inclusion criteria, information such as location, setting, type, duration, and description of the intervention or trial; number of participants; knowledge or behavior change target; and outcomes were extracted for a meta-analysis.

Results

23,459 papers were identified; of those 3,049 were relevant and included for final review and meta-analysis. Interventions described included introducing standardized treatment guidelines and rapid, point-of-care diagnostic technology; using mobile AMS applications; and implementing internal, prescriber-led clinical review boards. Most interventions followed randomized controlled trial, observational, or pre/post intervention methodologies. As expected, most studies were conducted in high-resource countries in human health settings.

Conclusions

In our presentation, we will describe the least and most effective interventions and outline a strategic, intersectoral approach to improving AMU and AMS across the human and animal health sectors in various settings and global contexts.

Notes:

P003 - Knowledge, Attitude and Practices (KAP) survey of farmers on antimicrobial use, antimicrobial resistance and use of the laboratory facilities

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Session: Antimicrobial use/resistance/stewardship, Dec. 6, 6:00 - 8:00 PM

Objective

Antimicrobial resistance (AMR) impacts public health, animal health, plants and environmental health, requiring the One Health approach to address it. To contain AMR, veterinary diagnostic laboratories play a key role in identifying pathogens and differential diagnosis. However, there is little published information available about the knowledge, attitudes, and practices of the dairy farmers regarding AMU, AMR and the use of laboratories in Pakistan. Therefore, this study aims to explore the knowledge, attitudes, and practices of the dairy farmers regarding AMU, AMR and the use of laboratories.

Methods

A cross-sectional survey was conducted. The selected dairy production system included smallholders, market-oriented farms and peri-urban farms. A questionnaire was developed containing different questions about AMU, AMR and the use of laboratories. The questionnaire was reviewed by local and international experts and was pretested before finalization. Descriptive statistics has been produced so far. Furthermore, Item response theory (IRT) models will be used to evaluate the overall levels of knowledge, attitude, and appropriate practices and associations among these three outcomes will be evaluated using multivariable models.

Results

Of 443 respondents, 88.7% were used to seek the veterinarian's advice before antibiotic use and 76% used to keep leftover antibiotics for future use. A total of 73.8% were unaware of AMR. The most frequent source of antibiotics was veterinary drug stores (98%). 53.7 % of the respondents reported that veterinary laboratories are not available in their area and 48% reported that biggest barrier for not using the laboratory is unawareness.

Conclusions

Assessing the antimicrobial usage behaviors of the farmers and providing them with best practice guidelines will be an effective strategy to reduce the global burden of AMR. The results of this study will assist in establishing the baseline data that will be used to set national action priorities in line with the global action plan to contain AMR and develop an antimicrobial stewardship program.

Notes:

P004 - Prevalence and antibiotic resistance of *Staphylococcus* spp. found in dairy systems from 10 states in Mexico

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Session: Antimicrobial use/resistance/stewardship, Dec. 6, 6:00 - 8:00 PM

Objective

The main objective of this cross-sectional study was to determine the presence of *Staphylococcus* spp. resistant to methicillin in beddings of the maternity areas, as well as periparturient cows and lactating calves from the major milk production areas of Mexico. The second objective was to determine the antibiotic resistant pattern of the isolated strains.

Methods

The sampling took place from October 2019 to January 2020 in 10 states of Mexico. Samples were taken directly from the rectum of asymptomatic calves, asymptomatic periparturient cows, and maternity floors with Q-swabs. The number of samples were at least six from each dairy for a total of 120 samples. The confirmation of *Staphylococcus* genus was done by PCR. Antimicrobial susceptibility tests were conducted by disk diffusion method. *Staphylococcus* spp. isolates were tested against five antibiotic groups: penicillin, aminoglycosides (gentamicin), tetracyclines (tetracycline), fluoroquinolones (ciprofloxacin, levofloxacin) and sulfonamides (sulfamethoxazole trimethoprim).

Results

From the 20 dairies sampled, 10 had at least one positive result, conforming a 50% prevalence with 23 isolates in total. Stratified analysis indicated that 5 calves were found positive in 3 dairies (n=38); 9 periparturient cows in 7 dairies (n=44); and 8 isolates were positive for maternity areas (n=38), in 6 dairies. According to PCR confirmation of *Staphylococcus* genus, 22 were *Staphylococcus aureus* and one *Staphylococcus epidermidis*. 23% of the *Staphylococcus aureus* isolates were resistant to at least one antibiotic tested (5 out of 22 isolates). Resistance to tetracycline and chloramphenicol was detected in 13.6% of the isolates.

Conclusions

The wide distribution of this pathogen may represent a risk for animal health and have underlined the requirements for monitoring MRSA in food-producing animals. The presence of *Staphylococcus* spp. in fecal samples and environment could play a significant role in transmission of MRSA as a zoonotic pathogen. To our knowledge, there is no other study of Staphylococcus spp. detection of this scale in Mexico.

Notes:

P005 - Antimicrobial activities of phytophenols against liver abscess causing pathogens in feedlot cattle

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Session: Antimicrobial use/resistance/stewardship, Dec. 6, 6:00 - 8:00 PM

Objective

Liver abscesses occur in finishing cattle fed high-grain, low-roughage diets. Liver abscesses are of significant economic concern to the feedlot industry. The causative agents include *Fusobacterium necrophorum* subsp. *necrophorum*, *F. necrophorum* subsp. *funduliforme*, *Trueperella pyogenes*, and *Salmonella enterica*. Tylosin, a macrolide antibiotic, is supplemented in the feed to reduce liver abscesses. Because of the concern with emergence of potential antimicrobial resistance, there is a need to find antibiotic alternatives. Plant based phenolic compounds, which have antimicrobial activity, have the potential to be an antibiotic alternative to control liver abscesses.

Methods

We investigated the efficacy of phenolic compounds extracted from sorghum (black, sumac, brown, and burgundy varieties), rosemary, grape seed, green tea, matcha tea, and yerba mate on liver abscess-causing bacterial pathogens. Phenolic compounds were extracted by using 75% aqueous acetone as a solvent, and total phenolic content was determined spectrophotometrically. Muller-Hinton broth (for *S. enterica* and *T. pyogenes)*, and anaerobic brain heart infusion broth (for *Fusobacterium)* with and without phenolic extracts (1 mg/ml) were used. Growth was measured at 24 and 48 hours by determining bacterial concentrations. A micro-broth dilution method was used to quantify the inhibition, if the compound was inhibitory.

Results

Black and sumac sorghum, grape seed, green tea, yerba mate and matcha tea phenolics inhibited growth of both *Fusobacterium* subspecies, *T. pyogenes* and *S. enterica* based on microdilution assay. On quantification, the phytophenols were inhibitory against *T. pyogenes* with minimum inhibitory concentration ranging from 6.25-12.5 µg/ml.

Conclusions

Further studies are ongoing to investigate different concentrations and stability of these plant based phenolic compounds at various temperature conditions on the liver abscess pathogens. Plant based phenolic compounds that inhibit the pathogens may have the potential to be supplemented in the feed to control liver abscesses.

Notes:

P006 - Estimating national-level antimicrobial consumption for Pakistan: The antibiotic footprint project

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Session: Antimicrobial use/resistance/stewardship, Dec. 6, 6:00 - 8:00 PM

Objective

The antibiotic footprint, modelled after the carbon footprint, seeks to estimate antimicrobial consumption and to visualize this as a communication tool to aid policy approaches to reducing consumption. With 220 million people and 1486 million poultry, there is an urgent need to quantify antimicrobial consumption to better inform policy aimed at use reduction in Pakistan.

Methods

Sales data from IQVIA was used to estimate human consumption and import data on the Pakistan Import Export Database (EXIM) was used to estimate animal consumption. The estimation for the human sector follows established methods as described elsewhere. Using the list of licensed veterinary medicines, the 2019 imports of antimicrobials on EXIM were downloaded onto EXCEL. Data was verified using a second subscription database, and cleaned. Import volumes for veterinary-only imports were converted from gross metric tons to net weights in kg by first calculating a package-weight, or directly from the item description's label. Net weight calculations were performed for active pharmaceutical ingredients, finished pharmaceutical products, and feed additives. Consumption in the animal sector was expressed as mg/Population Correction Unit (PCU).

Results

Human consumption was 35.46 Defined Daily Dose per 1000 inhabitants per Day (DID). By type, almost 40% belong to the World Health Organization's AWARE Watch group against ~20% in the Access group. The estimated veterinary import volume was: 1,152,000 kg. Animal consumption (work-in-progress).

Conclusions

This study presents the first attempt to estimate national-level antimicrobial consumption for Pakistan. Here, we have expanded a method for consumption estimation in the animal sector using import data that can be applied to other Low- and Middle-Income Countries. The main limitation with the use of import data is the fact that it may overestimate consumption. It is also difficult to separate human from animal use to avoid potential double-accounting. Results validation and visualization are on-going. The results would inform policy interventions.

Financial Support
The Fleming Fund Country Grant Pakistan

Notes:

P007 - Antimicrobial use on equine racetracks

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Session: Antimicrobial use/resistance/stewardship, Dec. 6, 6:00 - 8:00 PM

Objective

Antimicrobial use (AMU) has received relatively little attention in equine medicine, even though antimicrobial resistance is a growing problem in horses. Extremely limited data are available on antimicrobial use at equine racetracks, which are environments where large-scale movement of horses and people from many different locations occurs. The objective of this study was to characterize antimicrobial use on four racetracks in the eastern United States during the peak racing seasons of 2017-2018.

Methods

Handwritten daily treatment sheets provided by attending veterinarians listing treatments administered to horses stabled at the racetrack were obtained. Information contained in the treatment sheets included the date, name of the horse and its trainer, the type of treatment, and a brief (usually one-word) indication for treatment. The handwritten data listed on the racetrack treatment sheets were manually transcribed and analyzed.

Results

A total of 2,684 antimicrobial prescriptions were recorded, representing 7% of all drug treatments. The most frequently dispensed antimicrobials were enrofloxacin with 854 dispensations (32% of antimicrobial treatments), followed by gentamicin (570, 21%), ceftiofur (388, 14%), and penicillin (220, 8%). The relative frequencies of antimicrobial class and indication for treatment varied significantly by racetrack and by prescribing veterinarian. Limitations associated with the data precluded ascertainment of the proportion of horses treated or exact indications for treatment.

Conclusions

Antimicrobials appear to be prescribed relatively infrequent at racetracks relative to other types of drugs, but highly or critically important antimicrobials were most often used. The appropriateness of use of these drugs remains unknown.

Notes:

P008 - Rumination and activity patterns of cows with metritis treated with chitosan microparticles

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Session: Antimicrobial use/resistance/stewardship, Dec. 6, 6:00 - 8:00 PM

Objective

Chitosan microparticles (CM) have been shown to have a broad spectrum of antimicrobial activity. Our objective was to characterize the behavioral changes of cows diagnosed with metritis and treated with CM with those treated with ceftiofur (CEF).

Methods

Nulliparous Holstein cows (n = 311) were fitted with a neck-automated monitoring device (SCR Inc., Netanya, Israel) *from -21 to 60 d relative to calving.* Cows diagnosed with metritis (d 0), characterized by watery, fetid, pink/brown uterine discharge within 21 days in milk (DIM) were assigned randomly to: CEF (n = 47) – subcutaneous injection of 6.6 mg/kg ceftiofur crystalline-free acid on d 0 and 3; CM (n = 45) – intrauterine infusion of 24 g of CM dissolved in 40 mL of sterile distilled water on d 0, 2, and 4; CON (n = 39) – no treatment. Contemporary cows not diagnosed with metritis NMET (n = 180) were selected for comparison (d 0 matched to cows diagnosed with metritis according to the week of calving).

Results

Both activity and rumination were greater for NMET cows when compared with cows diagnosed with metritis regardless of treatment. When the different treatments of metritis were compared separately, treatment had no effect on activity and rumination before diagnosis. Post-diagnosis, activity was not different between treatments. Post-diagnosis, CM (437.1 ± 11.8 min/d) had lower rumination than CEF (486.3 ± 10.7 min/d) and there was a tendency compared with CON (467 ± 11.4 min/d), and CEF was not different from CON.

Conclusions

In summary, CM decreased rumination compared with CEF and CON, which indicates that CM hinders the recovery of cows with metritis.

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

Notes:

P009 - Knowledge, Attitude and Practices (KAP) survey on veterinary drug prescription

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Session: Antimicrobial use/resistance/stewardship, Dec. 6, 6:00 - 8:00 PM

Objective

Antimicrobial resistance (AMR) is a global emerging health problem that is a significant health concern for both animals and humans. Irrational antimicrobial use (AMU) in food animals is considered one of the contributing factors in the development of AMR. However, little is known about the attitudes of veterinarians towards AMR and AMU in Pakistan. This study aims to explore their knowledge, attitude and practices and the factors influencing their behaviors regarding AMU and AMR.

Methods

A questionnaire has been developed containing questions on antimicrobial use and the emergence of AMR in dairy animals. The questionnaire was reviewed by local and international experts and was pretested before finalization. A a cross-sectional survey was conducted by a team of data collectors. Descriptive analysis has been produced so far. Furthermore, Item response theory (IRT) models will be used to estimate overall levels of knowledge, attitude, and practice sections of the survey and associations among sections will be evaluated using multivariable models.

Results

Of 164 respondents, 98.7 % (139/164) believed that AMR is a significant problem and 99% (163/164) believed that antibiotics being used in dairy animals are contributing to the development of AMR. 72% (118/164) of the respondents were able to identify the Critical Important Antibiotics (CIAs) and 75% (123/164) felt that irrational use of antibiotics is due to difficulty in making the accurate diagnosis.

Conclusions

The data resulting from this study will be used to develop an antimicrobial stewardship program, evidence-based strategies and guidelines for the veterinarians on prudent antimicrobial use. It will support veterinarians, public health experts and policymakers to engage in collaborative One Health efforts to combat AMR at the national and global scale. The findings of the KAP survey will be transformed into a communication package for different target audiences that will serve as an advocacy tool for the development of key interventions in the animal health sector.

Notes:

P011 - Endotoxemia and the pathogenesis of lipolysis dysregulation in periparturient dairy cows

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Session: Dairy cattle physiology, immunology or disease, Dec. 6, 6:00 - 8:00 PM

Objective

During the periparturient period of dairy cows, adipose tissues (AT) lipolysis fulfills, in part, energy deficits driven by parturition and lactogenesis. Lipolysis induces a remodeling process within AT that is characterized by inflammation with infiltration of macrophages (ATM). In healthy cows, lipolysis rate decreases and AT inflammation resolves as lactation progresses. However, when lipolysis is dysregulated, AT inflammation is maintained leading to metabolic and infectious diseases. An additional factor that is strongly connected with the presentation of periparturient diseases is the sharp increase in circulating bacterial endotoxins. We propose that endotoxemia triggers lipolysis dysregulation through toll-like receptors signaling leading to proinflammatory polarization of ATM and adipocyte insulin resistance. Our hypothesis is that endotoxemia induces adipose tissue macrophage proinflammatory polarization and impairs adipocyte insulin sensitivity, leading to lipolysis dysregulation.

Methods

We will investigate this hypothesis by pursuing two specific aims, and utilizing models of AT lipolysis complemented with functional assays and single-cell RNAseq analyses. Aim 1 will determine how endotoxins enhance adipocytes' lipolytic responses through changes in adipocytes' insulin sensitivity and ATM phenotype. Aim 2 will determine how endotoxemia potentiates AT lipolytic response during negative energy balance and polarizes ATM to pro-inflammatory phenotypes.

Results

This proposal will elucidate the mechanisms by which endotoxemia induces lipolysis dysregulation and increases periparturient dairy cows' susceptibility to diseases.

Conclusions

Our long-term goal is to develop nutritional and pharmacological tools to prevent lipolysis dysregulation during catabolic periods. We expect these tools will benefit herd health, improve dairy cows' welfare, and increase the economic sustainability of dairy farming.

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

Notes:

P012 - Effect of mucosal immune stimulant on mammary gland immune responses during dry and lactating periods in dairy cows

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Session: Dairy cattle physiology, immunology or disease, Dec. 6, 6:00 - 8:00 PM

Objective

Mastitis is the main reason for the use of antimicrobials (AB) on dairy farms. The use of AB in animals has been implicated in the emergence of AB resistant bacteria. Identifying alternative treatments capable of upregulating the immune system can simultaneously contribute to control mastitis and enhance AB stewardship on dairy farms. Hence, our objectives are: 1) evaluate the impact of a novel mucosal immunotherapeutic (IT) on enhancing mammary gland immune responses during critical stages of lactation and, 2) determine whether this IT is effective in preventing experimental mastitis.

Methods

Two studies will be executed to address objective 1. Experiment 1: Twenty-four cows randomly allocated at dry off to: 1) intramammary (IMM) administration of diluent (PBS); 2) IMM IT; 3) IMM antibiotic (ABX); or 4) IMM ABX + IT. Experiment 2: Twelve early-lactation dairy cows without previous administration of IT randomly allocated to receive IMM infusion of PBS or IT, but not challenged with a pathogen. One experiment to address objective 2. Experiment 3: Twenty early-lactation dairy cows without previous administration of IT randomly allocated to: PBS or IT. Three days after treatment, cows in both groups will be challenged with IMM *Streptococcus uberis*. In all experiments milk and dry cow secretions will be cultured using standard techniques whereas milk somatic cell counts determined and immunoglobulin binding to the surface of bacteria analyzed by flow cytometry. Microbiome and resistome will also be analyzed in milk and dry cow secretions. Statistical models will evaluate the effect of IS in mammary gland immune response and bioinformatics analysis will be used to compare microbiome and resistome between groups.

Results

We hypothesize that the novel immunotherapeutic to be tested in our project will improve mammary gland immune responses and to ultimately reduce the overall usage of AB in the dairy industry.

Conclusions

This project was supported by Agriculture and Food Research Initiative Competitive Grant no. 2021-67015-34558 from the USDA National Institute of Food and Agriculture.

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

Notes:

P015 - Effect of intramammary infections on heifer mammary gland growth and development

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Session: Dairy cattle physiology, immunology or disease, Dec. 6, 6:00 - 8:00 PM

Objective

Intramammary infections (IMI) are common in primigravid dairy heifers. These IMI are expected to interfere with the growth and development of the mammary tissue, but this has been yet to be investigated. The objective of this study was to evaluate the growth and development of infected mammary glands experiencing a persistent IMI, compared to those that were uninfected, during rapid mammary growth that was artificially induced in a model setting via estradiol and progesterone administration.

Methods

Twelve-month-old nonpregnant Holstein heifers (n = 18) received daily injections containing supraphysiological doses of estradiol and progesterone for 14 consecutive days. On the 8th day of injections, 1 quarter of each heifer was randomly selected and infused with *Staphylococcus aureus* via the teat canal to establish a persistent IMI for the trial's duration. Half the heifers (n = 9) were euthanized on the last day of injections, and mammary tissues were collected from the *Staphylococcus aureus* challenged quarter and an uninfected quarter of each animal. Mammary tissues were similarly collected from the 9 remaining heifers 13 days after the last injection. Lacteal secretions were collected throughout the experiment. Mammary tissues will be histologically evaluated, and mammary secretions will be enumerated to determine differential somatic cell counts. A microbiome examination will also be conducted on the collected mammary tissues and lacteal secretions.

Results

Sixteen of the 18 *Staphylococcus aureus* challenged quarters had an IMI that persisted throughout the trial. The uninfected quarters remained uninfected throughout the trial. The collected mammary tissues are currently being histologically evaluated.

Conclusions

Results from this work will yield information as to how persistent IMI affect mammary gland growth and development in rapidly growing and developing mammary glands. The microbiome evaluation of the collected tissues will reveal how the microbiome adapts during lactogenesis in the presence or absence of an IMI.

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

Notes:

P016 - Effect of cholecalciferol, anti-SpA IgY and RP185 on the phagocytic action of HC11 cells on *Staphylococcus aureus*

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Session: Dairy cattle physiology, immunology or disease, Dec. 6, 6:00 - 8:00 PM

Objective

Infectious mastitis is a common disease of cattle of economic significance. Clinical mastitis affects between 20% to 25% of cows each year. *Staphylococcus aureus* (SA) causes an estimate of 10-11.7% of bovine infectious mastitis cases in the US, reducing milk production, causing significant morbidity and need for culling, and increasing antibiotic use. The cure rate of SA mastitis is of up to 50% (using antibiotics). SA is internalized by mammary epithelial cells, evading most of the cellular and humoral immune systems. This allows SA to persist subclinically and chronically, and leads to antibiotic therapy failure. Therefore we propose a treatment for the disease by preventing SA internalization into HC11 cells using a combination treatment of cholecalciferol, anti-SpA IgY and RP185.

Methods

HC11 cells will be grown in 96-well plates and assigned one of the following treatments: Y5, Y10 or Y150 with 5µg/ml, 10µg/ml or 150µg/ml of commercial anti-SpA chicken IgY, respectively; C20, C50 and C80, with 20, 50 or 80 nM cholecalciferol, respectively; R1, R5, R10, R100 with 1, 5, 10 or 100µg of RP185, respectively, and their possible combinations, and N (no treatment). Then the HC11 will be exposed to live SA. The HC11 will be washed twice with gentamicin solution to eliminate any non-internalized SA. The remaining HC11 will be lysed with distilled water, and 10µL samples will be used to determine the number of CFUs between groups.

Results

Preliminary data shows that anti-SpA chicken IgY inhibits the growth of SA in vitro at 5 and 25µg/ml respectively. IgY and cholecalciferol will both prevent internalization of SA synergistically. The effect of RP185 in internalization is to be determined.

Conclusions

This study will benefit the dairy industry.

Notes:

P017 - Tracing the source and route of uterine colonization in dairy cows

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Session: Dairy cattle physiology, immunology or disease, Dec. 6, 6:00 - 8:00 PM

Objective

The source and route of bacterial colonization of the uterus are still not established. The objective was to investigate the source and route of bacterial colonization of the uterus by exploring the genetic relationship among *E. coli* strains isolated from the gastrointestinal and the reproductive tract of dairy cows pre- and postpartum.

Methods

Cows (n = 34) had the rectoanal junction (RAJ), vulva, and vagina swabbed every three days starting six days before expected calving until nine days postpartum. The uterus was swabbed postpartum. A blood sample was collected at all time points, but cultures were negative. Whole-genome sequencing was performed on 44 isolates recovered from eight cows with growth on selective *E. coli* media from the RAJ, vulva and/or vagina and uterus.

Results

Clonal isolates were found in the RAJ or the vulva prepartum and in the vulva, vagina or uterus postpartum. Clonal isolates were also found in the RAJ, the vulva, the vagina and the uterus postpartum. Clonal isolates were found in individual cows and different cows. The absence of clustering based on virulence factor genes and all genes indicates no strain specificity to body site.

Conclusions

These findings indicate that the gastrointestinal tract is the likely source of bacteria that colonize the reproductive tract via ascending colonization of the uterus through the lower genital tract. Additionally, cow to cow transmission occurs, and strains are not specific to body site.

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

Notes:

P018 - Immunogenicity of the novel enterobactin conjugate vaccine in dairy cows

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Session: Dairy cattle physiology, immunology or disease, Dec. 6, 6:00 - 8:00 PM

Objective

Mastitis is highly prevalent and economically disastrous disease in dairy industry. Environmental bacteria *E. coli* is a common cause. However, the only commercial vaccine J5 does not prevent new cases, which is likely due to antigenic variation and accessibility issue of target sites. Recently, we developed a Keyhole limpet hemocyanin (KLH)-enterobactin (Ent) conjugate vaccine that targets conserved iron-binding molecule Ent. In this study, we evaluated the immunogenicity of KLH-Ent in dairy cows.

Methods

Twelve pregnant multiparous Holstein dairy cows at 2nd – 3rd lactations were randomly assigned to vaccine (KLH-Ent) and control (PBS) groups on the drying off day. Cows were vaccinated with 5 mL of KLH-Ent (200 µg) emulsified with Emulsigen®-D adjuvant at drying off (D+0), and boosted with 5 mL of KLH-Ent (200 µg) emulsified with Freund's incomplete adjuvant (FIA) at 21 and 42 days after drying off (D+21and D+42). The control cows were injected with the same regimen except without KLH-Ent. Serum and milk samples were collected at D+0, D+21, D+42, C (calving) and 14 and 30 days after calving (C+14 and C+30) for antibody (IgG, IgG1, IgG2 and IgA) level analysis using indirect ELISA. Fecal microbiota were analzed at D+0, D+21, D+42, and C+14 using 16S rRNA sequencing.

Results

KLH-Ent vaccine induced significantly higher antibody in serum and milk of vaccinates compared with control cows. In particular, in vaccinated cows, the IgG and IgG2 response against KLH-Ent conjugate and Ent reached the peak at calving (C+0), and continued increasing until 30 days after calving (C+30). Ent specific IgG1 and IgA peaked at D+42 and remained high at C and C+14, and then quickly declined. The microbiota structures at the phylum level for control and vaccined groups were similar on the same day at all time points.

Conclusions

KLH-Ent successfully triggered strong Ent-specific immune response in dairy cows without significantly affecting the microbiota diversity and gut health. Thus, Ent conjugate vaccine may serve as effective vaccine against *E. coli* mastitis in dairy cows.

Financial Support
UT Center of Excellence in Livestock Diseases and Human Health

Notes:

P019 - Elucidating the origin and impact of MAP infections in commercial dairy herds on milk production and longevity

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Session: Dairy cattle physiology, immunology or disease, Dec. 6, 6:00 - 8:00 PM

Objective

Johne's disease (JD) in cattle is caused by *Mycobacterium Avium subsp. Paratuberculosis* (MAP) and persistent infection manifests as chronic enteritis resulting in wasting due to drastic reduction in nutrient absorption. Neonatal calves born from Johne's-positive dams are at risk of transmission via the fecal-oral route or from contaminated colostrum. Youngstock represent a critical link in controlling the transmission chain and need to be at the forefront of Johne's control programs, as they can harbor Johne's disease from birth, and begin shedding by 8 months old. Previous studies have shown that over half of animals infected with MAP at less than 6 months of age later present with a positive diagnostic result or histopathological lesions. High incidence of Johne's within commercial dairies represents a significant economic burden as Johne's disease has been demonstrated to cause up to 25% reduction in milk production and increased rate of heifer replacements. We hypothesize that post-parturient neonatal MAP infections via vertical transmission disproportionately occurs in commercial dairies that administer raw colostrum.

Methods

We aim to perform a pilot study of 30 heifer calves born from Johne's positive dams whereby serum ELISA and fecal PCR testing will occur at day 0 and day 30 to determine incidence of neonatal MAP infection. Colostrum and environmental samples will be obtained from bedding, hutches, and water sources. PCR-positive samples will be utilized for MAP strain identification by next generation sequencing to determine origin of infection.

Results

Preliminary analysis of actual pounds of milk produced per lactation of 686 cattle with Johne's disease and approximately 5,000 Johne's negative cattle from 5 herds confirmed that Johne's positive cattle produce significantly less milk in their third lactation.

Conclusions

This study aims to elucidate the origin of youngstock MAP infections to better understand how infections early in life impact progeny performance.

Notes:

P020 - Alternative treatment of bovine mastitis

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Session: Dairy cattle physiology, immunology or disease, Dec. 6, 6:00 - 8:00 PM

Objective

The treatment of mastitis is based mainly on the use of antibiotics. However, in recent times, an increase in resistance phenomena and the presence of residues in milk and their derivatives has been reported. Few studies have focused on the treatment of mastitis by homeopathy elsewhere or in Algeria. The objective of this current study aimed to clarify in particular the interest of homeopathy in the treatment of mastitis.

Methods

The study was carried out on two dairy farms on a total of 14 cows located in Laghouat region (southern Algeria). Before the start of the experiment, a tolerance test was performed on two cull cows free from any apparent infection (except mastitis). Local and general reactions were noted at specific times. A total of 31 mammary quarters received 4 intramammary injections of a homeopathic preparation containing several natural products every 12h over 48h. A clinical examination and an analysis of the milk samples on D0, D7 and D14 were performed on all cows and the conclusion was made on the day 14.

Results

Data showed a very good tolerance to the homeopathic preparation and a 75% cure rate of the clinical mastitis. An improvement with a decrease in CMT score was also noted. In addition, healing rates of 51.85% for subclinical mastitis on D7 and 59.29% on D14 were also reported.

Conclusions

Ultimately, homeopathy could, in some cases, represent an alternative to antibiotic therapy and bring an advantage to breeders. Further investigations should be performed in the future.

Notes:

P021 - Slime production, VanA gene and antiseptic resistance genes in Staphylococci isolated from bovine mastitis

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Session: Dairy cattle physiology, immunology or disease, Dec. 6, 6:00 - 8:00 PM

Objective

Staphylococcus strains are frequently associated with clinical and subclinical bovine intra-mammary infection. The virulence factors
of staphylococcus have not been widely studied in Algeria. The objective of this study was to determine the frequency of slime production, VanA gene and antiseptic resistance genes in staphylococci strains isolated from bovine mastitis in Algeria.

Methods

The study examined 35 Staphylococci strains obtained from the inflammatory secretion of mammary glands of cows with mastitis. Slime production was determined by detecting the icaA and icaD genes using the polymerase chain reaction (PCR) method and Congo red agar (CRA) method. The presence of qacAB and qac C antiseptic resistance genes and the VanA resistance gene in these isolates was investigated by PCR.

Results

The results of the current study revealed that of the 35 Staphylococci isolates, 42.85% (15/35) and 17.14% (6/35) of the isolates harboured the slime production gene by analysing icaA and icaD genes, respectively and 71.42% (25/35) by the CRA method. However, VanA and antiseptic resistance genes (qacAB and qac C) were not detected in any of the isolates.

Conclusions

Therefore, the majority of Staphylococcus strains were capable of producing slime, and the CRA detection rate was higher than the
PCR method for the biofilm-producing capacity of Staphylococcus strains. Thus, the presence of the ica genes in Staphylococcus strains confirms its role as a virulence factor in the pathogenesis of bovine mastitis.

Notes:

P022 - *Ex vivo* evaluation of enzymatic degradation of egg yolk IgY in chicken gastrointestinal tract

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Session: Diagnostic testing/disease modeling/precision agriculture, Dec. 6, 6:00 - 8:00 PM

Objective

Egg yolk antibody (IgY) has been increasingly recognized as a promising alternative to antibiotics to prevent and control infectious diseases for enhanced biosecurity in livestock. Oral administration of egg yolk IgY is the most common and convenient route in many studies. However, the stability of egg yolk IgY in gastrointestinal (GI) tract, a critical issue for the success of this approach, is still largely unknown. Our recent chicken study indicated that the egg yolk IgY was not stable in GI tract. Here, we aimed to further comprehensively evaluate the stability of egg yolk IgY in chicken GI tract using a well controlled *ex vivo* system.

Methods

A total of 15 broiler chickens were raised and euthanized at age of 2, 4, and 6 weeks, respectively, for the collection of gizzard and small intestinal contents from each individual chicken. Each GI content sample was subjected to pH measurement, slight dilution using saline, and being spiked with hyperimmune egg yolk. After the incubation at 42 °C for different lengths of time, each sample was subjected to ELISA, SDS-PAGE, and immunoblotting analyses to examine the titer and integrity of IgY.

Results

The pH in gizzard slightly increased with age (from 2.4 to 3.0) while consistently kept around 5.8 in small intestine. Despite subtle decrease in the specific IgY titer (2 fold) upon 30 min of treatment in small intestinal content, the IgY titers were drastically reduced by an average of 256, 64, and 32 fold in the gizzard contents from the chickens at age of 2, 4, and 6 weeks, respectively. Consistent with this finding, both SDS-PAGE and immunoblotting using IgY-specific antibody clearly demonstrated that the IgY was almost completely degraded upon as short as 5 min of treatment in gizzard content; however, the IgY was relatively stable in small intestinal content with up to 30 min of treatment. Immunoblotting also showed that treatment of IgY with HCl (pH 3.0) for 60 min did not affect the integrity of IgY, further supporting the enzymatic degradation of IgY in gizzard.

Conclusions

Egg yolk IgY could be substantially degraded in chicken gizzard, raising a significant challenge for its practical application in livestock.

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

Notes:

P023 - Development of swine immune reagents for analysis of immune correlates for vaccines, infection, and in biomedical research

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Session: Diagnostic testing/disease modeling/precision agriculture, Dec. 6, 6:00 - 8:00 PM

Objective

Our USDA-NIFA Swine Immune Toolkit Initiative has been involved in generating priority immune reagents based on inputs from veterinary immunology researchers worldwide, and pipeline them for marketing.

Methods

In our efforts we express soluble proteins and use them for production of panels of monoclonal antibodies (mAbs) using collaborations with commercial partners for protein expression and mAb production.

Results

So far recommended, we generated new panels of mAbs reactive to porcine IL-6, IL-13, IFN-γ, IL-17A, IL-28B, CXCL10, and BAFF and screened for their reactivity in multiple immune assays. Reactivity tests of labeled anti-IL-6 and anti-IL-13 mAbs for intracellular staining of porcine immune cells using flow cytometry assay are in progress. Our results have been confirmed for porcine IL-17A, IFN-γ and CXCL10 mAbs. A sensitive sandwich ELISA is now available for IL-17A, IL-13 and CXCL10; other targets are being screened for best mAb pairs for such assays. Planning has been initiated for the generation of IL-5 and IL-21 mAbs, and SLA-I & -II tetramers to identify swine CD4 and CD8 T cells specific for influenza virus peptides.

Conclusions

For each target, our goal is to provide the veterinary community with new commercial reagents and standardized assay techniques using these reagents for their research efforts. Tools and reagents generated by this project will undoubtedly advance swine immune, disease, vaccine and biomedical research efforts.

Notes:

P024 - Remote body temperature sensing for early disease detection

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Session: Diagnostic testing/disease modeling/precision agriculture, Dec. 6, 6:00 - 8:00 PM

Objective

We propose developing a novel data collection system to provide technologies needed to understand and improve animal agriculture and wildlife management through environmental, welfare, health, efficiency, and sustainability efforts. These tools are necessary for establishing evidence-driven livestock management practices and providing researchers, veterinarians, and producers with more decision-making power.

Methods

We are developing and validating a digital platform that collects, integrates, and interprets environmental, physical, and physiological data in various temporal and agricultural contexts. This unified technology platform (UTP) will simultaneously use inputs from multiple sensors that detect environmental, health, behavior, welfare, and performance parameters in real-time. Our proposed project will investigate the feasibility, utility, and efficacy of developing and validating the UTP system for use in both traditional and precision livestock management and wildife mangement. Initial efforts will focus on colelcting body temperature and acceleraometer data Soon, UTP's will be modified to integrate data from additional existing and novel sensors.

Results

Results are pending. By early fall, we will integrate collar-based sensors for body temperature, location, accelerometer, and ambient temperature data to identify animal health, performance, and welfare dynamics. Initially, the UTP will describe febrile responses, estrous patterns, environmental heat stress, and exertional distress.

Conclusions

Validation of the UTP will be addressed by the following goals: 1) Achieving predictive power and agreement of internal body temperature sensors with patterns of ambient temperature and traditional methods of body temperature measurement, 2) Achieving real-time data transmission at a variety of distances in free-ranging and captive animals, and 3) Designing and enhancing protocols for sensor development and integration, implementation for spatial and environmental-physiological response modeling. To provide agricultural context to the functions of the proposed UTP, we will view the data and processes described by the UTP through the scope of two critical animal agriculture phenomena-stress and disease.

Notes:

P026 - Improving detection of digital dermatitis in beef cattle using computer vision and portable devices

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Session: Diagnostic testing/disease modeling/precision agriculture, Dec. 6, 6:00 - 8:00 PM

Objective

Digital Dermatitis (DD) is an infectious claw disease that affects beef and dairy cattle feet and causes lameness worldwide. As DD is infectious, it can spread quickly within a herd and becomes increasingly difficult to manage, especially with intermingling exposure of beef cattle in a feedlot. Computer vision (CV) models can be used for real-time detection of DD, but improvements are needed to create more portable devices and increase utility for on-farm use. The aim of the study is to develop a YOLOv4-tiny model that can be used to quantify DD prevalence in feedlot beef cattle.

Methods

With a database of 1,832 images, a model was trained to classify 3 M-stages of DD (M0, M2, and M4) using YOLOv4-tiny architecture and Darknet framework. Different deployment strategies were tested using a Raspberry Pi microcomputer and laptop computer to run the CV model in real-time with a wireless camera. A treatment list was created by combining DD classification with radio frequency cattle identification (RFID) signals and manual input of identification.

Results

The YOLOv4-tiny DD model detected with a mean average precision (mAP) of 66.03%. Internal validation to quantify the agreement between the CV and human evaluator results in a Cohen's kappa of 0.5466, which quantifies as 'moderate' agreement. Real-time object detection (external validation) was used on-farm with unknown hooves and achieved a Cohen's kappa of 0.4454, which quantifies as 'moderate' agreement. Streaming video wirelessly from a cellular phone and running the model on a laptop computer was presented as a usable framework for real-time detection DD at 15-20 frames per second.

Conclusions

Computer vision is a growing field in veterinary medicine that helps clients monitor and measure traits of interest. Our improved CV model will allow better identification of beef cattle with DD to receive prompt treatment and therefore has the potential to decrease DD prevalence in feedlots.

Financial Support
University of Wisconsin; USDA NIFA Animal Health

Notes:

P027 - Comparative analysis of RT-QuIC, ELISA, and immunohistochemistry for chronic wasting disease diagnostics

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Session: Diagnostic testing/disease modeling/precision agriculture, Dec. 6, 6:00 - 8:00 PM

Objective

Chronic wasting disease (CWD) is a prion disease affecting free-ranging and captive cervids. The approved diagnostic methods for CWD in United States relies on demonstration of the misfolded CWD prion protein (PrP^{CWD}) in the brain or retropharyngeal lymph nodes (RLNs) by ELISA and/or immunohistochemistry (IHC). Real-time quaking-induced conversion (RT-QuIC) assay offers a newer, high-throughput approach, that can detect minute amounts of prions in tissues and secretions of CWD-infected animals. In the current study, ELISA, IHC and RT-QuIC were evaluated on tissues collected from white-tailed deer in Michigan.

Methods

RLNs were collected from a total of 1300 animals at necropsy, during routine CWD surveillance in Michigan from 2017 to 2021. Samples were screened for evidence of PrP^{CWD} by ELISA and all ELISA-positive RLNs (n= 171) were also analyzed by IHC. A standardized RT-QuIC assay was performed on all 1300 RLNs using the recombinant Syrian hamster prion protein (residues 90–231) as a substrate. RT-QuIC results were compared against those obtained by conventional ELISA and IHC.

Results

ELISA detected 184 RLNs positive for PrP^{CWD} of 1300 samples tested. From the 184 ELISA positive RLNs, 176 were also IHC positive for CWD. RT-QuIC assay detected PrP^{CWD} in 178 RLNs (of the 1300 samples tested). There were six discordant results when comparing ELISA and RT-QuIC results. Of the six discordant results, all were ELISA positive and RT-QuIC negative. IHC analyses revealed 5 of the 6 samples matched the RT-QuIC outcomes. When comparing RT-QuIC with IHC, one sample did not have matching results between the two techniques (negative by IHC, but positive by RT-QuIC). All the negative ELISA samples were also negative by RT-QuIC.The percentage of agreement between ELISA and RT-QuIC, and between IHC and RT-QuIC was 99.5 % and 99.7 %, respectively, and the *kappa* value 0.98 and 0.99.

Conclusions

Taken together, RT-QuIC is comparable to ELISA and IHC for CWD detection. Further work is needed to validate the assay assess RT-QuIC for the early-stage CWD diagnosis, as well as CWD strain differentiation.

Financial Support
Michigan Department of Natural Resources

Notes:

P028 - Alternative expression cassettes generate affordable and stable mRNA vaccines for animals.

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Session: Diagnostic testing/disease modeling/precision agriculture, Dec. 6, 6:00 - 8:00 PM

Objective

mRNA vaccines, as demonstrated by Sars-CoV-2 vaccines, have shown significant efficacy against viral pathogens in humans. However, manufacture of these types of vaccines are very labor intensive and quite costly to generate. Moreover, these vaccines are not highly efficient in entering cells despite lipid nanoparticle delivery, require costly 5' methyl caps, expensive modified nucleotides, and additional enzymes to attach a stabilizing 3' poly(A) tail. Couple these issues with the additional hurdle of deep cold chain storage required by these types of vaccines and use for vaccinating production animals is impractical. Redesign of mRNA vaccines that could overcome these issues would be highly favorable to future vaccins for production animals.

Methods

We have developed alternative expression cassettes that require none of the typical costly modifications to mRNA. Our expression cassettes are based on plant and insect viruses where RNA structures overcomes in vitro transcribed RNA modifications previously mentioned. Furthermore, we have developed techniques to generate mRNA and package them into exosomes for delivery. Finally, incorporation of our mRNA constructs into polyanhydride nanoparticles or rods instills heat resistant characteristics and eliminates the need to be kept in cold storage.

Results

Immunization tests using influenza HA or bovine RSV prefusion F demonstrate efficacy in test animals. Animals exhibited strong antibody responses to the vaccines. In addtion, we found the antibody responses to be diverse thus capable of neutralizing multiple strains of influenza from groups 1 and 2.

Conclusions

Our vaccine expression cassettes represent an opportunity to use mRNA vaccine technology in production animals and realize the benefits of these vaccines for the myriad of animal viral diseases. Further optimization could further lower costs below the predicted costs of $0.58 a dose.

Notes:

P029 - Cross-sectional study of sample characteristics and PCR result associated with successful Senecavirus A isolation

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Session: Diagnostic testing/disease modeling/precision agriculture, Dec. 6, 6:00 - 8:00 PM

Objective

The aim of this study was to evaluate the association between successful Senecavirus A (SVA) isolation and characteristics of each sample at the California Animal Health and Food Safety (CAHFS) Laboratory. At the beginning of the SVA outbreak, virus isolation (VI) was performed followed by real-time PCR, in order to grow sufficient virus for follow-up research. However, some samples grew successfully in cell culture while others did not. Therefore, the purpose of this study was to evaluate field and laboratory factors that are significantly associated with successful SVA isolation.

Methods

The data was obtained from the CAHFS laboratory from 2017 to 2019. A total 719 samples from California were used in this analysis. A cross-sectional study design was used as the time points were coded, therefore prevalence odds ratio (PR) was determined. Data storage and analysis were performed by RStudio (Version 1.4.1103) and Microsoft Excel (Version 2109 Build 16.0.14430.20224). Logistic regression was used to evaluate the significant association between variables and the outcome.

Results

In the final model, pooled PCR results, sampling sites (coronary and oral), and interaction due to pooled coronary and oral samples were chosen as independent variables. Using each coronary samples (PR=2.7 (95%CI: 1.6-4.5), p = 0.0001) and oral samples (PR=3.1 (95%CI: 1.7-5.5), p = 0.0002) were significantly associated with successful VI. However, using both types of samples reduced the odds of successful VI (PR=0.3 (95%CI: 0.1-0.8), p = 0.02). Pooled PCR result (p >0.05), on the other hand, was not significantly associated. The area under the ROC curve (AUC) was 0.57 (95% CI: 0.53-0.62).

Conclusions

Sampling from the coronary and oral sites were positively associated with successful VI. However, pooling two types of samples was negatively associated with successful VI. Pooling samples from different sites might dilute the virus concentration, leading to the failure of detectable virus growth. The final model in this study can moderately predict the outcome. However, further studies are needed to establish causality.

Notes:

P030 - Distribution of cattle brucellosis in various regions of Armenia in 2020

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Session: Epidemiology and/or public health, Dec. 6, 6:00 - 8:00 PM

Objective

Cattle brucellosis one of the infectious diseases of livestock in Armenia that registered every year nearly in all regions of the country and have serious impact of livestock breeding sector in country. The main goal of the current study is to identify the distribution of cattle brucellosis in various regions depending on number of cattle population and brucellosis cases and prevalence.

Methods

The data for analysis were taken from official sources of the Food Safety Inspection Body of the Republic of Armenia for all 10 regions. The data about cattle population have been taken from State Statistic Agency of the republic of Armenia.

Results

During 2020, a total of 1,924 brucellosis cases among cattle were registered in Armenia (912 cases in spring and 1,012 cases in fall). Most cases were registered in the region of Aragatsotn (450), followed by Gegharkunik (353), Kotayk (280), Shirak (263) and Tavush (4). The 4 regions with the highest cases accounts for 69.9% of all brucellosis cases in Armenia and house 45% of the cattle population.
The brucellosis prevalence for the entire country is 0.35% (n=541,772). Regions with higher total prevalence: Kotayk (0.71%), Aragatsotn (0.59%), Ararat (0.53%), and Gegharkunik (0.36). The Tavush region has the lowest prevalence at 0.01%.

Conclusions

In 2020, while there were more cases of brucellosis in the Aragatsotn region (450) we found a higher prevalence in the Kotayk (0.71%) region. Three regions in Armenia (Aragatsotn, Kotayk and Gegharkunik) have more case numbers and prevalence of cattle brucellosis which corresponds to the regions where pastures are widely used in cattle breeding systems. The pasture system allows increased direct and indirect contact with other cattle, sheep and goats. In total, 69.9% of all brucellosis cases in (2020) are in four regions which maintain 45% of total population of cattle. These regions should be considered as a high-risk area for cattle brucellosis in Armenia. These results will allow us to modify and improve brucellosis control strategies depending on location.

Notes:

P031 - Associations and predictability of feedlot pen factors on bovine respiratory disease in the first 45 days on feed

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Session: Epidemiology and/or public health, Dec. 6, 6:00 - 8:00 PM

Objective

Bovine respiratory disease (BRD) is the leading cause of morbidity in feedlot cattle. Research has identified risk factors associated with BRD; yet, knowledge gaps remain. Our objective was to evaluate the associations and predictive ability of pen-level factors towards BRD morbidity risk in the first 45 days on feed (DOF). These factors include: pen/bunk space available per head, total and shared waters sources between pens, and number of neighboring pens.

Methods

Pen factors were combined with retrospective data from 10 U.S. feedlots which included sex, arrival weight, total received head, and arrival date quarter. Generalized linear mixed models were used to evaluate potential associations between the factors of interest, cattle demographics, and BRD morbidity in the first 45 DOF. Five predictive classification models were trained to predict BRD risk of incoming cattle groups. Groups were classified into high (≥ 15% BRD morbidity 45 DOF) or low (<15%) risk classes. Each model's predictive ability was evaluated with receiver operating characteristic curves and area under the curve (AUC).

Results

Pen area per head, bunk space per head, number of neighboring pens, and total number of water sources, and their interactions with cattle demographics, were significantly associated with BRD morbidity in the first 45 DOF ($P < 0.05$). For example, heavy cattle with pen area per head greater than 32.51 sq. m had lower BRD morbidity compared to those with less available pen space. Accuracy in discriminating BRD risk between predictive models ranged from 10.9% to 79.4%, and AUC ranged from .682 to .789. A random forest (RF) model performed best with an AUC of .789, correctly identifying 42 of 47 high-risk cohorts and 233 of 386 low-risk cohorts.

Conclusions

The pen management factors investigated were statistically significant, but only pen area per head displayed meaningful differences that may potentially impact BRD morbidity risk. The RF model was able to correctly classify high-risk groups that were truly high-risk, but were poor at correctly predicting low-risk groups that were truly low-risk.
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 for Food and Agriculture

Notes:

P033 - Seroprevalence of severe fever with thrombocytopenia syndrome virus among companion, farm and wild animals in the Republic of Korea

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Session: Epidemiology and/or public health, Dec. 6, 6:00 - 8:00 PM

Objective

Severe fever with thrombocytopenia syndrome (SFTS) is caused by *Dabie bandavirus* and it has been found in Asian countries including China, Japan, Korea, Vietnam, Taiwan, Thailand, Pakistan, and Myanmar. SFTS virus (SFTSV) has been detected from various animals and humans. The aim of this study was to detect antibody of SFTSV from animals such as dogs, cats, cattle, goats, horses, wild boar, Korean water deer, roe deer, chickens, ducks, geese, and wild geese in the Republic of Korea (ROK).

Methods

From 2018 to 2020, 1,056 sera from dogs, 325 sera from cats, 841 sera from cattle, 988 sera from goats, 787 sera from horses, 768 sera from wild boar, 225 sera from Korean water deer, 23 sera from roe deer, 296 sera from chickens, 246 sera from ducks, 3 sera from geese, and 26 sera from wild geese were collected in the ROK. For detection of SFTSV antibody, Enzyme-linked immunosorbent assay (ELISA) was performed with above sera to detect SFTSV specific antibodies. The optimal density was measured at 450 nm using a microplate reader.

Results

Of a total of 5,584 sera, 229 (21.7%) sera in dogs, 25 (7.7%) sera in cats, 194 (23.1%) sera in cattle, 88 (8.9%) sera in goats, 183 (23.3%) sera in horses, 221 (28.8%) sera in wild boar, 47 (20.9%) sera in Korean water deer, 6 (26.1%) sera in roe deer, 84 (28.4%) sera in chickens, 81 (32.9%) sera in ducks were seropositive for SFTSV using ELISA. SFTSV antibodies were not detected in geese and wild geese sera.

Conclusions

The result of this study indicates that there is a possibility on transmission or exposure evidence of SFTSV in various species of animals. Thus, it is necessary to consider the strategies of prevention against SFTSV infection among these animals.

Financial Support
Government-wide R&D Fund for Infectious Diseases Research (HG18C0021)

Notes:

P034 - Molecular prevalence of severe fever with thrombocytopenia syndrome virus in companion, farm and wild animals from the Republic of Korea

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Session: Epidemiology and/or public health, Dec. 6, 6:00 - 8:00 PM

Objective

Severe fever with thrombocytopenia syndrome (SFTS) is viral infectious disease caused by SFTS virus (SFTSV), and ticks are thought to be a potential vector of this virus. This study was carried out to investigate the prevalence of SFTSV in companion, farm, and wild animals from the Republic of Korea (ROK).

Methods

In order to investigate the SFTSV infection in animals, sera were collected from a total of 86 animal species (2 companion animals, 7 industrial animals, 77 wild animals) and total 7,700 animals from environments exposed to ticks from 2018 to 2020. Viral RNA was extracted from sera using viral RNA extraction kit and one-step RT-nested PCR was performed to amplify the S segment of the SFTSV. The sequence data were analyzed using Chromas and were aligned using CLUSTAL X. The phylogenetic analysis was constructed using the maximum-likelihood method using MEGA7.

Results

Antigen infection rate of SFTSV was investigated, 14 animal species, 172 animals were positive and the total infection rate was 2.2%. Sera were collected from a total of 1,532 companion animals, and the SFTSV infection rate was 1.1% (17/1,532). Sera were collected from a total of 3,719 farm animals, and the overall infection rate was 2.4% (88/3,719). Sera were collected from a total of 2,449 wild animals, SFTSV was detected in 5 of the animal species, and the overall infection rate was 2.7% (67/2,449). The annual infection rate of SFTSV is showing an increasing trend of 0.8% in 2018, 2.2% in 2019, and 3.2% in 2020.

Conclusions

This data confirmed the detection of SFTSV in companion, farm and wild animals in the ROK, and epidemiological investigations from more various wild animal species should be evaluated. Therefore, since zoonotic SFTSV has been detected in 14 animal species, secondary infection from animals to humans should be managed in public health.

Finanical Support
Government-wide R&D Fund for Infectious Diseases Research (HG18C0021)

Notes:

P035 - Seroprevalence of Crimean-Congo hemorrhagic fever virus infections in humans and livestock in West Africa

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Session: Epidemiology and/or public health, Dec. 6, 6:00 - 8:00 PM

Objective

Crimean-Congo hemorrhagic fever virus (CCHFV), the causative agent of Crimean-Congo hemorrhagic fever (CCHF), is a zoonotic, tick-borne pathogen endemic in parts of Africa, Asia, Middle East and Europe. CCHF outbreaks pose a significant threat to public health with a high case fatality ratio of up to 40%. Treatment is primarily supportive and there is no vaccine available. This study aimed to investigate the seroprevalence and seroepidemiology of CCHFV in at-risk populations in endemic settings of West Africa.

Methods

A convenience sample of 486 serum samples were collected from both healthy and febrile participants between August 2010 and March 2018 from three major regions in Nigeria and tested for the presence of CCHFV antibodies against the viral nucleocapsid protein using a validated dual antigen enzyme linked immunosorbent assay (DA ELISA) kit. In a related ongoing study to investigate the risk of zoonotic transmission of CCHF, serum samples were collected from sheep (n = 437) and goats (n = 583) in environmental settings of close human-livestock interactions at various locations in The Gambia/West Africa. The samples are similarly being tested for antibodies against CCHFV.

Results

Of the 486 human serum samples collected from individuals in Nigeria, 8 tested positive for CCHFV antibodies indicating a seroprevalence of 1.65%. Risk factors for CCHF infection included age, gender, and febrile illness, with 50% of the CCHFV antibody positive individuals within an older age range of 40 – 80 years, 62.5% representing female and 87.5% presenting with fever.

Conclusions

Our results support previous epidemiological studies conducted in Nigeria indicating serological evidence and possible endemicity of CCHFV infection within the country; and also suggest that manifestation of febrile symptoms may be associated with CCHFV infection in endemic settings. Conclusions of our CCHFV seroprevalence studies in The Gambia will be reported once the studies are completed.

Notes:

P037 - Assessing gastropods as parasite vectors: Reducing risks on Maine farms

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Session: Epidemiology and/or public health, Dec. 6, 6:00 - 8:00 PM

Objective

Gastropods can act as intermediate hosts and vectors of important parasites of livestock and people (e.g. liver flukes [*Fasciola spp*.] rat lungworm [*Angiostrongylus cantonensis*] and meningeal worm [*Parelaphostrongylus tenuis; P.tenuis*]). In areas where wildlife habitat overlaps with livestock grazing areas, risk for gastropod-born parasite transmission increases. The purpose of this research is to identify the diversity of parasites within gastropods across agricultural landscapes in Maine, create a likelihood model for gastropod-borne parasite transmission risk, and develop preventive management strategies to reduce risk of gastropod- borne parasite transmission to livestock.

Methods

Following a survey to assess awareness of gastropod-borne parasitic risk to small ruminants, we designed a study of farm-specific risks using six farms in Maine using rotational or continuous pasture systems. Field-collected gastropods were digested in the laboratory using pepsin and evaluated for the presence of helminth larvae and evidence of environmental microplastics. Field-collected fecal samples from white-tailed deer (*Odocoileus virginianus; P.tenuis* definitive host), were evaluated using a Baermann technique for the presence of helminth larvae. Morphology was used to classify larvae as to stage and genus, and a subsample of larvae were used for DNA extraction, PCR using an ITS-2 rDNA primer set, and sequencing. Ecological observations, collected over multiple visits to each farm, were compared to gastropod and parasite data. Interventions, including mowing and use of pastured poultry, were studied as gastropod mitigation measures. Incidental livestock mortalities were evaluated for likelihood of *P. tenuis* infection.

Results

Preliminary results evaluating over 1300 gastropods suggest that *P. tenuis* prevalence is consistent with the literature (~4%).

Conclusions

We can conclude that gastropod-borne parasites are present on the Maine farms studied, and that parasite- related health issues are likely to have economic and management consequences for farmers, wildlife managers, and public health officials.

Notes:

P040 - Modeling swine movement patterns and disease surveillance at the U.S. national scale

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Session: Epidemiology and/or public health, Dec. 6, 6:00 - 8:00 PM

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 results for USAMM-Swine to provide the first predictions of the numbers and sizes of swine shipments at the national scale incorporating data from Interstate Certificates of Veterinary Inspection and commuter agreements. 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

Notes:

P041 - Assessing biosecurity knowledge and practices among Illinois swine producers

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Session: Epidemiology and/or public health, Dec. 6, 6:00 - 8:00 PM

Objective

There is a growing risk to the health and productivity of the Illinois and US swine population from foreign (e.g., African swine fever) and endemic (e.g., porcine reproductive and respiratory syndrome) infectious diseases. Effective on-farm biosecurity practices play a pivotal role in preventing these high-consequence pathogens from affecting swine farms. However, there is a gap in existing literature on biosecurity knowledge, awareness, and practices of Illinois swine producers. Additional research is warranted to identify these knowledge gaps and to assess Illinois swine producers' preparedness for a potential foreign animal disease outbreak. Focusing on this objective, our study assesses the knowledge and perception of Illinois swine producers related to foreign and endemic diseases and evaluates the biosecurity practices implemented on their hog farms.

Methods

An online questionnaire was designed using Qualtrics^{XM} software and was sent out via email to 406 swine producers that were registered with the Illinois Pork Producers Association.

Results

In total, 17 swine producers opened the questionnaire, out of which 13 producers (3.2 % of total producers) completed the survey and were included in this study. These 13 producers owned a total of 82 swine farms, distributed across Illinois. The preliminary analysis revealed that the most common business arrangement was the independent hog producer (54%) followed by contract hog producer (31%) and contractor/ integrator (15%). More than half of the producers (62%) either had a Secure Pork Supply Plan or have had a biosecurity assessment of their farms. Almost all swine producers (92.3%) reported that prevention of disease is the most cost-effective practice, and 85% of them spent between $2-15 per hog annually for disease prevention and control methods on their farms.

Conclusions

Despite some biosecurity awareness among responders, the need for a biosecurity-related outreach program was evident from the survey results.

Financial Support
U.S. Department of Agriculture, Animal and Plant Health Inspection Services

Notes:

P042 - West Nile virus in companion animals, susceptibility and epidemiological role

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Session: Epidemiology and/or public health, Dec. 6, 6:00 - 8:00 PM

Objective

West Nile virus (WNV) is a Flavivirus, normally circulating in a mosquito–bird–mosquito cycle. WNV currently circulates in Africa, Europe, Asia and Australia. In 1999, the first North American human case of WNV was reported in New York, then and then the virus spread in Canada, Latin America and the Caribbean. Companion animals have also been shown to be susceptible, having disease and eventually die, being able to transmit WNV to other species, including humans. This study aims to review the role of companion animals in the dynamics of WNV spreading, as well as their possible role as sentinels for WNV circulation.

Methods

A comprehensive review of scientific literature on WNV in companion animals was performed on the major literature search engines. Companion animals included were dog, cat, rabbit, hamster, guinea pig, laboratory mice/rats, squirrels, geese, chickens, pigeons, ducks, doves, lemurs and lake frogs.

Results

Most vertebrate hosts are susceptible to WNV infection, however, little is known about clinical manifestations on non-conventional pets, such as reptiles, amphibians and other mammals. Indeed, lemurs and lake frogs may develop suitable viraemia levels to support arthropod – borne transmission. Dogs and cats have been shown to be susceptible to WNV infection, showing also high seroprevalences, without developing clinical disease. Most companion animals, however, develop very low or ephemeral viraemia, insufficient for infecting mosquitoes to complete the transmission cycle.

Conclusions

Considering the higher seroprevalence in cats and dogs and the fact they do not develop sufficient viraemia to infect the vector, this may suggest that they could play a role as indicators of WNV circulation in their living area and in humans sharing the same environment. More attention should be given to non-conventional pets susceptible to WNV, including birds, mammals, amphibians and reptiles, given that little is known about the clinical manifestation on these species. More investigations are needed to better understand the role played by some companion animal species in WNV epidemiology.

Notes:

P045 - Senecavirus A in the environment of sow slaughter plants

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Session: Epidemiology and/or public health, Dec. 6, 6:00 - 8:00 PM

Objective

Vesicular disease caused by Senecavirus A (SVA) is clinically indistinguishable from other vesicular diseases of swine. A foreign animal disease investigation (FADI) is required every time a vesicular lesion is observed. Since 2015 there has been an increase in the number of FADIs and SVA positive samples at slaughter plants in the U.S. The objectives of this study were to: 1) understand the environmental burden of SVA in select sow slaughter plants, 2) correlate PCR, VI and swine bioassay results, and 3) sequence 2020 SVA isolates for comparison to previously sequenced SVA isolates.

Methods

Environmental swabs were collected from four sow slaughter plants (Plants 1-4) from June to December 2020. All swab samples were tested for SVA by PCR and virus isolation (VI). Eighteen samples of various Ct values were selected to inoculate individually housed pigs (n=2/sample). Fecal and oral swabs and serum samples were tested for SVA by PCR to determine infectivity to swine. Finally, two isolates from Plants 1-3 (n=6 total) were selected for next-generation sequencing and analysis.

Results

SVA PCR positive samples were consistently found at Plants 1-3, while Plant 4 had no positive samples. The greatest percentage of SVA positive samples was found in the summer. Among 450 samples taken from Plants 1-3, 308 samples were PCR positive. Of the samples with a Ct value below 30, 76.5% were VI positive, while only 11.2% of samples with a Ct value greater than 32 were VI positive. Samples for the swine bioassay had Ct values ranging from 24 to 33 and only one sample (Ct value = 24) infected pigs and had detectable nucleic acid in samples collected. Genome sequences of the 2020 isolates generally clustered together and with other contemporary US isolates.

Conclusions

The work in this study demonstrated that SVA is in the environment of sow slaughter plants, but the virus detected may not be likely to be infectious to swine. A better understanding of the epidemiology of SVA in the marketing chain may help reduce the number of FADIs and aide in the development of control measures to reduce the spread of SVA.

Notes:

P046 - Swine influenza virus and porcine reproductive and respiratory syndrome virus antibodies in market pigs in Uganda

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Session: Epidemiology and/or public health, Dec. 6, 6:00 - 8:00 PM

Objective

Swine influenza virus (SIV) is a type A influenza virus that causes an acute and highly contagious respiratory disease in pigs, while porcine reproductive and respiratory syndrome virus (PRRSV) is an enveloped RNA virus that leads to mortality and reproductive failure in sows, and respiratory disease in weaned and growing pigs. These two viruses can cause clinical signs and lesions like those caused by African swine fever virus (ASFV) and little is known about their occurrence in Uganda which is an African swine fever (ASF) endemic country. The objective of this study is to determine the seroprevalence of SIV and PRRSV in market pigs in Uganda.

Methods

At least 1200 pigs will be sampled from six abattoirs (Wambizzi, Lusanja, Budo, Katabi, Buwate and Kyetume) over a 13-month period (May 2021- June 2022). So far, 276 serum samples have been analyzed for the presence of antibodies against SIV and PRRSV using the Ingenasa's SIV and PRRS indirect ELISAs respectively. For each pathogen, the ELISA positivity rate and 95% confidence interval was calculated.

Results

Of the 276 pig serum samples analyzed, 241 (87.3%, 95%CI: 82.84%, 90.77%) had antibodies against SIV and six (2.2%, 95%CI: 0.89%, 4.77%) had antibodies against PRRSV. One of the pigs that was seropositive for SIV was also seropositive to ASFV. Another pig that was seronegative to ASFV, was seropositive to PRRSV, and had hemorrhages in the skin of ears, legs, and abdomen, markedly enlarged and darkened spleen, enlarged and diffusely hemorrhagic gastro-hepatic, renal and mesenteric lymph nodes which are also clinical signs and lesions observed in ASF. Active infection by ASFV cannot be ruled out in this pig until further testing using qPCR is completed.

Conclusions

Early data suggests a high-level of exposure to SIV and low exposure to PRRSV in pigs in Uganda. The presence of these viruses in Uganda could make early diagnosis of ASF difficult because in the early stages of ASF, the clinical signs and lesions maybe non-specific and similar to those observed in infections with these viruses.

Financial Support
U.S. Defense Threat Reduction Agency

Notes:

P047 - Prevalence of SARS-CoV-2 antibodies in companion dogs and cats, USA

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Session: Epidemiology and/or public health, Dec. 6, 6:00 - 8:00 PM

Objective

This study aims to provide data on the exposure of dogs and cats from around the USA to SARS-CoV-2.

Methods

In this study, we tested convenience samples of sera from dogs (n=1336) and cats (n=956) across 48 states of the USA in 2020. We screened the sera with a commercial double antigen sandwich ELISA kit, and then selected samples were further tested with a commercial sVNT and a classical VNT method.

Results

An ELISA targeting the antibody against nucleocapsid identified eleven positive and two doubtful samples in cats and five positive and five doubtful samples in dogs. A surrogate neutralization assay detecting antibodies blocking the attachment of the spike protein to ACE2 was positive with three of the ELISA positive and doubtful samples and one of 463 randomly selected ELISA negative samples. These four positive samples were further confirmed using SARS-CoV-2 virus neutralization testing. All positive samples were from cats in New York ((n=1), Florida (n=1), and New Jersey (n=2).

Conclusions

The serosurvey results, one of the largest yet completed on dogs and cats globally, indicate that dogs and cats in the U.S. appear to be infrequently infected with SARS CoV-2, and this supports other evidence that companion animals are not a significant source of human infection. Companion animals provide multifaceted health benefits to their owners, including increased emotional well-being, significant stress reduction, and increased physical activity. Such benefits are particularly important to senior citizens during isolation and stress induced by the COVID-19 pandemic. Media should thus be encouraged to refrain from emotive reporting on the role of pets in COVID-19. The current OIE, CDC, and AVMA recommendations relating to companion animal infections should be followed closely. Companion animal veterinarians need to be alerted to the possibility, albeit low, of SARS-CoV-2 in their patients and the most appropriate treatments and methods to prevent the spread of infection to other animals and people in the household.

Notes:

P049 - Proteomic analysis of native *Mycobacterium avium* subspecies *paratuberculosis* membrane vesicles

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Session: Host or pathogen genomics or proteomics, Dec. 6, 6:00 - 8:00 PM

Objective

Nanosized membrane vesicles (MVs) derived from Gram-positive bacteria as one of their strategies for manipulating their host immune response and increasing their survivability during infection have been considered an excellent candidate in vaccine development and pathogenesis study. To explore the roles of MVs in *Mycobacterium avium* subspecies *paratuberculosis* (MAP) in infection, MVs released from a virulent strain MAP K-10 were analyzed by tandem mass spectrometry techniques (TEM) for protein composition analysis.

Methods

MAP K-10, an isolated bovine strain, was cultured in minimal medium (KH_2PO_4 1g/L, Na_2HPO_4 2.5 g/L, asparagine 0.5 g/L, ferric ammonium citrate 50 mg/L, $MgSO_4 \cdot 7H_2O$ 0.5 g/L, $CaCl_2$ 0.5 mg/L, $ZnSO_4$ 0.1 mg/L, Mycobactin J 2 mg/L, Tyloxapol 0.05% (v/v), glycerol 0.1% (v/v), pH 7.0) for 6-8 weeks. Culture medium containing MVs from MAP K-10 were collected by centrifugation and filtration with PVDF membrane. Ultracentrifugation (100,000 xg, 2h, 4°C) was followed by gradient ultracentrifugation (100,000 xg, 16h, 4°) for MVs purification and the purified MVs were suspended in PBS for the following TEM and proteomic analysis.

Results

Duplicate MVs preparations and cellular fractions from MAP K-10 were analyzed on TEM and mass spectrometry, with 317 overlapping proteins identified in both duplications. Most proteins in K-10 MVs are localized in the cytoplasm (65.0%), followed by plasma membrane (17.7%), unknown location (12.9%), extracellular (3.5%), and cell wall (0.9%). In K-10 MVs, 73 proteins (23%) were considered as virulent factors predicted by VirulentPred. Genome functional annotation revealed that most proteins equipped in MVs might involve in carbon and fatty acid metabolic pathways, antibiotic and amino acids biosynthesis, and fatty acid degradation.

Conclusions

We have successfully isolated from our MAP culture and identified protein composition. Future work to identify and illustrate the contribution of MVs to virulence and benefit for MAP infection in the dynamic environment of the host is needed.

Financial Support
Agriculture and Food Research Initiative

Notes:

P050 - Transcriptomics and genomic CRISPR knockout to delineate host factors involved in coronavirus transmission

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Session: Host or pathogen genomics or proteomics, Dec. 6, 6:00 - 8:00 PM

Objective

Porcine deltacoronavirus (PDCoV) has been identified as a potential zoonotic pathogen. Genomic technologies can rapidly identify factors mediating host species susceptibility to infection, allowing for the identification of potential therapeutic targets. Objectives of these studies are to identify critical host proteins for PDCoV infection in human and porcine cell lines, in addition to critical signaling pathways mediating infection through RNA-sequencing (RNAseq) and genomic CRISPR knockout (GeCKO) approaches.

Methods

Cell lines derived from intestinal lineages were used to reproduce primary sites of viral infection. Porcine intestinal epithelial cells (IPEC-J2) and human intestinal epithelial cells (HIEC) were infected with PDCoV. At 24 h post infection, total cellular RNA was harvested and analyzed using RNA-seq. HIEC/PDCoV RNA-seq data was compared to human bronchial epithelial cells (NHBE) /SARS-CoV-2 RNA-seq data[SL1] . Newborn pig trachea (NPTr) Cas9 expressing cells were assessed for PDCoV susceptibility for GeCKO knockout experiments.

Results

We identified 7,486 differentially expressed genes (DEGS) in HIEC and 1,134 DEGs in IPEC-J2 upon infection with PDCoV. These DEGs primarily resided in the NF-kappa-B transcription factor family, interferon (IFN) family, protein kinase family, and signaling pathways such as apoptosis, JAK-STAT, inflammation/cytokine, Toll-like receptor, NOD-like receptor, Ras, and cytosolic DNA-sensing pathways. Comparison to the betacoronavirus SARS-CoV-2 shows many similarities for coronavirus infections. In this case, we found that most pathways affected by PDCoV infection in HIEC cells were also affected by SARS-CoV-2 infection in NHBE cells. For our next experiments utilizing genomic CRISPR knockout, we have shown that NPTr cells are susceptible to PDCoV infection and can be utilized to create the porcine GeCKO library.

Conclusions

The newly discovered human zoonotic pathogen PDCoV elicits distinct transcriptional profiles in human and pig cells that have conservation between delta- and betacoronaviruses. NPTr cells are susceptible to PDCoV infection making them an appropriate host for screening porcine GeCKO libraries.

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

Notes:

P051 - Investigation of host genetic role in PCV2 and PRRSV infections

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Session: Host or pathogen genomics or proteomics, Dec. 6, 6:00 - 8:00 PM

Objective

PCV2 and PRRSV are etiological agents that impact production efficiency and can lead to mortality. Previous genome-wide association studies (GWAS) identified a common genomic region on chromosome 7 that influence susceptibility to PCV2 and PRRSV. The objective of this study was to fine map genomic regions, identify genes, DNA variants and pathways that could predict susceptibility to PCV2 and PRRSV.

Methods

Previous GWAS using Porcine SNP60 BeadArray (53,529 SNPs) identified a major genomic region located next to Swine Leukocyte Antigen Complex (SLA) associated with susceptibility to PCV2 (n=974) and PRRSV (n=167). Since the BeadArray had limited DNA markers located in this region, a custom genotyping array (SowPro91, 105,601 SNPs) was designed and used for genotyping a subset of the samples (n=215) representing the tails of the distribution for PCV2 viral load from the PCV2 dataset (n=974). A Bayesian method (BayesIM) that utilizes haplotypes instead of individual SNPs was used to fine map regions associated with PCV2 viral load. Haplotypes were assigned to individuals based on similarity using a hidden Markov model and used as covariates in GWAS.

Results

With the development of the SowPro91 array, there was an ~100X increase in the number of SNPs located in the SLA locus. The average distance between SNPs on SLAI and II was ~646bp, while across the chromosome the distance was ~10Kbp. Different BayesIM approaches that take in account haplotype size, SNP density, distance between SNPs, etc., were evaluated. In the extreme subset of samples, taking in account SNP density, the position of the QTL was dependent on the prior haplotype size (1.2 to 25 SNPs/haplotype). The QTL position became stable at a haplotype size of 15 SNPs/haplotype. The largest model frequency for the SSC7 QTL, an indicator of the QTL effect, was observed at a larger prior haplotype size (25 SNPs/haplotype). We expect that precision in genome assembly and improvement in gene annotation in the SLA region, accompanied by an increase in the size of the phenotype/genotype data will improve the consistency of the QTL location, providing potential genes and DNA variants associated with PCV2 but also PRRSV susceptibility.

Conclusions

This research aids in the development of mapping approaches to identify DNA variants associated with PCV2 and PRRSV susceptibility that could lead to improved health and welfare of pigs.

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

Notes:

P052 - Transmission bottlenecks during adaptation of human influenza A virus to pigs

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Session: Host or pathogen genomics or proteomics, Dec. 6, 6:00 - 8:00 PM

Objective

Influenza is a prevalent respiratory disease of swine, leading to significant losses in pork production. Transmission of seasonal human influenza viruses to swine is relatively frequent and has contributed to the genetic and antigenic diversity of influenza A viruses (IAV) circulating in pigs. However, only a subset of these human spillover viruses become established in the swine host. Our understanding of the necessary molecular features required for interspecies transmission and subsequent persistence of novel viruses is limited, but previous events appeared to be associated with mutation in surface proteins and reassortment to acquire endemic swine IAV lineage genes. The purpose of this study was to investigate the molecular factors and bottlenecks during the adaptation of IAV following human to swine transmission.

Methods

To evaluate within and between host evolution of human IAV in pigs, we will perform serial transmissions of a reassortant virus containing human seasonal HA and NA genes with a swine-origin backbone in pigs to mimic a wildtype event that led to the 2010.1 H3N2 lineage in swine. Low passage virus stocks were prepared, titrated, and sequenced for the reassortant human IAV and a swine-adapted control virus containing the same backbone. The infectious dose 50 (ID50) for each virus will be determined in a pilot study. Pigs will then be inoculated intranasally with 10 ID50 of each virus in sets of 3, each pig kept in a separate enclosure. Two days later, contact pigs will be introduced in each enclosure. The inoculated pigs will be euthanized, and new contacts introduced, following with 5 more consecutive transmissions. Nasal swabs will be collected and sequenced using high-throughput Illumina MiSeq platform.

Results

Sequence analysis will be performed and viral diversity within and between hosts quantified.

Conclusions

During these studies we will identify the molecular signatures that facilitated the emergence and transmission of human influenza viruses in swine.

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

Notes:

P053 - Amplicon-based whole genome sequencing of genotype II clinical African swine fever virus samples.

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Session: Host or pathogen genomics or proteomics, Dec. 6, 6:00 - 8:00 PM

Objective

African Swine Fever Virus (ASFV) causes a highly transmissible and fatal disease in pigs that is currently devastating global swine production. While originating in Africa, in 2007 a genotype II ASFV isolate was detected in the Caucasus and has subsequently spread throughout Europe and Asia, resulting in significant economic losses. Accurate reporting of ASFV genomic data collected from field isolates is essential to understand the genetic make-up of the virus causing ongoing ASF outbreaks, and to implement effective mitigation strategies to control its spread. Unfortunately, whole genome sequencing of ASFV from clinical samples is inherently difficult and costly, due to the large size of the genome (170-190 kb) and extensive host DNA contamination of the clinical samples. We therefore developed a simple and cost-effective method to sequence almost the entire ASFV genome from clinical samples.

Methods

Genomic amplification of ASFV clinical samples was performed in two separate reactions using tiled primers to amplify 19 different overlapping segments of the ASFV genome using a LongAmp Taq (NEB) polymerase. The amplicons were then subjected to library preparation and sequenced with barcoding using the following next generation sequencing methods: MiSeq/iSeq (Illumina) and nanopore MinION (Oxford Nanopore).

Results

Nearly the entire ASFV genome was successfully amplified and sequenced to a high depth of coverage using both next generation sequencing methods.

Conclusions

These data demonstrate that nearly the entire genome of an ASFV genotype II isolate can be successfully amplified from clinical samples using an efficient and cost-effective method. Moreover, the ASFV amplicons can be barcoded and sequenced using various next generation sequencing methods. These methodologies provide flexible and rapid procedures to improve the ASFV sequencing depth and at the same time mitigate issues with host genome contamination. This approach could therefore significantly improve the scale of ASFV whole genome sequencing to monitor this important agricultural disease and control its continued spread.

Notes:

P054 - Deconstructing the role of SYNGR2 in viral disease susceptibility in livestock

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Session: Host or pathogen genomics or proteomics, Dec. 6, 6:00 - 8:00 PM

Objective

Viral diseases pose a constant threat to the economic sustainability of livestock producers due to detrimental effects on animal growth and welfare. With the help of recent advancements in the capabilities of genomic analyses, we were able to identify a candidate gene (SYNGR2) and missense mutation (SYNGR p.Arg63Cys) associated with susceptibility to PCV2, a prevalent swine DNA virus. SYNGR2 has also been shown to promote replication of an human RNA virus (SFTSV), indicating this gene may influence a broad range of viral pathogens. The objective of this study was to validate and characterize the effects of SYNGR2 and its alleles on susceptibility to PCV2 and to other DNA and RNA viruses that infect livestock.

Methods

CRISPR-Cas9 gene editing was utilized to generate SYNGR2 knock-out (KO) and SYNGR2 p.Arg63Cys variant clones from two cell lines permissive to various viral pathogens, PK15 and Vero. Specifically, cells were transfected using a lipid-based delivery approach with fluorescently-tagged ribonucleotide (RNP) complexes comprised of guide RNA targeting the second exon of SYNGR2 and the Cas9 enzyme. To generate the SYNGR2 p.Arg63Cys variants, a ssDNA template encoding the 63Cys allele was simultaneously transfected into cells with the RNP complexes. Successfully transfected single cell clones were acquired by fluorescence-activated cell sorting and screened for desired editing events. PK15 cells and clones were seeded into 12 well plates and after 24 hours were infected with PCV2b inoculate. Cell and supernatant samples were collected at multiple time points post infection and viral copy number was quantified by qPCR specific for PCV2 Capsid DNA.

Results

We generated a PK15 KO clone (E1) homozygous for a 106bp deletion within the second exon of SYNGR2 predicted to cause a shift in the reading frame and a truncated protein product. Infection of PK15 cells and the E1 clone with PCV2b validated the involvement of SYNGR2 in facilitating viral proliferation as indicated by a significant reduction in viral copy number as early as 24 hours post infection in E1 compared to PK15 ($P<0.05$). Several heterozygous PK15 clones (63Arg/63Cys) and one clone heterozygous for the missense variant and an 11bp deletion (63Arg_Del/63Cys) have also been obtained. In order to evaluate the effects of SYNGR2 on other viral pathogens besides PCV2, we generated a Vero clone (F6) homozygous for a 111bp in-frame deletion within the second exon of SYNGR2. However, upon confirming the KO status of this clone at the RNA level, other SYNGR2 isoforms not observed in parental cells were detected, one missing exon 2 and another missing exons 2 and 3. Whether the presence of these alternate isoforms in the F6 clone is due to compensatory expression or an increased ability to capture them is not yet clear.

Conclusions

This research will generate knowledge that could be applied to improve animal health and welfare as well as reduce economic losses.

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

Notes:

P055 - Whole genome sequencing for genomic epidemiological studies of tuberculosis in Asian elephants of Nepal

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Session: Host or pathogen genomics or proteomics, Dec. 6, 6:00 - 8:00 PM

Objective

Tuberculosis (TB) in elephants is caused by *Mycobacterium tuberculosis (M.tb)* and sometimes by *M. bovis*. TB has also been confirmed in both captive and free ranging elephants across Asia and Africa which threatens their survival. The objective of this study is to establish a pipeline for genomic epidemiology that will enable understanding of origins of infecting strains, their diversity within these populations, and defining the role of interspecies transmission that endangers this species in captivity and the wild.

Methods

M.tb complex bacteria were isolated from two animals from lung tissues at necropsy. Both isolates were confirmed as *Mycobacterium tuberculosis* by IS*6110*-RD4 multiplex PCR, *gyrB* sequencing, spoligotyping and multi-locus variable number of tandem repeat analysis (MLVA). Whole genome sequencing was undertaken on the two *M. tb* isolates using Illumina NovaSeq and *de novo* genome assembly was conducted using shovill and annotated using PGAP. Comparative genomic analysis was performed for comparisons across the full extent of diversity described for *M.tb* to establish potential sources of infection in these animals.

Results

Whole genome analyses revealed that the total sequence length of strain S1 genome is 4,475,258bp, with a GC content of 65.57%. Similarly, the total sequence length of strain S3 genome is 4,398,381bp with a GC content of 65.59%. Sequencing returned high genomic coverage (359x for isolate S1, 385x for isolate S3). The SNP barcoding of the two isolates contained SNPs supporting an unusual inconclusive lineage of sublineage 1.2.2. and sublineage 4.9. Sublineage 4.9, close to *M.tb* H37Rv, has not yet been reported in humans or elephant populations in Nepal which may lead to speculate that SNP-based barcode may not be suitable to classify the clinical isolates in animal species.

Conclusions

This is first study of *M. tb* genomes from endangered Asian elephants in Nepal. It is expected that whole genome sequencing of TB isolates from Asian elephants will allow us to better understand the genomic epidemiology including outbreak investigation and occurrence of interspecies transmission that will eventually help in the conservation of this important endangered species.

Notes:

P056 - How does analytic approach impact pathogen population structure when analyzing whole genome sequence data?

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Session: Host or pathogen genomics or proteomics, Dec. 6, 6:00 - 8:00 PM

Objective

Whole-genome sequencing is commonly performed for foodborne illness surveillance and outbreak detection. However, the bioinformatic pipelines used to analyze these data are not yet standardized. The overall objective was to quantitatively evaluate whether different analytic approaches to WGS analysis impact phylogenetic results for isolates without a known epidemiological relationship. Secondary objective was to support an accurate, reproducible, and transparent approach to WGS analysis for outbreak investigations.

Methods

We used an *in silico* experiment to generate WGS datasets from the NCBI pathogen database for *Salmonella enterica*, *Escherichia*/Shigella, and *Listeria monocytogenes* isolates. Datasets represented the following metadata variables: sample type, sequence quality, geographical source, host species, and a random selection of genomes. Each dataset was analyzed using core- and pan-genomes, and each of the following approaches: SNP-based; k-mer-based; gene-by-gene allelic comparison, and a novel comparison of virulence factor domains. For each approach and dataset, phylogenetic trees were generated. Concordance and discordance in phylogenetic relatedness and cluster membership of outbreak genomes were evaluated.

Results

Multivariable modeling indicated that important WGS decision points (pipeline, core- or pan-genome, sample type, and host species) were significantly associated with differences in tree structure and shape. Nearly all pairwise tree comparisons exhibited large Robinson-Fould values, corresponding to large differences in tree topologies for most trees. Ongoing and near-future computing challenges in analyzing large genomic datasets were reported, including how they hamper WGS-based outbreak investigations.

Conclusions

The results of this project contribute to a deeper understanding of the limitations of current WGS analyses for foodborne outbreak detection, and demonstrate that different pipelines can produce discrepant results about relationships between a given set of pathogen isolates with unknown epidemiological links.

Financial Support
Foundation for Meat and Poultry Research and Education; Minnesota Agriculture Research, Education and Extension Technology Transfer Program

Notes:

P057 - Characterization of previously unannotated coding region of bovine herpesvirus 1

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Session: Host or pathogen genomics or proteomics, Dec. 6, 6:00 - 8:00 PM

Objective

Bovine Respiratory Disease (BRD) is a complex condition for the cattle industry due to the cost of treatment and extensive losses due to morbidity. BRD is caused by the combined effects of stress and the combined infection by one or more viral and bacterial agents. One virus of significance is Bovine Herpesvirus 1 (BoHV-1), which has the ability to cause lifelong recurrent infections that trigger BRD. This objective of this study is to further understand the complexities of the BoHV-1 genome by characterizing new coding sequences that may prove relevant to herpesvirus infection.

Methods

In our previous study, tandem mass spectrometry (MS/MS) was used to identify BoHV-1-encoded peptides that aligned to previously unannotated regions of the genome. Here, bioinformatic, RACE and RT-PCR analysis were utilized to characterize a transcript produced from the opposite strand of a coding region. Western blot and immunofluorescence were done using an antibody produced against the original MS/MS peptide.

Results

RACE and RT-PCR verified the presence of a transcript that potentially encodes the peptide discovered via MS/MS, since it overlaps this sequence. The potential new protein-coding gene is encoded in the negative strand, located antisense to the viral helicase gene. RNA expression kinetics indicate this is a late gene. Western blot and immunofluorescence verified the presence of a protein. Recombinant mutant viruses further aid in the study the protein's involvement in viral infection.

Conclusions

These results add further evidence to the recent consensus that viral genomes, especially large DNA genomes, have far more genomic complexity and coding potential than previously thought. As a result, previously unnoted peptides and small proteins could prove relevant in the search for vaccine or treatment targets.

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

Notes:

P059 - Hematological network analysis defines mechanisms associated with bovine respiratory disease and performance

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Session: Host or pathogen genomics or proteomics, Dec. 6, 6:00 - 8:00 PM

Objective

Co-analysis of hematological and gene expression changes would allow for advancements in clinical bovine respiratory disease (BRD) diagnostics and prediction. This study illustrates co-expressed networks of genes which correspond with changes in hematological and clinical traits.

Methods

RNA-Seq (NovaSeq 6000; ~45M reads/sample) and complete blood count analyses were performed on at-arrival blood samples from 23 beef cattle. Weighted gene co-expression network analysis (WGCNA) was utilized for expression network and module construction. 12,795 genes were constructed into co-expression modules according to signed Pearson correlations, with a minimum of 30 genes in each module, merging modules with intermodular correlations above 0.75, and assigning unique color identification. Module-trait Pearson correlations were considered weak or strong at $p<0.1$ and $|R|>0.3$ or $p<0.05$ and $|R|>0.4$, respectively. Cross-population module preservation was evaluated with GSE161396, utilizing Zsummary and medianRank cutoffs of >10 and <5, respectively. Preserved modules were evaluated for Gene Ontology (GO) and Reactome pathway enrichment, utilizing over-representation analysis within WebGestalt API (FDR<0.05).

Results

Five expression modules (“black”, “purple”, “lightgreen”, “tan”, and “steelblue”) were identified. Steelblue was strongly associated with clinical BRD, days-at-risk, and increased red blood cell concentration, and weakly associated with decreased neutrophil-lymphocyte ratio; steelblue enriched for antigen receptor-mediated signaling and alpha-beta T-cell receptor complexes. Purple was weakly associated with increased eosinophils and weight gain, but not BRD; purple enriched for aerobic metabolism, proteolysis, and rRNA binding.

Conclusions

These results illustrate associations between gene expression and hematological changes that underlie BRD development, and highlight co-expression modules which may independently influence BRD and weight gain outcomes.

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

Notes:

P060 - Early weaning stress shapes long-term immune responses in a sex-specific manner in pigs

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Session: Host or pathogen genomics or proteomics, Dec. 6, 6:00 - 8:00 PM

Objective

Two major factors known to influence immune function and mortality in animals and people are early life stress and biological sex. How these two factors interact to shape long-term immune development and later life disease risk is poorly understood. Here we conducted experiments in pigs to address the hypothesize that immune system development and subsequent immune responses to stressors later in life are shaped by both independent and interactive effects of early weaning and biological sex.

Methods

Ten-week-old female (F), intact-male (IM) and castrated-male (CM) pigs that were previously early-weaned (EW) and later-weaned (LW) (at 15 or 28 d of age, respectively), were intramuscularly injected with either saline vehicle, or lipopolysaccharide (LPS) to induce a systemic inflammatory response. Blood samples were obtained at 0h (basal), 2h, and 4h post LPS-challenge. Plasma cortisol and complete blood count were assayed to assess the systemic inflammatory and hypothalamic pituitary adrenal (HPA) axis response to LPS.

Results

Early weaning affected later life LPS-induced elevations in plasma cortisol levels only in EW-IM pigs which exhibited greater ($P<0.05$) cortisol levels compared with LW-IM pigs. Blood neutrophil to lymphocyte ratios (NLR), an index of physiologic stress, were greatest in EW-F pigs compared with other experimental groups and was due to increased neutrophil numbers. Early-weaned CM pigs exhibited higher NLR compared with EW-IM pigs. However, in contrast to EW-F, increased NLR in CM were due to decreased lymphocyte numbers which indicates that both early weaning and male gonadal status impact LPS-induced alterations in immune cell populations.

Conclusions

These studies show that early weaning, biological sex, and male gonadal status have significant impacts on later life LPS-induced HPA axis reactivity and immune responses. Understanding how immune development is altered by early life stress and sex could reveal new targets and strategies for optimizing lifetime immune function and stress and disease resilience.

Financial Support
U.S. Department of Agriculture; U.S. National Institutes of Health

Notes:

P061 - Tracing viral transmission and evolution of BLV through long read Oxford Nanopore sequencing of the proviral genome

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Session: Host or pathogen genomics or proteomics, Dec. 6, 6:00 - 8:00 PM

Objective

Bovine leukemia virus (BLV) causes Enzootic Bovine Leukosis (EBL), a persistent life-long disease resulting in immune dysfunction and shortened lifespan in infected cattle, severely impacting the profitability of the US dairy industry. Our group has found that 94% of dairy farms in the United States are infected with BLV with an average in-herd prevalence of 46%. This is partly due to the lack of clinical presentation during the early stages of primary infection and the elusive nature of BLV transmission.

Methods

This study sought to validate a near-complete genomic sequencing approach for reliability and accuracy before determining its efficacy in characterizing the sequence identity of BLV proviral genomes collected from a pilot study made up of 14 animals from one commercial dairy herd. These BLV-infected animals were comprised of seven adult dam/daughter pairs that tested positive by ELISA and qPCR.

Results

The results demonstrate sequence identity or divergence of the BLV genome from the same samples tested in two independent laboratories, suggesting both vertical and horizontal transmission in this dairy herd.

Conclusions

This study supports the use of Oxford Nanopore sequencing for the identification of viral SNPs that can be used for retrospective genetic contact tracing of BLV transmission.

Notes:

P062 - Proteomic analysis of outer membrane vesicles of Aeromonas hydrophila ML09-119

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Session: Host or pathogen genomics or proteomics, Dec. 6, 6:00 - 8:00 PM

Objective

Aeromonas hydrophila ML09-119 is an important fish pathogen that severely affects channel catfish aquaculture. To better understand this strain's virulence factors, outer membrane vesicles (OMVs) were isolated, and their proteome was assessed.

Methods

Using transmission electron microscopy and dynamic light scattering, OMVs were shown to be monodispersed particles with an average diameter of 120.33 nm. OMV proteins were identified using mass spectrometry, and analysis of the resulting proteome of 74 proteins revealed that many originated from the cytoplasm, but there was an enrichment of outer membrane, periplasmic, and extracellular proteins compared to the total proteome.

Results

The majority of the functional classifications were associated with bacterial metabolism. Of the predicted virulence factors, several had a putative function in adherence, and there were type III secretions system proteins as well as three secreted exotoxins.

Conclusions

Overall, our data reveal new insights into *A. hydrophila* OMVs and their potential roles in physiology and virulence.

Financial Support
U.S. Department of Agriculture

Notes:

P064 - Evaluating reference-free variant callers on metagenomic data

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Session: Host or pathogen genomics or proteomics, Dec. 6, 6:00 - 8:00 PM

Objective

Variant calling is often a fundamental step in genomic analyses. Despite this, variant calling remains problematic for metagenomic data due to a lack of callers specifically designed for it. Even the variant callers that *are* designed for metagenomic data either require the use of a reference database or haven't been adequately benchmarked against other callers. The goals of this project are to establish a list of relevant variant callers and to benchmark their resource usage and variant prediction accuracy on metagenomic data.

Methods

This ongoing project benchmarks many variant callers including well-established reference-based callers such as GATK and Samtools as well as newer and poorly evaluated reference-free callers designed for metagenomic data. In order to evaluate variant caller performance, we are using in silico-generated metagenomic datasets that have been injected with known SNPs. Additionally, we are varying several data "demographics", e.g., read depth and genome coverage, to evaluate their impacts on runtime, memory, and accuracy metrics for each variant caller.

Results

Popular tools such as GATK and Samtools exhibited low or inconsistent precision on bacterial WGS data. Strelka2 and BactSNP, however, showed high overall accuracy, but may worsen when used on mixed bacterial genomes. Specialized reference-free tools like LueVari, DiscoSNP++, and ebwt2snp are fast and accurate, but usability is limited due to installation difficulties and poor documentation. Analysis is ongoing, but we expect that reference-free metagenomic callers will have superior accuracy to generalist reference-based callers but will have higher computational requirements.

Conclusions

There are dozens of variant callers available for use, including several specifically designed for metagenomic data. However, there is currently little evidence to guide proper selection of these tools, as there has not yet been a comprehensive study using metagenomic datasets with varying characteristics. We expect that this work will help inform appropriate selection of variant callers for use on metagenomic data.

Financial Support
Agricultural and Food Research Initiative grant no. 2019-67017-29110 from the USDA National Institute of Food and Agriculture

Notes:

P065 - Is sequencing of pooled samples an efficient method for characterizing the microbiome of animal groups?

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Session: Host or pathogen genomics or proteomics, Dec. 6

Objective

Sequencing composited samples (either by pooling matrix or pooling extracted DNA) could be a more efficient method of appropriately characterizing group-level microbiome composition compared to sequencing large numbers of samples from individual animals. The purpose of this study was to compare microbiome and resistome results of individual fecal samples with those obtained through compositing of sample matrix or extracted DNA.

Methods

Fecal samples (2g) were collected per rectum from 50 calves that were born and raised at an organic dairy. Fifteen different composite groups were created, each consisting of 10 different samples, and (Pool A – Pool O), where each individual sample was included in 3 pools each composite contained 10 samples and each individual sample is represented in 3 different composites. Both composited feces and composites of DNA extracted from individual samples were processed for sequencing. The microbiome of samples was characterized using 16S rRNA gene sequencing, and the resistome was characterized using target-enriched shotgun sequencing. Microbiome classification was conducted with QIIME2, resistome data were classified using the AMR++ pipeline, and statistical analyses were conducted in the R platform using phyloseq.

Results

The composition of microbiome and resistome for both composite types were similar to the mean community structure of individuals. The largest difference in microbiome composition between individuals and composites was differing abundances of Proteobacteria and Bactroidetes. Both composite types had similar resistome composites when compared to individuals within the same pool, except for lower abundance of tetracycline resistance determinants when compared to individual samples.

Conclusions

Microbiome and resistome composition of both types of composite samples could be useful for group-level sampling of large populations. Additionally, composite samples contained all major taxonomic and resistome taxa that were found in individual samples, suggesting that pooling would have a similar sensitivity for detection of important features.

Financial Support
U.S. Department of Agriculture; U.S. Department of Agriculture, Center for Epidemiology and Animal Health

Notes:

P066 - Pathogenesis and control of emerging reoviral arthritis in turkeys

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Session: Microbiome and bacteriology, Dec. 6, 6:00 - 8:00 PM

Objective

Turkey arthritis reoviruses (TARVs) are responsible for significant economic losses in the turkey industry. Yet their pathogenesis is poorly understood, and autogenous vaccines measures have failed to control the disease and prevent the emergence of antigenic variants and new pathotypes. Although a large amount of genomic data is being accumulated, antigenic and pathogenic determinants of TARVs are largely unknown.

Methods

Specific-pathogen-free turkeys were infected with TARV O'Neil and assessed for arthritis through gait-scoring, gross pathology, and histology. Further, dysbiosis of gut microbiota in TARV-infected turkeys and commercial turkeys was determined through 16S rRNA gene sequencing and bioinformatic analysis in QIIME2 and various R packages.

Results

To aid studies on TARV pathogenesis and TARV vaccine development, we have established a specific-pathogen-free turkey model that recapitulates the features of arthritis observed in commercial turkeys including clinical signs (lameness), gross pathology, and histological inflammation of gastrocnemius and digital flexor tendons. Furthermore, our field and experimental observations in commercial and specific-pathogen free turkeys suggest that gut microbiota may play a role in initiation and establishment of TARV infection and subsequent induction of arthritis, a variable that has not been accounted for in previous research.

Conclusions

Further experiments are needed to understand how viral, host, and intrinsic microbial factors contribute toward the onset, development, and severity of reovirus-associated arthritis in turkeys.

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

Notes:

P067 - Isolation of potential lytic bacteriophage for *Mycoplasma ovipneumoniae (M. ovi)*

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Session: Microbiome and bacteriology, Dec. 6, 6:00 - 8:00 PM

Objective

M. ovi causes respiratory disease in domestic and bighorn sheep. Morbidity in domestic sheep is considered modest, however, its introduction into naïve bighorn sheep appears quite lethal. We recently demonstrated that antibiotic treatment was unable to clear experimental *M. ovi* infection in domestic lambs. In the absence of effective countermeasures, current management practices restrict grazing locations for domestic sheep, which causes hardships for the industry. Our long-term goal is to identify novel approaches to eliminate infection in domestic sheep.

Methods

This "Seed Project" is focused on identifying potential lytic bacteriophage (phage) for *M. ovi* that could be used alone or in combination with other treatments to clear infection. In year one, we established broth cultures of different *M. ovi* isolates and developed a flow cytometry-based screening assay for potential phage. We collected tissue washes and samples from infected animals as potential sources of phage. In the past year, we continued our sample collection, flow cytometry-based screens, and on developing and implementing a plate-based screening assay.

Results

A SYBR green based fluorescent assay for viable bacteria employing flow cytometry was used to identify inhibitory tissue samples and fluids for *M. ovi*. Zones of inhibition were also detected on lawns of *M. ovi* (Y98) using a new "plaque-type" plate-based assay. Using these assays, putative phage preparations were selected, enriched, clarified and used in analysis by transmission electron microscopy (TEM). The TEM analysis showed phage particles at a concentration of approximately $1x10^6$ virus particles per ml. The virus particles average 31 nm in diameter and appear to form T=3 icosahedral particles.

Conclusions

We have succeeded in identifying the first lytic phage for *M. ovi.* These results are intended to provide the tools and preliminary data in support of a much larger project focused on testing the following overarching hypothesis: Lytic phage therapy in combination with innate immune stimulation or antibiotics will clear domestic sheep of *M. ovi* infection.

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

Notes:

P068 - Mechanistically connecting the immune system and microbiome to beneficial effects of anti-IL-10 antibody during coccidiosis in broilers

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Session: Microbiome and bacteriology, Dec. 6, 6:00 - 8:00 PM

Objective

Pathogen-induced upregulation of interleukin-10 (IL-10) is a well-recognized strategy to evade host defenses through immunosuppression. Parasitic coccidiosis (*Eimeria spp*) leading to bacterial necrotic enteritis via *Clostridium perfringens* costs the broiler industry $6 billion/year, a devastating 20% of the market value. Current control strategies are encountering pathogen resistance, and no new drugs have entered the market for 30+ years. We intend to advance basic mechanistic knowledge of the immune response and microbiota community changes when the broiler is allowed to respond to *Eimeria* and downstream sequelae necrotic enteritis with an immune response unhindered by pathogen upregulation of IL-10.

Methods

To accomplish this goal, we proposed to dissect differences in immunological responses and intestinal microbial community and function using a broiler coccidiosis challenge treated with control or oral anti-IL-10 antibody. In summer 2021, we began preparing for the live animal trial, including laying hen vaccination, egg collection, and yolk freeze drying (negative control), and received commercial anti-IL-10 egg powder. We obtained *Salmonella typhimurium*, *Eimeria maxima* M6, and *Clostridium perfringens* isolates from University of Arkansas Hargis Lab, performed 16S rRNA gene amplicon sequencing on *Salmonella typhimurium* and *Clostridium perfringens,* and confirmed the identity and purity of each isolate to ensure a robust coccidiosis and necrotic enteritis model. We then started two concurrent groups of broiler chicks staggered by 7d to determine *Eimeria maxima* dose needed to achieve 25% loss in body weight gain without clinical signs of disease to ensure all disease parameters are met in this pilot trial prior to the full trial scheduled this fall 2021.

Results

We expect full trial results to point towards both immune and microbiome-mediated mechanisms that allow for the control of infections that manipulate host production of IL-10.

Conclusions

In the full animal trial, we will model how the alteration of IL-10 protein expression alters normal innate and adaptive immune defenses against pathogens and show how disruption of pathogen immune evasive strategies can be defeated by immunotherapeutics that target key host immune processes.

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

Notes:

P069 - Microbial communities in the Salmonidae family, a meta-analysis

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Session: Microbiome and bacteriology, Dec. 6, 6:00 - 8:00 PM

Objective

The microbiome plays a critical role in health and disease across farmed species, but microbiomes associated with farmed salmonids are not well understood. Further, salmon farms employ diverse management practices, and it is unknown whether this heterogeneity impacts the fish microbiome. The aim of this study was to describe the mucosal microbiomes of Salmonidae species based on relevant existing datasets.

Methods

A literature search was conducted through MEDLINE and Scopus databases (2010 to January 2021). Studies were restricted to English and screened in two stages: abstract screening and full-text screening. Studies were included in the analysis if they met these criteria: were peer-reviewed, presented data on salmonid species, performed high-throughput 16S rRNA gene sequencing, and made the sequence data publicly available. Additional studies were screened from NCBI's Short Read Archive (SRA). A meta-analysis will be performed using the publicly available datasets from studies that met the inclusion criteria.

Results

A total of 523 studies were screened, 112 were eligible for full-text screening, and 52 were included in the meta-analysis (MEDLINE and Scopus: 40; SRA: 12). Of those, 83% used Illumina as their sequencing platform, 36% targeted the v3-v4 region, and 25% the v4 region. No studies were included from 2010-2014, while 12 studies were included from 2020. The most represented species were the Atlantic salmon (*Salmo salar*) (53%) and the Rainbow trout (*Oncorhynchus mykiss*) (34%). Sample type was dominated by gut content (65%), while others such as skin and gill were scarce (5 and 4% respectively). Analysis of the hypervariable regions and factors explaining different microbiome patterns is ongoing.

Conclusions

Microbiome studies of farmed Salmonidae species have increased recently, with a focus on gut samples. Further research should characterize the microbiome of other Salmonidae species and other sample types like gill or skin, as these mucosal surfaces are critical against pathogens. In addition, there should be an effort to publish sequence data to repositories such as the SRA.

Financial Support
University of Minnesota; Signature Program Research Funding, College of Veterinary Medicine, University of Minnesota

Notes:

P070 - Does the fecal microbiome vary based on individual, composite individual, and pooled DNA samples?: A pilot study

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Session: Microbiome and bacteriology, Dec. 6, 6:00 - 8:00 PM

Objective

Processing of individual samples during large field-based microbiome studies is often resource-intensive. The primary objective of this study was to characterize and compare the piglet fecal microbiome obtained from samples processed using both individual and pooled workflows.

Methods

A total of 20 litters reared in a single commercial swine facility were enrolled in the study. Half of the litters (N=10) contained piglets ~2 days of age, while the other 10 contained piglets ~20 days of age. All of the piglets (N=258) in each litter were sampled individually by inserting a sterile cotton-tipped swab into the rectum. Additionally, 3 composite samples were collected from each litter by swabbing the pen floor with a cotton-tipped swab. Swabs were stored at -80°C and then processed using four methods: 1) individual raw samples were processed for DNA extraction ("individual", N=258); 2) raw material from individual samples was pooled into subpools (4 subpools per litter), and then DNA was extracted from each sub-pool ("fecal subpools", N=80); 3) raw material from individual samples was processed for DNA extraction and then the DNA was pooled into subpools (4 subpools per litter), ("DNA subpools", N=80); and 4) composite floor samples (3 per litter, N=60). All DNA samples (N=478 samples) were sequenced (16S rRNA, v4 region) for microbiome analysis. Amplicon sequence variants will be identified using the DADA2 pipeline, and microbiome composition and diversity will be compared between the four workflows.

Results

All libraries have been submitted for sequencing. The results will inform the sampling design for a larger project, the goal of which is to understand whether diet and/or management strategies can be used to mitigate the development and persistence of antimicrobial resistance after metaphylactic antibiotic use in swine.

Conclusions

Our study will evaluate whether litter-level pooling of raw material or extracted DNA can be used to represent the full diversity and composition of the litter microbiome, by comparing to individual piglet samples as the "gold standard".

Financial Support
U.S. Department of Agriculture; Agricultural and Food Research Initiative grant no. 2021-68015-33499 from the USDA National Institute of Food and Agriculture

Notes:

P071 - Longitudinal survey of the bovine teat microbiome

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Session: Microbiome and bacteriology, Dec. 6, 6:00 - 8:00 PM

Objective

Bovine mastitis is an important disease of dairy cows, with an etiology that has been studied for over a century. The teat epithelium is thought to provide an important physical and microbial barrier for mastitis prevention. The main objective of this longitudinal study was to survey the teat epithelial microbiomes of organically reared primiparous cows during a critical period of mastitis risk.

Methods

To address this objective, we prospectively followed 503 primiparous cows on 5 organic-certified U.S. dairy farms over a three-month period. Teat epithelial samples were collected weekly beginning 8 weeks prior to calving and for 5 weeks after calving. DNA was extracted from teat epithelial samples and subjected to PCR amplification of the 16S rDNA gene and sequenced on an Illumina MiSeq. Microbial diversity was measured using Shannon Diversity and changes assessed using LOESS regression. Ordination analysis was used to compare microbial community composition and structure between prepartum and postpartum samples.

Results

In total, we collected over 5,000 samples from the teat epithelium of primiparous cows, with results available for approximately half of these samples; analysis is ongoing. Based on this subset of samples, microbial diversity remained relatively constant throughout the prepartum period and began to shift as animals approached calving. Bacteria belonging to the *Acinetobacter, Corynebacterium, Romboutsia, Staphylococcus, and Turcibacter* genera were consistently recovered from the teat epithelium, though distinct taxonomic profiles were observed between farms and prepartum and postpartum timepoints.

Conclusions

The teat epithelium is a rich source of microbial cells, with population structures that vary within and across farms. The microbial diversity of these populations appears to be in a stable state throughout the prepartum period and then transitions into an alternative state, characterized by sharp increases or decreases in diversity. Continued analysis of this sample set may yield new information about microbial risk factors that impact mastitis risk in primiparous cows.

Financial Support
U.S. Department of Agriculture

Notes:

P072 - Feasibility of *Galleria mellonella* invertebrate model to study Leptospira

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Session: Microbiome and bacteriology, Dec. 6, 6:00 - 8:00 PM

Objective

The *Galleria mellonella* larvae infection model is emerging as a valuable tool for studying various characteristics of infectious agents and host-pathogen interaction. This system has been widely recognized as a high throughput, ethical, and cost-effective invertebrate infection model to study the virulence and pathogenesis of various bacterial pathogens. In this study, we evaluated the feasibility and potential of using *Galleria mellonella* larvae to study *Leptospira* an important bacterial pathogen of global zoonotic importance.

Methods

We inoculated *G. mellonella* larvae with *Leptospira interrogans* serovar Copenhageni (pathogenic) or *Leptospira biflexa* serovar Patoc (saprophytic) strains at doses ranging from 1 x 10^1 to 1 x 10^6 through the intrahaemocoelic route. The health index scoring system and survival data was calculated. PCR and bacterial culture were used to detect presence of *Leptospira* in the inoculated samples.

Results

We observed significant pathologic changes such as decreased mobility and complete melanization in *G. mellonella* larvae infected with a pathogenic strain *L. interrogans* serovar Copenhageni compared to those infected with a nonpathogenic strain *L. biflexa* serovar Patoc. The larvae infected with the pathogenic strain exhibited a lower survival rate and low health index score.

Conclusions

Our study demonstrates the feasibility and the potential of using *G. mellonella* larvae as an alternative model to study virulence mechanisms and pathogenesis of *Leptospira* strains. Once optimized, *G. mellonella* infection model can be a potential substitute for hamsters to explore various host and pathogen-related mechanistic events in *Leptospira* infection.

Notes:

P073 - *Fusobacterium* is key to alter uterine microbiota structure and function in metritic cows

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Objective

Ceftiofur is a preferred antibiotic in the US for the treatment of metritis, a microbiota-associated uterine disease characterized by high abundance of *Fusobacterium*, *Bacteroides*, and *Porphyromonas*. How ceftiofur affects uterine microbiota and leads to cure is still to be found. Herein, we aimed to investigate ceftiofur effects on uterine microbiota in metritic cows.

Methods

We collected uterine swabs from 20 cows that were diagnosed as having metritis. After sampling, 10 cows immediately received a 2.2 mg/kg of ceftiofur hydrochloride (Excenel®, Zoetis) via i.m. injection and the rest remained untreated. Two days after metritis diagnosis/treatment, uterine swabs were again collected from the same individuals. Bacterial genomic DNA was isolated from uterine swabs, which were used for qPCR and Illumina sequencing targeting the V4 hypervariable region of the bacterial 16S rRNA gene.

Results

A single dose of ceftiofur resulted in reductions in uterine bacterial load, relative abundance of *Fusobacterium*, genes involved in LPS biosynthesis, and rectal temperature, whereas uterine microbiota diversity and genes involved in pantothenate and coenzyme A biosynthesis increased. The relative abundance of *Bacteroides* and *Porphyromonas* were unaffected by ceftiofur treatment, which may lead to delay or failure to cure.

Conclusions

We conclude that the efficacy of ceftiofur to control uterine pathogens is limited to *Fusobacterium*, but it can result in alteration in uterine microbiota structure and function towards health.

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

Notes:

P074 - Detection of pathogenic *Leptospira* spp. in fish

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Session: Microbiome/Bacteriology, Dec. 6, 6:00 - 8:00 PM

Objective

Leptospirosis is an important zoonotic disease that accounts for significant morbidity and mortality in animals and humans, and is maintained in a population due to chronic kidney infection of reservoir mammals. Earlier work from our lab has shown that rodents, voles, shrews, chipmunks and several species of amphibians and reptiles are reservoirs of *Leptospira* spp. in the Cumberland Gap Region of KY, TN and VA. The aim of this study is to determine if fish contribute to the maintenance of leptospirosis in the aquatic environment.

Methods

To that end, we will be collecting 300 fish belonging to various species from the Powell River in Harrogate, TN, and their kidneys will be screened with a highly sensitive and specific Taq-Man quantitative PCR (qPCR). So far, we have collected 110 fish belonging to the following species: bass (*Ambloplites* (17)*, MIcropterus* (7)), stone rollers (*Campostoma* (16)), darters (*Percina* (5), *Etheostoma* (2)), chubs (*Erimystax* (2)*, Semotilus* (5)*, Nacomis* (5)), sunfish (*Lepomis* (22)), catfish (*Pylodictus* (1)), hogsuckers (*Hypentelium*) (3)), shiners (*Luxilus* (9)*, Notemigonus* (6)*, Cyprinella* (1)), longnose gar (*Lepisoteus* (3)), minnow (*Pimephales* (1))*,* Redhorse (*Moxostoma* (5)). Genomic DNA from kidneys of 81 fish were extracted and screened by qPCR.

Results

Only one kidney was positive for leptospiral DNA and contained 1 x 10^4 GE/g (genomic equivalents per gram) of kidney tissue. Additionally, multi locus sequence typing (MLST) will be used to genotype leptospires present in positive kidney. Multiple gene loci will be PCR amplified, sequenced and compared with available homologous gene sequences in the databases.

Conclusions

This study will provide information on the role of fish in the epidemiology of leptospirosis in the region.

Notes:

P075 - Assessing the microbiota of recycled bedding sand on a Wisconsin dairy farm

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Session: Microbiome/Bacteriology, Dec. 6, 6:00 - 8:00 PM

Objective

Bedding sand is often recycled on dairy farms to reduce costs. However, the bacterial communities of recycled bedding sand and how they might impact dairy cattle health are understudied. Here the bacterial community of recycled bedding sand, and its dynamics, were characterized across several stages of the recycling process and different seasons.

Methods

Sand and grey water samples were collected from several stages of a sand recycling system on a single Wisconsin dairy farm during the summer and winter of 2018, using sterile wooden spoons. DNA was extracted from each sample using phenol-chloroform and the V4 variable region of 16S rRNA genes were PCR amplified. The products were sequenced on an Illumina MiSeq and sequences were processed and analyzed using mothur and R.

Results

Bacterial community compositions differed by both season and stage. Summer samples had higher richness and distinct community compositions compared to winter samples. Diversity of recycled sand decreased with time post-recycling in both seasons. In summer samples, operational taxonomic units (OTUs) classified to the genera *Acinetobacter* and *Pseudomonas* increased in abundance with time post-recycling while no OTUs were found to drive the differences in diversity observed across winter stages. A core microbiome of 141 OTUs were found across all stages and seasons which represented 68.5±10.3% SD of the relative abundance of each stage. *Acinetobacter, Psychrobacter*, *Corynebacterium*, *Pseudomonas*, and *Enterococcus* were the 5 most abundant genera of the core microbiome.

Conclusions

The bacterial community composition of a bedding sand recycling system is dynamic across both season and stage of the sand recycling process, with a large core microbiome that accounts for most of the bacterial community reads at each stage. The abundant core microbiota and the presence and enrichment of several genera containing known mastitis pathogens suggest sand recycling systems may serve as bacterial reservoirs that warrant further investigation.

Financial Support
Walter and Martha Renk Endowed Laboratory for Food Safety; UW-Madison Food Research Institute

Notes:

P076 - Virulence typing of *Escherichia coli* isolated from clinical cases compatible with avian colibacillosis in Peru

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Session: Microbiome/Bacteriology, Dec. 6, 6:00 - 8:00 PM

Objective

Avian pathogenic *Escherichia coli* (APEC) is the causative agent of avian colibacillosis. Several genes have been associated with virulence in APEC, however, they have been also identified in non-pathogenic isolates. Evaluating these genes in *E. coli* isolates obtained from avian colibacillosis outbreaks would help to understand their potential pathogenicity. Thus, this study aimed to evaluate 7 APEC virulence genes in *E. coli* isolates obtained from clinical cases compatible with avian colibacillosis in Peru.

Methods

Eleven *E. coli* isolates were obtained from unrelated clinical cases in 10 broilers and 1 turkey, characterized by airsacculitis, perihepatitis, peritonitis, fibrinous pericarditis and enterocolitis. Tissue samples were cultured in MacConkey agar at 37°C for 24 hours. Lactose positive colonies were selected and submitted for biochemical testing to classify them as *E. coli*. Virulence gene typing was carried out by two multiplex PCR (mPCR) reactions: the first one assessed the *fimC* (type 1 fimbriae), *iucD* (aerobactin synthesis) and *tsh* (temperature-sensitive haemagglutinin) genes, and the second one *iss* (increased serum survival), *papC* (pilus), *irp-2* (iron repressible protein) and *vat* (vacuolating autotransporter toxin) genes.

Results

At least 4 virulence genes were simultaneously detected in 6 out of 11 isolates, 3 virulence genes in 4/11 isolates and 1 virulence gene in 1/11 isolate. *fimC* gene was detected in all the isolates while *vat* gene in none. *iucD*, *tsh*, *iss*, *papC* and *irp-2* genes were detected in 10/11, 8/11, 8/11, 1/11 and 1/11 different isolates respectively.

Conclusions

The virulence genes commonly identified among this small set of isolates were *fimC, iucD, tsh* and *iss*. The adhesins *fimC* and *tsh* genes are known to contribute to the development of air sacs lesions in early stages of infection, whereas *iucD* gene is involved in iron and manganese uptake from the host and *iss* gene has been significantly associated with highly pathogenic APEC strains. Further studies with more clinical isolates are needed to confirm the importance of these genes in the pathogenicity of APEC.

Notes:

P077 - Changes in the nasopharyngeal bacterial communities of recently received stocker cattle

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Session: Microbiome/Bacteriology, Dec. 6, 6:00 - 8:00 PM

Objective

The composition of the nasopharyngeal (NP) bacterial community is highly diverse and plays an integral part in maintaining health. We aimed to characterize temporal changes in NP bacterial community due to naturally occurring bovine respiratory disease (BRD) in stocker cattle.

Methods

This study included auction-market-derived, recently-weaned beef steers (n = 40) at a local, privately owned stocker operator facility. Based on clinical signs of BRD, calves were categorized retroactively based on the number of antibiotic treatments (NumTrt) received (0x: healthy; 1x: received antibiotic once; 2x: received antibiotics twice). Deep NP samples were collected from each calf at d 0, 7, 14, and 21, and microbial16S rRNA gene sequences were processed using phyloseq and dada2 in R. Differential abundances at the genus level based on day and NumTrt were tested using a repeated measure ANOVA (Proc GLIMMIX; SAS). A repeated measure ANOVA was used day or NumTrt differences in alpha diversity metrics.

Results

Mycoplasma, *Histophilus*, *Geobacillus*, *Saccharococcus*, *Lactobacillus, Pasteurella* were the most abundant genera. In healthy calves *Mycoplasma* relative abundance was significantly greater ($P = 0.01$) at d 7 (0.30 ± 0.05) compared to day 0 (0.06 ± 0.05). *Histophilus* and *Mannheimia* were not detected until d 14 and d 21, respectively, in healthy calves. A NumTrt by day interaction was observed for *Mycoplasma* ($P = 0.03$), where 0x calves had significantly reduced bacterial relative abundances at d 7 compared to calves receiving treatment, and no differences were observed among NumTrt statuses on other days. The relative abundance of *Lactobacillus* and *Bacillus* did not differ temporally ($P > 0.05$); however, numeric relative abundance decreased over time. Alpha diversity matrices did not differ based on day or NumTrt.

Conclusions

The relative abundance of various BRD pathogens exhibited temporal variation in recently received stocker cattle. The effects of animal variation, weaning, age, breed, and transportation distance likely played a role in these changes and should be further described.

Financial Support
University of Tennessee; U.S. Department of Agriculture, National Institute for Food and Agriculture; This project was supported by the state of Tennessee through the University of Tennessee Institute of Agriculture AgResearch, the Department of Animal Science, and the United States Department of Agriculture National Institute of Food and Agriculture Multistate project NC1192. Funding was acquired through the UTCVM Center of Excellence in Livestock Diseases and Human Health competitive grant.

Notes:

P078 - Elucidation of mechanism of action of GI-7, a novel small molecule inhibitor of avian pathogenic *E. coli* (APEC)

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Session: Microbiome/Bacteriology, Dec. 6, 6:00 - 8:00 PM

Objective

Avian pathogenic *Escherichia coli* (APEC), a subgroup of extraintestinal pathogenic *E. coli* (ExPEC), causes colibacillosis in chickens, and shares similarities with ExPECs that are implicated in human urinary tract infections and meningitis. However, the emergence of antibiotic-resistant strains and diverse APEC serotypes causing disease limits the use of available vaccines and antibiotics. We recently identified a novel small molecule growth inhibitor of APEC, GI-7 that showed efficacy in chickens against APEC infection. GI-7 works by inducing changes in the APEC membrane. The purpose of this study is to elucidate the mechanism(s) of action on GI-7 and identify the potential targets for GI-7.

Methods

We evaluated the effects of GI-7 on the expression of outer membrane proteins essential for maintaining OM integrity by RT-qPCR. To support the gene expression findings, we investigated the levels of total membrane proteins after treating with GI-7 by SDS-PAGE and LC-MS/MS analysis. In addition, thermal proteome profiling (TPP) assay was used to determine other potential targets of GI-7 in APEC.

Results

Expression of genes essential for OM integrity was downregulated in GI-7-treated APEC. The downregulation of lpt genes was higher than the other OM genes (bamA, lolB, mlaA, and pbgA). Furthermore, LC-MS/MS analysis showed a depleted level (or low exponentially modified protein abundance index [emPAI]) of LptE in GI-7-treated APEC (emPAI of 8.46) compared to the level (emPAI of 13.84) in untreated APEC. To further support that GI-7 affects Lpt levels and, thereby, the LPS transport, we observed lower LPS level in the OM of APEC treated with GI-7. In addition, the TPP assay also uncovered the lptD as a potential target for GI-7 and revealed other potential targets.

Conclusions

GI-7 targeting the APEC OM can overcome the resistance problem, and thus represents a novel approach to control APEC infection in poultry. Furthermore, with APEC being genetically similar to human ExPECs, our findings can have profound implications in developing new antibacterial drugs against human ExPECs.

Financial Support
U.S. Department of Agriculture

Notes:

P079 - The impact of carbadox on the swine intestinal microbiota and intraepithelial T cell populations post-weaning

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Session: Microbiome/Bacteriology, Dec. 6, 6:00 - 8:00 PM

Objective

The interaction of intestinal intraepithelial T cells (IETs) and the microbiota is thought to play an important role in shaping gut development. We describe corresponding changes in the gut microbiome and IETs in post-weaning piglets on different diets to further characterize the relationship between the microbiota and host immunity.

Methods

Thirty-two freshly weaned nursery pigs were fed a diet with or without carbadox (50 g/ton) and necropsies were performed at 14 or 28 days post-weaning (dpw). We conducted 16S rRNA gene sequencing of cecal contents and mucosal swabs of the ileum, jejunum, and cecum. Epithelial-enriched cell fractions were isolated from the same tissues, and flow cytometry was used to evaluate T cell populations and their activation states.

Results

There was no significant effect of carbadox on the microbiome of similarly aged pigs. Rather, a shift was detected between 14 and 28 dpw, and the shift was larger in pigs receiving carbadox. IET community structure significantly differed by intestinal location and time, but a treatment effect was detected only in the jejunum at two wpw. Considering samples across all treatments and intestinal locations, amplicon sequence variants of *Lachnospiraceae* and *Akkermansia* were positively correlated with a subset of $CD27^-$ effector-like $CD2^+CD8a^+$ gamma-delta (gd) and $CD4^-CD8a^+CD8b^+$alpha-beta (ab) IETs and negatively correlated with less terminally differentiated $CD27^+CD2^+CD8a^-$ gd T cells. *Clostridium*, *Fusobacterium*, and *Leptotrichia* had an opposite pattern of correlation with the same IETs. Findings suggest association of $CD27^-$ effector-like gd and CD8ab IETs with microbes linked to gut health, while less terminally differentiated gd IETs may be associated with bacteria commonly linked to disease.

Conclusions

We identified disparate changes in the gut microbial and IET community structures associated with age and in-feed carbadox. Multivariate models for integrating datasets hold promise for clarifying relationships between the microbiota and IETs, which could be useful in developing strategies to improve swine production and health.

Notes:

P080 - Manipulation of the *Dermacentor andersoni* microbiome to mitigate pathogen transmission

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Session: Microbiome/Bacteriology, Dec. 6, 6:00 - 8:00 PM

Objective

Non-pathogenic microbes make up the majority of tick microbial communities. Our interest is in how the microbiome affects the ability of ticks to acquire and transmit the cattle pathogen *Anaplasma marginale (Am)*. In an earlier study we showed that two populations of *Dermacentor andersoni* ticks differed in their microbiome composition, with the population from Lake Como, MT having an endosymbiont (*Rickettsia bellii; Rb*) not present in the population from Burns, OR. The presence of Rb was correlated with a reduced ability to acquire *Am*. The goal of this study is to determine whether Rb affects transmission of *Am*.

Methods

Our rationale is to use the Burns ticks, which were devoid of Rb, and introduce Rb to a cohort of this population to establish genetically matched cohorts that are + or - for Rb to use as control matched groups in a transmission experiment. We collected ticks from Burns and assessed the Rb status using ddPCR. We attempted to breed Rb- colonies by rearing eggs from females that tested negative for Rb. We introduced Rb to a cohort of ticks by feeding on Rb containing blood in an artificial feeding system. We are examining competition between Rb and Am in Dermacentor (DAE100) cell culture.

Results

We found that after a four year hiatus of sampling that *Rb* is present in the current Burns population, albeit at very low levels – ~30% of the males have $<3x10^3$ *Rb*/tissue (midgut and salivary gland). Attempts to breed Rb negative tick subpopulations failed, as did attempts to increase the levels of Rb in the cohort. The ticks acquired Rb, but also fungus and had to be discarded. Because our goal of establishing a genetically matched *Rb* + and - population has proved difficult, we acquired flourescently marked strains of *Am* and Rb, and are testing thier growth in DAE cells.

Conclusions

While the Burns populations exhibited a stable microbiome during our initial study, it shifted in the intervening time. Manipulation has proven difficult, and has been complicated by the pandemic. We have moved to a cell culture approach, which will answer mechanistic questions.

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

Notes:

P081 - Evaluation of *Amblyomma americanum* vector competence for *Anaplasma marginale*

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Session: Parasitology and/or ticks, Dec. 6, 6:00 - 8:00 PM

Objective

Changes in climate, ecosystems, and increased animal transport have contributed to geographic expansion of various disease-transmitting tick species, including the Lone Star Tick (LST) (*Ambylomma americanum*), a species commonly found on cattle. Bovine anaplasmosis is a tick-transmitted, production-limiting disease and a major obstacle to profitable cattle production in the U.S. The intracellular rickettsial pathogen and agent of anaplasmosis, *Anaplasma marginale* (*Am*), is primarily transmitted by *Dermacentor* tick species in the U.S.; however, LST are more commonly found on cattle in many anaplasmosis endemic areas. LST are not thought to be involved in *Am* transmission; however, the frequency of LST infestation on cattle warrants specific examination into whether LST contributes to *Am* transmission. Additionally, genetic diversification of expanding LST populations could influence vector competence. Thus, the objective of this study was to determine and compare the vector competence of LST derived from historic or expanded LST geographic ranges for *Am* in a controlled transmission experiment.

Methods

The vector competence of LST for *Am* was evaluated by comparing the ability of two geographically distinct LST strains to acquire and transmit *Am* compared to a known *Am* vector, *Dermacentor variabilis*, using an established tick-calf *Am* transmission model. The *Am* bacterial levels were monitored throughout the transmission process in calf blood and tick midgut and salivary gland tissues using a quantitative PCR assay targeting the single copy Msp5 gene to track *Am* levels.

Results

Transmission of *Am* by LST sourced from either a historic or expanded range population of LST was not observed. Further, *Am* was not detected in any LST midgut or salivary gland tissues. In contrast, *D. variabilis* did successfully acquire and transmit *Am* from infected to naïve calves.

Conclusions

These results provide direct evidence that LST are unlikely to contribute to *Am* transmission in the U.S. Critical review of vector competence for pathogens vectors are commonly exposed to is important to confirm disease risk factors.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture; Foundation for Food and Agricultural Research

Notes:

P083 - Garlic Mustard (*Alliaria petiolata*) an invasive plant species as a risk factor for tick abundance

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Session: Parasitology and/or ticks, Dec. 6, 6:00 - 8:00 PM

Objective

Prior research has shown that invasive plants can increase risk to human and livestock health by increasing the proliferation of disease-transmitting arthropods. This study seeks to determine the association between different invasive plant functional groups (woody shrub, herbaceous forb, and grass) and ticks and tick-borne diseases.

Methods

Research plots with the shrubs autumn olive (*Elaeagnus umbellata*) and amur honeysuckle (*Lonicera maackii*), the forb Japanese chaffflower (*Achyranthes japonica*), garlic mustard (*Alliaria petiolata*), and grass Japanese stiltgrass (*Microstegium vimineum*) have been established as the representative invasive plants.
Weather stations were deployed to record relative humidity and temperature at each plot. Standard protocols for tick sampling and parasite testing were conducted using drag and flag sampling. Samples were transported to a BSL-2 facility where collected ticks were identified to species using standard taxonomic keys, enumerated by life stage and then transferred into cold storage at <4°C. Ticks will be tested individually using next-generation sequencing with barcoded, universal 16S rRNA primers.

Results

Preliminary results indicate that compared to uninvaded plots, plots invaded with garlic mustard have a significant increase in tick abundance (Wilcoxon rank sum p=0.012). Plots invaded by Garlic Mustard may be more likely to harbor ticks but at this time in the sample collection, this difference in not statistically significant. ($p < 0.08$, OR = 2.1 CI=1.66, 2.69). Tick sampling will continue throughout the fall of 2021, results of microclimate analysis and pathogen prevalence will be presented .

Conclusions

Garlic mustard has been shown to secrete chemicals from its roots that interfere with the growth of soil fungi such as *Beauveria bassiana*. Inhibition of *B. bassiana* by garlic mustard may promote tick abundance and increase survival. Control of this invasive plant species may be an effective tool for reducing tick abundance and the risk of tick borne disease in humans and livestock.

Financial Support
Dudley Smith Initiative University of Illinois Extension

Notes:

P084 - Phenotypic selection of dairy cattle with BLV demonstrates immunogenetic resilience through genotyping of MHC genes

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Session: Physiology or Immunology, Dec. 6, 6:00 - 8:00 PM

Objective

Characterization of the bovine leukocyte antigen (BoLA) DRB3 gene has shown specific alleles associate with susceptibility or resilience to the progression of bovine leukemia virus (BLV), measured by proviral load (PVL). Through surveillance of multi-farm BLV eradication field trials, we observed differential phenotypes within seropositive animals that persist from months to years. Repeated diagnostic observations defined two distinct phenotypes in our study population, ELISA-positive animals that do not harbor detectable levels of provirus and those who do have persistent proviral loads. We sought to assess the relationship between BLV phenotype and two BoLA genes.

Methods

BLV phenotpyes were determined using longitudinal results from milk ELISA screening and subsequent blood draw on seropositive animals for PVL determination using a novel BLV Proviral Load multiplex qPCR assay. A multiplex next-generation sequencing workflow (NGS-SBT) capable of genotyping 384 animals per run was developed. In total, 558 cows-168 BLV susceptible (ELISA-positive/PVL-positive) and 390 BLV resilient (ELISA-positive/PVL-negative) from nine Midwest dairy farms were selected for NGS-SBT.

Results

Three BoLA-DRB3 alleles, including one novel allele, were shown to associate with disease resilience, **009:02*, **044:01*, and **048:02* were found at rates of 97.5%, 86.5%, and 90.3%, respectively, within the phenotypically resilient population. Alternatively, *DRB3*015:01* and **027:03*, both known to associate with disease progression, were found at rates of 81.1% and 92.3%, respectively, within the susceptible population.

Conclusions

Phenotyping prior to genotyping agrees with cited associations between BLV and BoLA genotype. This study helps solidify the immunogenetic relationship between BoLA-DRB3 alleles and BLV of these two phenotypic groupings of US dairy cattle.

Notes:

P085 - Interactions of LenC, a leptospiral protein, with host extracellular matrix

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Session: Physiology or Immunology, Dec. 6, 6:00 - 8:00 PM

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 LenC with laminin and fibronectin, ELISA wells were coated with laminin or fibronectin and incubated at 4ºC 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 between each step. TMB substrate was then added to each well, stopping the reaction after 5 minutes. Laminin or fibronectin binding with three different truncations of LenC (LenC-N, LenC-M, and LenC-C) was studied using the above protocol. The ability of heparin (Sigma-Aldrich) to compete for the binding of laminin to LenC was assayed similarly as previously described. rLenC was immobilized to 96-well ELISA plates (100 ng/well) and 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 with PBS-T, then incubated with a laminin-specific polyclonal antiserum (1:2,500) for 1 h at 37°C. Reaction products were analyzed as described above.

Results

LenC binds to mammalian laminin and fibronectin in a dose dependent manner with observed dissociation constants of <0.1µM. LenC-directed antibodies inhibited the LenC binding to laminin. Heparin inhibited LenC-laminin binding, suggesting interactions through the collagen-binding domains of laminin. Fibronectin had the greatest binding to the LenC-N truncation of LenC.

Conclusions

LenC, a leptospiral membrane protein, binds with host laminin and fibronectin in a dose dependent manner and could facilitate establishment of infection through adhesion of Leptospira to the host extracellular matrix components.

Notes:

P086 - A novel approach to vitamin A and zinc supplementation to support mucosal immunity and promote resistance to bovine respiratory disease

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Session: Systemic and mucosal immunology, Dec. 6, 6:00 - 8:00 PM

Objective

Bovine respiratory disease (BRD) is a leading cause of morbidity and mortality in the United States cattle industry. Proper nutrition is essential for maintaining optimal health, and micronutrient status is a key factor affecting the incidence of BRD. Vitamin A (VA) and zinc (Zn) are micronutrients required for appropriate immune function. Stress and illness clearly contribute to an increased need for both VA and Zn; yet, the micronutrient requirements of the morbid animal are poorly defined. In this project, we will investigate the interplay between Zn and VA on immune function during a respiratory infection and explore practical intervention strategies. We hypothesize VA and Zn deficiency will increase the susceptibility and severity of BRD, and that acute BRD will negatively impact the micronutrient status of vitamin- and mineral-replete animals. We further hypothesize therapeutic VA/Zn co-supplementation will improve the outcome of transport stress and acute respiratory infection in newly received feedlot calves.

Methods

In Objective 1, we will investigate the mechanistic basis of VA and Zn utilization in the calf, including: 1) the effect of VA/Zn co-deficiency on the outcome of acute respiratory infection and 2) in turn, the effect of acute respiratory infection on VA and Zn status. VA and Zn are required for optimal immune function. However, acute infection significantly alters the host's ability to properly metabolize the micronutrients and limits their availability to target organs, even in well-nourished animals. In Objective 2, we will investigate the applied aspects of VA/Zn supplementation in the context of transport stress and BRD, with the goal of positively influencing disease outcome and recovery.

Results

We will begin these studies in the Fall of 2021.

Conclusions

Our studies will contribute to the development of a therapeutic micronutrient supplementation strategy that will provide optimum benefit to the diseased animal, thus expediting disease recovery and maximizing the animal's inherent potential.

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

Notes:

P087 - Identification of antileukoproteinase as a marker for the immune status of horses during equine herpesvirus type 1 infection

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Session: Systemic and mucosal immunology, Dec. 6, 6:00 - 8:00 PM

Objective

Equine herpesvirus type 1 (EHV-1) is a widely spread respiratory pathogen of the horse, which infects the nasal mucosa of the upper respiratory tract leading to respiratory disease. Entry of the virus into nasal epithelial cells, followed by infection of local lymphoid tissues allows for the establishment of cell- associated viremia, which can lead to abortion or neurologic disease. This study investigated the nasal transcriptomic profile of both immune and susceptible horses over the course of EHV-1 infection, to identify markers that differ in horses based on their immune status.

Methods

RNA sequencing was performed on nasal swab samples from EHV-1 immune (n=4) or susceptible horses (n=4), to assess differences in gene expression at the site of viral entry. Samples were taken before, early (d1pi and d3pi), mid (d8pi and d10pi) and late (d18pi) during infection, allowing us to determine genes that were differentially expressed over the course of infection and between groups. To further quantify some of the identified markers, novel equine monoclonal antibodies (mAbs) were developed following the immunization of mice with recombinant equine protein.

Results

We identified 30 genes that were significantly different between groups over the course of infection. For this work, we selected one protein, antileukoproteinase (SLPI), which was highly expressed only at the early time point in immune horses, while increasing in expression over time in susceptible horses. To better characterize the role of SLPI in EHV-1 infection, mAbs against equine SLPI were developed. Six clones specific to recombinant SLPI were identified and are currently further characterized using both flow cytometry and ELISA.

Conclusions

SLPI is secreted at mucosal surfaces where it inhibits serine proteases to protect the epithelium. In humans it is seen at high levels in both the respiratory and genital tract and has been shown to play a role in viral infection. Here we identified SLPI as a potential marker of immunity against EHV-1 infection and are in the process of developing mAbs to further investigate its role at the nasal mucosa of the horse.

Financial Support
Harry M. Zweig Memorial Fund for Equine Research; USDA-NIFA Food Animal Residue Avoidance Databank

Notes:

P088 - Oxidative stress compromises lymphocyte function in neonatal dairy calves

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Session: Systemic and mucosal immunology, Dec. 6, 6:00 - 8:00 PM

Objective

Dairy calves are unable to mount an effective immune response during their first weeks of life, which contributes to increased disease susceptibility during this period. Oxidative stress (OS) diminishes the immune cell capabilities of humans and adult cows, and dairy calves also experience OS during their first month of life. However, the impact that OS may have on neonatal calf immunity remains unexplored. Thus, we aimed to evaluate the impact of OS on newborn calf lymphocyte functions.

Methods

We conducted two experiments. First, we assessed the association of OS status throughout the first month of age and the circulating concentrations of the cytokines interferon-gamma (IFN-gamma) and interleukin (IL) 4, as well as the expression of cytokine-encoding genes IFNG, IL2, IL4, and IL10 in peripheral mononuclear blood cells (PBMCs) of 12 calves. Subsequently, we isolated PBMCs from another 6 neonatal calves to investigate in vitro the effect of OS on immune responses in terms of activation of lymphocytes, cytokine expression, and antibody production following stimulation with phorbol 12-myristate 13-acetate or bovine herpesvirus-1. The results were compared statistically through mixed models.

Results

Calves exposed to high OS status in their first month of age showed higher concentrations of IL-4 and expression of IL4 and IL10 and lower concentrations of IFN-gamma and expression of IFNG and IL2 than calves exposed to lower OS. In vitro, OS reduced lymphocyte activation, production of antibodies, and protein and gene expression of key cytokines.

Conclusions

Collectively, our results demonstrate that OS can compromise some immune responses of newborn calves. Hence, further studies are needed to explore the mechanisms of how OS affects the different lymphocyte subsets and the potential of ameliorating OS in newborn calves as a strategy to augment the functional capacity of calf immune cells, as well as enhance calves' resistance to infections.

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

Notes:

P089 - Pro-inflammatory cytokine responses to bacteria antigen LPS differ between young adult and old horses in vitro

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Session: Systemic and mucosal immunology, Dec. 6, 6:00 - 8:00 PM

Objective

Old horses show signs of diminished immune function (immunosenescence) apparent from reduced vaccination response and lymphocyte proliferation. Data on whether further aspects of immune function are impaired is currently sparse. Therefore, it was the goal of this study to determine if the pro-inflammatory cytokine response to Gram-negative bacteria antigen LPS differs between immune cells from young adult and old horses.

Methods

Whole blood samples of ten female old (≥20 years) and seven female young adult (4-6 years) horses were incubated with a low dose of LPS (L-LPS; 0.01ug/mL), a high dose of LPS (H-LPS; 1ug/mL), or without LPS (negative control) for 6h. Gene expression of pro-inflammatory cytokines (IL-1β, IL-6, IL-8, IL-18 and TNFα) was quantified in the samples using RT-qPCR. Gene expression was normalized to a reference gene (β-GUS), and expressed as fold change relative to the corresponding non-LPS stimulated negative control sample. Between group and dose differences were identified using ANOVA. Gene expression data was ln transformed prior to analysis and results were considered significant at $P \leq 0.05$ and trending at $P < 0.10$.

Results

Gene expression of IL-1β, IL-6, IL-8, and TNFα was significantly higher in whole blood samples incubated with H-LPS compared to L-LPS, irrespective of horse age group. LPS dose did not affect IL-18 gene expression in samples from young adult or old horses. In old horse samples incubated with L-LPS, gene expression of IL-8 and TNFα was significantly lower, and expression of IL-1β tended to be lower ($P = 0.08$), than in young adult horse samples treated with L-LPS. Old horse samples incubated with H-LPS showed significantly lower TNFα expression, and a trend to lower IL-18 expression ($P = 0.09$), compared to young adult horse samples incubated with H-LPS.

Conclusions

In line with the concept of immunosenescence, the in vitro pro-inflammatory response to LPS appears diminished in old horses. As a reduced response to LPS might increase susceptibility to Gram-negative bacteria infections, further study in vivo is warranted.

Notes:

P090 - Development of anti-equine IL-1β monoclonal antibodies

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Session: Systemic and mucosal immunology, Dec. 6, 6:00 - 8:00 PM

Objective

Interleukin-1β (IL-1β) belongs to the IL-1 cytokine family and is produced in a variety of cell types. IL-1β is a potent mediator of innate immune responses and has a key role in inflammatory responses in both humans and horses. Specific antibodies against equine IL-1β will contribute to characterizing this important innate immune mediator on the protein level for multiple equine diseases. In this study, we describe the development of novel anti-equine monoclonal antibodies that specifically detect native equine IL-1β.

Methods

A black C57BL/6J mouse was immunized with recombinant IL-1β, produced in ExpiCHO cells. After a series of injections, mouse spleen cells were fused with myeloma cells to create hybridoma cell lines. ELISA and flow cytometry were used to select hybridoma clones based on recombinant and native antigen recognition and absence of cross-reactivity with other cytokines.

Results

Monoclonal antibodies from 3 selected cell lines were purified. A fluorescent bead-based assay was developed. The analytical sensitivity of the assay was 10 pg/ml. The antibodies were also conjugated to fluorescent dye and used in flow cytometry. Both techniques were used to analyze IL-1β expression in PBMC after *in vitro* stimulation with different mitogens, LPS, or PMA and ionomycin.Time and dose-dependent secretion of IL-1β in LPS stimulated PBMC was measured in the bead-based assay. The kinetics of IL-1β expression in peripheral blood monocytes was analyzed by intracellular staining and flow cytometry.

Conclusions

We have developed three new equine IL-1β mAbs and confirmed their specificity for equine IL-1β. We have characterized IL-1β expression and secretion kinetics from equine PBMC. The mAbs can be used to detect native IL-1β in secretions by bead-based assays or ELISA and intracellularly by flow cytometry. These IL-1β mAbs provide valuable new tools for evaluating inflammatory immune responses during different experimental and biological conditions and diseases of the horse.

Notes:

P091 - Role of equine herpesvirus-1 genes for induction of respiratory immunity and establishment of viremia

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Session: Systemic and mucosal immunology, Dec. 6, 6:00 - 8:00 PM

Objective

Equine herpesvirus 1 affects horses worldwide and causes respiratory disease, abortions, and equine herpesvirus myeloencephalopathy (EHM). Following initial infection of the nasal epithelium, the virus enters peripheral blood mononuclear cells (PBMCs) and a cell-associated viremia is established, which is central in the pathogenesis of EHM and abortions. There is mounting evidence that the early events at the respiratory tract determine immediate defense, transfer of the virus to PBMCs and incidence of secondary disease. For this reason, our hypothesis was that infection of equine respiratory explants with EHV-1 and EHV-1 mutants can be used to identify viral genes involved in modulation of respiratory immunity and establishment of viremia.

Methods

All viruses were propagated in equine dermal cells. Equine tracheal explants were collected from 4 respiratory healthy horses euthanized for unrelated reasons and inoculated with EHV-1 or EHV-1 mutants (Ab4 N752, Ab4 gB4, Ab4ΔgI-gE, Ab4ΔOrf1, Ab4ΔORF2, Ab4 gD4 and Ab4ΔUS3). Tissues were collected at 24, 48, 72 and 96 hours post inoculation (hpi) to assess viral replication and transfer of virus to local lymphocytes by immunohistochemistry and real-time quantitative PCR. Tissues were also collected at 3, 6, 12, and 24 hpi for determining interferon responses and cytokine/chemokine mRNA expression.

Results

Inoculation with Ab4 gD4 and Ab4ΔUS3 resulted in lower viral titers and slower viral growth in equine dermal cells, while other mutants exhibited viral kinetics comparable with the wild-type virus. Using respiratory explants we observed horse-to-horse variability, but overall Ab4ΔgI-gE, Ab4 N752, and Ab4ΔORF2 showed attenuated replication, while inoculation with Ab4ΔOrf1 showed an increased rate of infection. Transfer of virus to local lymphocytes and interferon, cytokine and chemokine responses are currently being evaluated.

Conclusions

Our results highlight the value of using primary equine respiratory explants in combination with viral mutants for the selection of viral candidate genes that are involved in EHM pathogenesis and immune modulation.

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

Notes:

P092 - Methods to evaluate engineered mRNA-expressed antibodies for treatment of *Tritichomonas foetus* infection in bulls

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Session: Systemic and mucosal immunology, Dec. 6, 6:00 - 8:00 PM

Objective

In bulls limited immunity to *Tritrichomonas foetus* (Tf) results in untreatable chronic genital infection. Immunity could be improved by synthetic mRNA application to preputial epithelium to induce expression of antibody against Tf surface antigen TF1.17. To establish conditions for this novel treatment, the objectives were: 1) optimize synthetic mRNA transfection of bovine primary preputial keratinocytes (PPK) for expression of antibodies against TF1.17, and 2) assess adhesion and viability of Tf co-cultured with antibody-expressing PPK.

Methods

PPK were transfected with 1, 2, or 4 ug mRNA encoding antibody to TF1.17 for 6, 12, 24, or 48 hours. Kinetics of antibody expression were assessed by immunofluorescence of transfected PPK and Tf treated with transfected PPK supernatants. For attachment and viability assays Tf were labeled with CellTrace™ CFSE at 5 uM, 10 uM, or 20 uM. Attachment and viability of Tf applied to transfected or control PPK was assessed at 6, 12, 24, and 48 hours.

Results

Intracellular antibody expression by PPK was identified at 6 hours and all later times. Transfection with 1 ug mRNA led to reliable expression not improved by 2 or 4 ug mRNA. PPK supernatants at all times contained Tf-binding antibody. Ten uM CFSE labeled Tf, facilitating their quantification on PPK monolayers. Tf attachment to transfected cells was not clearly decreased, but attachment variability complicated assessment.

Conclusions

PPK can be transfected with mRNA leading to antibody production as early as 6 hours post transfection. Work is ongoing to optimize assessment of the effect of expressed antibody on Tf attachment and viability.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture; U.S. Department of Agriculture, Animal Health Formula Funds

Notes:

P093 - Characterization of anti-porcine CXCL10 monoclonal antibodies

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Session: Systemic and mucosal immunology, Dec. 6, 6:00 - 8:00 PM

Objective

C-X-C motif chemokine ligand 10 (CXCL10) is secreted by cell types, such as monocytes, endothelial cells and fibroblasts, and facilitates chemoattraction of immune cells to tissues. For the USDA-NIFA Swine Immune Toolkit Initiative, our goal is to provide the veterinary community with commercial immune reagents and standardized assays for future research efforts. For this chemokine target, we aimed to characterize anti-porcine monoclonal antibodies (mAbs) to identify phenotypic markers unique to cell populations expressing CXCL10 protein.

Methods

A panel of aPoCXCL10 mAbs were produced against yeast expressed, recombinant porcine CXCL10 (rPoCXCL10). Each of the aPoCXCL10 mAbs was assessed by ELISA using cross-inhibition analyses of biotinylated mAbs, and direct binding to orthologous yeast expressed CXCL10 proteins. Distinct epitope groups for the generated aPoCXCL10 mAbs were assigned and select mAbs fluorescently tagged. Labeled aCXCL10 mAbs were screened for intracellular staining of pig immune cells under various stimulation conditions.

Results

Of the 9 produced mAbs, 2 detected intracellular CXCL10 expression in PMA/ionomycin or IFNg-stimulated cells. Further, cell characterization assays verified CXCL10+ cells as CD3-CD4-CD172+, with occasional CD4+ subsets. Our results showed that aPoCXCL10-1.4 mAb is the best mAb clone for intracellular staining. A sandwich ELISA was also developed to quantitate CXCL10 protein expression and verified for reactivity with native porcine CXCL10.

Conclusions

In summary, the majority of CXCL10 expressing cells are CD3-CD4-CD172+, with a limited subset of CD3-CD4+CD172+, likely dendritic cells. CXCL10 is induced by IFNg or PMA/ionomycin stimulation of pig cell cultures. aPoCXCL10-1.4 is recommended for future use in analysis of intracellular signaling to detect receptor interactions that regulate cell migration during pig immune responses. The tools identified will be useful for evaluating the role of CXCL10 in swine infectious disease responses.

Financial Support
USDA-NIFA AFRI grant # 2019-67015-29815 for the Swine Immune Toolkit Initiative

Notes:

P094 - Canine serum anti-parvovirus IgG titer is impacted by colostrogenesis

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Session: Systemic and mucosal immunology, Dec. 6, 6:00 - 8:00 PM

Objective

Canine nomograph analysis involves determination of vaccinal antibody titers in breeding bitches to estimate the duration of maternal antibody blockage of immunization. The nomograph also allows establishment of a timepoint for follow-up titer testing of the litter to ensure active vaccinal response. No studies to date have examined the role of colostrogenesis on circulating titers in the gravid bitch. While best timing for nomograph sample collection has been uncertain, the standard suggestion has been to avoid the two-week window around whelping due to colostrogenesis. The objective of this study was to determine if active transport of IgG into mammary tissue (colostrogenesis) induces a significant drop in circulating antibody titers, with the further goal of determining the best timing for collection of serum for nomograph analysis.

Methods

Sera was collected from 56 gravid beagle bitches over a periparturient period of 6 weeks and tested for specific antibody against CPV-2 via hemagglutination inhibition (HI) assay. Resulting titers were analyzed via repeated measures, one-way ANOVA (with Tukey's multiple comparisons) analysis. Significance was set at 0.05.

Results

At 2-weeks pre-whelp or at whelp, seven of 56 bitches (12.5%) showed a decrease in titer beyond the standard deviation inherent to the HI assay. Repeated measures, one-way ANOVA with Tukey's multiple comparisons *post hoc* analysis found significance of $p = <0.0001$.

Conclusions

Although 87.5% of the bitches tested maintained their CPV-2 titer throughout the periparturient period, a significant number of bitches showed a decline in titer at whelp ($p = <0.0001$). Sample collection for nomograph analysis should be avoided during the height of colostrogenesis. When sera for nomograph testing must be collected at whelp, an adjustment to the analysis must be applied. The preferred timing for serum collection for nomograph analysis of the breeding bitch is early in pregnancy or two weeks post whelp.

Financial Support
Winston's Challenge Fund Grant

Notes:

P095 - Use of virus-like protein cages to reduce *Mycoplasma ovipneumoniae* infection in domestic sheep

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Session: Systemic and mucosal immunology, Dec. 6, 6:00 - 8:00 PM

Objective

Respiratory infections are a major source of animal disease but many lack a specific vaccine. The innate immune response can avert this problem by eliciting nonspecific protection. Our previous work in murine models leveraged this mechanism and showed that non-infectious virus-like protein cages (VLPCs) enhanced clearance of pathogens for which no effective vaccines exist. *Mycoplasma ovipneumoniae* (*M. ovi*) is a common vaccineless respiratory pathogen in domestic sheep. While *M. ovi* rarely directly results in disease in domestic sheep, it may increase the severity of *Mannhemia* and *Pasteurella* infections through immunosuppression. In this study we utilize our established VLPC system to promote clearance of *M. ovi* infections in sheep.

Methods

To determine whether VLPC treatment reduced *M. ovi* burden, naturally-infected sheep were administered VLPCs or mock intranasally on 3 consecutive days. Nasal swabs were collected on days 0 and 3. Microbial DNA was extracted and quantitative PCR (qPCR) was used to determine the *M. ovi* genome copy number (GC) in samples using a standard curve.

Results

Before treatment, 4/8 sheep had sufficient bacterial DNA to quantify (VLPC = 2, mock = 2). Of these, 3/4 had levels of *M.ovi* below the range of our standard curve (< 17 GC). Following treatment, *M. ovi* was detected in all sheep. Of the sheep with pre-treatment analyses, each mock-treated sheep had increased burden at day 3. Of VLPC-treated sheep, 1 sheep showed reduced burden in 1 nare and 1 sheep cleared *M. ovi*. Notably, VLPC-treated sheep had a lower net endpoint burden than mock-treated sheep (VLPC: 0 – 38,000 GC; mock: 1,320 – 2,400,000 GC).

Conclusions

These data suggest that VLPC treatment may promote *M. ovi* clearance. As severe *M. ovi* infections typically feature multiple pathogens, a follow-up experiment is underway to study the same effect in infections by another ovine pathogen, Influenza D virus (IDV) either with or without *M. ovi*. These studies will enhance our understanding of the roles of *M. ovi* and IDV in respiratory infections so that non-pathogen-specific treatment options may be developed.

Notes:

P096 - Impact of *Mycoplasma ovipneumoniae* on the phagocytic ability of alveolar macrophages

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Session: Systemic and mucosal immunology, Dec. 6, 6:00 - 8:00 PM

Objective

Mycoplasma ovipneumoniae (M. ovipneumoniae) is a sheep respiratory pathogen associated with mild atypical pneumonia in domestic sheep and severe pneumonia in wild sheep. In many instances however, *M. ovipneumoniae* results in chronic asymptomatic colonization that is resistant to clearance by the immune system. The mechanism by which *M. ovipneumoniae* evades clearance and contributes to polymicrobial pneumonia is currently unknown, therefore the goal of this study is to determine whether *M. ovipneumoniae* infection impairs macrophage phagocytosis which would contribute to the immune evasion of the pathogen.

Methods

In pursuit of this, bronchioalveolar lavage (BAL) fluid samples were taken from sheep that were both PCR and serologically negative for *M. ovipneumoniae* and sheep experimentally infected with *M. ovipneumoniae*. These BAL samples were taken at two times post-infection to study the phagocytic ability and function of the resident alveolar macrophages (AMs) between the two groups. To determine whether direct interactions between *M. ovipneumoniae* and AMs altered phagocytic activity of the AMs, BAL fluid samples taken from healthy sheep, were exposed to a field isolate of *M. ovipneumoniae in vitro* for 24 hours and then the phagocytic ability of those AMs were compared to unexposed AMs. The phagocytic ability was quantified using imaging cytometry and exposure to 1 µm fluorescent polystyrene beads.

Results

This *in vitro* treatment led to a significant ($p=0.013$, Student T Test) decrease in phagocytic activity from 51±19% AMs in unexposed cells to 36±10.5% in *M. ovipneumoniae* exposed cells.

Conclusions

These observations indicate that the exposure of AMs in the pulmonary environment inhibits their phagocytic ability, which may contribute to immune evasions and predispose *M. ovipneumoniae*-infected sheep to infection with other respiratory pathogens.

Financial Support
Montana State University College of Agriculture; Montana Agricultural Experiment Station

Notes:

P097 - Generation of SERCA2a-specific TCR transgenic mice

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Session: Systemic and mucosal immunology, Dec. 6, 6:00 - 8:00 PM

Objective

Atrial fibrillation (AF), a common cause of arrhythmia, affects ~1% of the global population, and AF can coexist with dilated cardiomyopathy (DCM). Although inflammation is regarded as one of the important underlying mechanisms of AF, its primary triggers are unknown. We recently identified sarcoplasmic/endoplasmic reticulum Ca^{2+} adenosine triphosphatase (SERCA)2a as an autoantigen in the mediation of atrial myocarditis and DCM through the generation of autoreactive T cells. Since no suitable inflammatory animal models are available for AF, we sought to create the T cell receptor transgenic mice for SERCA2a 971-990 to evaluate the role of SERCA2a-specific T cells in the disease pathogenesis.

Methods

We generated a panel of T cell hybridomas specific to SERCA2a 971-990. Using 5' RACE PCR, we deciphered the composition for TCRα (V and J) and TCRβ (V, D, and J) chains of five hybridoma clones. After generating the genomic constructs of two clones (2 and 17), we determined the surface expression of TCR using 58αβ-/- cells that lack the endogenous TCRs to determine their functionalities.

Results

By using Major Histocompatibility Complex (MHC) class II dextramers as screening tools, we generated 92 SERCA2a 971-990-specific T cell hybridomas in a short period of three weeks. We transfected 58αβ-/- cells with two of these clones (2 and 17), which were found to have similar TCR α and β compositions with respect to their gene segments. Both the transfectants responded to anti-CD3 stimulation suggesting that both TCRα and β transgenes were expressed on the cell surface.

Conclusions

The use of MHC class II dextramers obviated the need to use the limiting dilution analysis to generate antigen-specific T cell hybridomas. After confirming the antigen specificity of 58αβ-/- cells transfected with the genomic constructs of clones, 2 and 17, we propose to use the constructs for one of these two clones for injections into the pronuclei of zygotes from C57Bl/6 mice. The resulting founder mice will be used to expand the transgenic colony for further investigations.

Financial Support
U.S. National Institutes of Health

Notes:

P098 - Determination of autoantibody repertoires by PhIP-Seq analysis in the mouse model of Coxsackievirus B3 infection

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Session: Systemic and mucosal immunology, Dec. 6, 6:00 - 8:00 PM

Objective

Various infectious and non-infectious agents can cause myocarditis that may lead to dilated cardiomyopathy. Among the infectious causes, viruses are commonly implicated. Mechanistically, autoantibodies have been demonstrated for various antigens, but their role continues to be enigmatic. Using the Coxsackievirus B3 (CVB3) infection model in mice, we adapted the phage immunoprecipitation sequencing (PhIP-Seq) as a versatile and high-throughput antibody analysis platform to evaluate antibodies for various antigens comprehensively.

Methods

Groups of mice were infected with or without CVB3. After 21 days, sera were collected for PhIP-seq analysis, and the select candidates were later validated by ELISA. Additionally, hearts and pancreata were collected for histological evaluation of inflammatory changes by Haematoxylin and Eosin staining.

Results

The PhIP-seq analysis revealed antibody reactivity to only CVB3 in the infected group but not in controls, thus validating the technique. Likewise, we noted antibody reactivity to 77 mouse proteins only in the CVB3 group. We then sought to confirm antibody reactivities to a few select antigens leading us to note that antibodies to phosphoinositide-3-kinase adaptor protein 1 (PIK3AP1) were consistently detected in infected animals as determined by ELISA. Histologically, pancreas was affected in all infected animals, and the disease severity was also greater than myocarditis, suggesting that antibody reactivities to various self-antigens might have resulted from tissue damage in both organs.

Conclusions

Our data suggest that PIK3AP1 may be an autoimmune target in the development of viral myocarditis, the translational relevance of which will be discussed.

Notes:

P099 - Impacts of vitamin A deficiency on mucosal immunity to respiratory syncytial virus infection

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Session: Systemic and mucosal immunology, Dec. 6, 6:00 - 8:00 PM

Objective

Bovine respiratory syncytial virus (BRSV) is a primary cause of lower respiratory tract disease in young cattle. It is epidemiologically known that vitamin A (VA) deficiency (VAD) leads to increased susceptibility to severe respiratory infections. The role of retinoic acid, an active form of VA, has been studied extensively in the gastrointestinal tract. However, less is known about the effects of VAD on lung immune function, specifically during BRSV infection. Therefore, to determine the impact of VAD on RSV infection, innate and adaptive immune responses were investigated using a mouse model of RSV infection.

Methods

Retinol concentrations were verified in the liver and the lung of non-infected or RSV-infected VA sufficient mice. Lung cells were isolated from VA sufficient or deficient mice infected with RSV. Antigen-specific CD8 T cell responses were identified using tetramers and intracellular cytokine staining, and the interferon-gamma production was measured in cell supernatants of an antigen recall assay by ELISA. The frequency and phenotype of gamma delta T cells, neutrophils, and CD103+ dendritic cells were also evaluated via multi-color flow cytometry. Cytokine production in lung tissue lysates of VA sufficient and deficient mice was analyzed by the Luminex assay.

Results

Although no differences in morbidity and mortality were observed between groups, gamma delta and CD8 T cells from VA deficient animals showed impaired production of interferon-gamma on days 3 and 8 post-infection of RSV, respectively. Changes in cytokines and chemokines levels were identified between VA deficient and control groups.

Conclusions

VAD may affect the Th1 balance of the lungs after a virus infection. VA appears to modulate inflammatory cytokines and alter the frequency and the phenotype of both adaptive and innate cell numbers in the lung, suggesting VA may play an essential role in lung immunity. Additional experiments are needed to better understand downstream effects on the innate and adaptive immune systems in the lungs during VAD after RSV infection.

Notes:

P100 - Vitamin A supplement to PEDV infected pregnant sows enhances primary lactogenic immunity and protection of piglets

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Session: Systemic and mucosal immunology, Dec. 6, 6:00 - 8:00 PM

Objective

We tested whether Vitamin A (VA) supplement, a key regulator of gut immune responses, to gestating and lactating sows can enhance the gut-mammary gland-sIgA axis, boosting passive immunity in piglets against porcine epidemic diarrhea virus (PEDV)

Methods

PEDV naïve sows were assigned VA deficient (VAD, 6) or sufficient (VAS, 3) diets on gestation day (GD) 30. Three VAD sows were given oral retinyl palmitate (30,000IU) daily from GD76 (VAD+VA). Sows were inoculated with PEDV, 1×10^5 PFU (2 VAS; 2 VAD; 3 VAD+VA;) or MEM (mock, 1 VAD, 1 VAS) at GD90, all piglets were challenged at 5 days old (PCD0). PEDV signs was assessed in sows and piglets post challenge. Primary lactogenic immune responses in sows and passively acquired immunity in piglets were evaluated

Results

Mean fecal scores were variable in sows, with VAS/VAD+PEDV sows developing soft feces while VAD+VA+PEDV sows had normal feces despite similar viral shedding, indicating VA enhances gut integrity. VAD+VA+PEDV sows had improved protection of piglets compared with VAD+PEDV sows as evident by increased weight gain, survival rates, reduced mean fecal scores post PEDV challenge. PEDV-specific IgA/IgG ASCs were highest in blood, milk and tissues of VAS/VAD+VA+PEDV sows, with their litters having highest IgA/IgG Ab titers in gut contents and highest PEDV neutralizing Ab titers in serum at PCD7, suggesting VA regulates these Ab responses. In VAD+PEDV (±VA) sows compared with VAS+PEDV sows, there was a trend for increased frequencies of B cell phenotypes in blood and milk; elevated $\alpha4\beta7^+$ MNCs in milk and higher mean areas positive for pIgR in mammary gland. Thus, while adequate levels of VA are important for B cell homeostasis, other IgA-producing/gut-homing B cells may be increased in VAD animals as a compensatory mechanism

Conclusions

Oral VA supplementation to pregnant and lactating PEDV infected VAD sows improved the function of gut-MG-sIgA axis, reducing diarrhea, virus shedding and mortality in neonates. Our findings may lead to more efficacious maternal vaccines enhancing active primary immunity and protection to piglets

Financial Support
U.S. National Institute of Child Health and Human Development

Notes:

P101 - Anticancer activity of milk fat rich in conjugated linoleic acid against Ehrlich ascites carcinoma cells

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Session: Systemic and mucosal immunology, Dec. 6, 6:00 - 8:00 PM

Objective

The major conjugated linoleic acid (CLA) isomers have anticancer effect, especially breast cancer cells, inhibits cell growth and induces cell death. Also, CLA has several health benefits in vivo, including antiatherogenesis, antiobesity, and modulation of immune function. The present study aimed to assess the safety and anticancer effects of milk fat CLA against in vivo Ehrlich ascites carcinoma (EAC) in female Swiss albino mice. This was based on acute toxicity study, detection of the tumor growth, life span of EAC bearing hosts, and simultaneous alterations in the hematological, biochemical, and histopathological profiles.

Methods

One hundred and fifty adult female mice were equally divided into five groups. Groups (1-2) were normal controls, and Groups (3-5) were tumor transplanted mice (TTM) inoculated intraperitoneally with EAC cells (2×106/0.2 mL). Group (3) was (TTM positive control). Group (4) TTM fed orally on balanced diet supplemented with milk fat CLA (40 mg CLA/kg body weight). Group (5) TTM fed orally on balanced diet supplemented with the same level of CLA 28 days before tumor cells inoculation. Blood samples and specimens from liver and kidney were collected from each group. The effect of milk fat CLA on the growth of tumor, life span of TTM, and simultaneous alterations in the hematological, biochemical, and histopathological profiles were examined.

Results

For CLA treated TTM, significant decrease in tumor weight, ascetic volume, viable Ehrlich cells accompanied with increase in life span were observed. Hematological and biochemical profiles reverted to more or less normal levels and histopathology showed minimal effects.

Conclusions

The present study proved the safety and anticancer efficiency of milk fat CLA and provides a scientific basis for its medicinal use as anticancer attributable to the additive or synergistic effects of its isomers.

Notes:

P102 - Characterization of pig respiratory epithelial cells and their stimulation with bacterial and viral ligands.

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Session: Systemic and mucosal immunology, Dec. 6, 6:00 - 8:00 PM

Objective

In an earlier study we established primary respiratory epithelial cell cultures from pig nasal turbinate and trachea. This study is focused on characterizing immortalized pig epithelial cells from primary nasal turbinate and tracheal epithelial cell cultures and studying gene expression of primary cells upon stimulation with bacterial and viral ligands.

Methods

Primary nasal turbinate and tracheal cells were transfected with a plasmid vector containing SV40 large T antigen. Cell immortalization was confirmed by PCR. Indirect immunofluorescence assay was also used to further verify the immortalization. Immunocytochemistry (ICC) was performed to confirm the phenotype of cells and expression of cell specific markers. Bacterial and viral ligands were used to stimulate primary cells and study the changes in gene expression of various pattern recognition receptors (PRRs) and cytokines.

Results

SV40 immortalized epithelial cells were established. ICC showed cytokeratin expression validating original epithelial cell characteristics after immortalization. The presence of DNA band parallel to SV40 plasmid verified the successful transfection. Immunofluorescence based staining of SV40 protein further verified successful immortalization. We stimulated cells with bacterial ligands LPS, PGN, FLA, MDP, iE-DAP and inulin and viral ligands Poly I:C, Poly I:C with lyovec, and Imiquimod. IL-6, IL-10, TNF-alpha, beta defensins, NOD-2, and few TLRs showed significant changes upon bacterial ligand stimulation. Poly I:C with lyovec stimulation caused upregulation of genes like IL-1beta, TNF-alpha, NOD-1. Striking outcome was consistent downregulation of cytokines, RLRs and TLRs gens by Inulin nanoparticles.

Conclusions

Overall, pig immortalized respiratory epithelial cells were established and characterized and primary nasal turbinate and tracheal cells modulated gene expression of specific PRRs and cytokines upon stimulation with viral and bacterial ligands.

Notes:

P103 - Enterobactin-based immune intervention to control colibacillosis in poultry

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Session: Vaccines and vaccinology, Dec. 6, 6:00 - 8:00 PM

Objective

Colibacillosis, caused by avian pathogenic *Escherichia coli* (APEC), is one of the most significant infectious diseases in poultry. Recently, an enterobactin (Ent) conjugate vaccine has been successfully developed and is promising for controlling APEC. Here, we aimed to conduct a chicken trial to evaluate protective efficacy of the vaccine against systemic APEC infection.

Methods

Specific-pathogen-free White Leghorn chicks were subjected to a crossed design with two variables, vaccination (with or without) and APEC challenge (APEC O1, O78, or PBS), leading to 6 groups (9 or 10 birds per group). Chickens were subcutaneously injected with PBS or KLH-Ent conjugate vaccine (100 μg per bird) at 7 days old, followed by booster immunization at 21 days old and subsequent intratracheal APEC challenge (10^8 CFU/bird of O1 strain, O78 strain, or PBS control) at 28 days old. At 5 days post-challenge, all chicks were subjected to necropsy to examine lesions and APEC load in major organs. Blood samples were collected at different time points and used for ELISA to monitor specific immune responses. Ileal and cecal samples were collected from each bird for microbiome analysis.

Results

Ent conjugate vaccine elicited strong specific immune responses, leading to approximately 1,024 fold increase in the titer against the whole conjugate vaccine and 128 fold increase specifically directed against small Ent molecule when compared to unvaccinated groups. Upon APEC challenge, the O1 strain only caused sporadic lesions in lung, air sac, heart, liver, and spleen in control chicks, which were similar to those observed in vaccinated chicks. However, the highly virulent O78 strain caused extensive lesions in unvaccinated chicks; the KLH-Ent vaccination alleviated the lesions in different organs and significantly reduced the accumulative lesion scores. The vaccination did not reduce the APEC load of both strains in lung, liver, or spleen.

Conclusions

The Ent conjugate vaccine could elicit strong specific immune responses in chickens and confer protection against APEC infection.

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

Notes:

P104 - Effects of concurrent administration of RB51 and modified live viral vaccines on immune responses

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Session: Vaccines and vaccinology, Dec. 6, 6:00 - 8:00 PM

Objective

Cell mediated immunity (CMI) is an important component of the immune response associated with protection for *Brucella abortus* and bovine viral diarrhea virus (BVDV). Brucellosis and modified live viral vaccine are licensed individually, but may be used concurrently in the field. Concurrent administration of these vaccines has led to concerns about immune interference contributing to lack of protection. This information has largely been based on anecdotal evidence in the field and a controlled study to evaluate both *Brucella*-specific and BVDV-specific CMI responses has not been performed in the face of concurrent vaccination. Therefore, the goal of this study was to characterize response to vaccination and determine the effect of concurrent vaccine administration.

Methods

Peripheral blood mononuclear cells (PBMC) from cattle vaccinated with either *Brucella abortus* strain RB51, a viral modified-live (MLV) vaccine containing BVDV, both RB51 and a viral MLV vaccine containing BVDV, or unvaccinated controls were utilized to evaluate the frequency of CD4+, CD8+, γδ+ T cells and NK cell populations and the frequency of interferon gamma (IFNγ) production within these predominant cell types via flow-cytometry. This was accomplished utilizing two distinct assays to help evaluate both the *Brucella*-specific and BVDV-specific CMI responses independently.

Results

Our data demonstrated the lack of vaccine interference following concurrent administration of these MLVs. The data may even suggest some level of enhanced IFNγ production within some cell types with concurrent administration of RB51 and a viral MLV containing BVDV.

Conclusions

While concurrent administration of individually licensed vaccines may contribute to vaccine interference, the data from this study would suggest there is not interference of these two vaccines.

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

Notes:

P106 - Novel vaccinology platform-assisted development of a broadly protective vaccine for porcine post-weaning diarrhea

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Session: Vaccines and vaccinology, Dec. 6, 6:00 - 8:00 PM

Objective

Enterotoxigenic *Escherichia coli* (ETEC) strains producing K88 (F4) or F18 fimbriae and enterotoxins are the predominant cause of post-weaning diarrhea (PWD) in pigs, a common disease associated with significant economic losses to swine producers worldwide. A vaccine that induces antibodies to protect against adherence of K88 (F4) and F18 fimbriae and enterotoxicity of ETEC toxins would be effective against PWD. However, a broadly protective PWD vaccine is not yet available, because of virulence heterogeneity and the inability to identify antigens for protective antibodies against F18 fimbriae and enterotoxins.

Methods

In this study, we applied novel epitope- and structure-based vaccinology platform multiepitope-fusion -antigen (MEFA) along with identification of neutralizing epitopes of fimbriae K88 and F18, heat-labile toxin (LT), heat-stable toxin type I (STa) and type II (STb) and Shiga toxin 2e (Stx2e). Using this strategy, we generated a polyvalent fimbria-toxin MEFA immunogen for broad immunity to both ETEC fimbriae and four toxins, and evaluated its potential in development of a broadly protective PWD vaccine.

Results

Mice or pigs immunized with this fimbria-toxin MEFA protein developed strong IgG antibody responses to K88, F18, LT and STb and moderate responses to toxins Stx2e and STa. Importantly, antigen-induced antibodies inhibited adherence of K88-fimbrial or F18-fimbrial bacteria to pig intestinal cells and neutralized enterotoxicity of LT, STa and STb toxins and cytotoxicity of Stx2e toxin.

Conclusions

These results indicated that this fimbria-toxin MEFA induced broadly protective antibodies against two ETEC fimbriae and four toxins, strongly suggesting a potential application of this MEFA protein in developing an effective multivalent vaccine for PWD.

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

Notes:

P107 - Swine biomarkers of influenza vaccine-associated enhanced respiratory disease

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Session: Vaccines and vaccinology, Dec. 6, 6:00 - 8:00 PM

Objective

Adjuvanted whole inactivated virus (WIV) vaccines reduce clinical disease against homologous influenza A virus (IAV), but when challenged with antigenically drifted IAV may lead to vaccine-associated enhanced respiratory disease (VAERD). Currently VAERD can only be diagnosed by pathology at necropsy, and the discovery of a biomarker would be beneficial. The objective of this study was to evaluate hematology, plasma chemistry, serum acute phase proteins, and cytokines of bronchoalveolar lavage fluid (BALF) in a search for potential biomarkers for VAERD.

Methods

Pigs were immunized twice three weeks apart with WIV from a pre-pandemic human seasonal H1N1 virus, challenged with a 2009 pandemic H1N1 virus, and necropsied 5 days post infection (dpi). Blood for hematology and chemistry was collected at 0, 1, 3, and 5 dpi. At necropsy BALF was collected and tissues for pathology scored to compare vaccinated-challenged (V/C) to challenge only (NV/C). Blood and serum were submitted to clinical pathology laboratories for processing. C-reactive protein (CRP) and BALF were assessed by a commercial ELISA and multiplex bead-based kits.

Results

The V/C group developed VAERD with significantly higher macroscopic lung lesions and microscopic lesions in lung and trachea compared to NV/C. V/C pigs had elevated total white blood cells and neutrophils at 1 and 3 dpi, and elevated mean platelet volume at 3 dpi. V/C also had significantly decreased plasma protein, creatinine kinase, and calcium at 1 dpi, and significantly increased CRP at 3 and 5 dpi. In BALF, IL-1β, IL-6, IL-8, and TNF-α were increased and IFN-α decreased in V/C.

Conclusions

The blood and BALF changes were consistent with increased inflammation in V/C pigs. CRP may be useful as a biomarker at 3 and 5 dpi to evaluate the development of VAERD after challenge. BALF cytokines may also be useful, but the invasiveness of antemortem BALF collection may limit its utility for biomarkers.

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

Notes:

P108 - Development of a broadly protective vaccine against swine influenza A virus

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Session: Vaccines and vaccinology, Dec. 6, 6:00 - 8:00 PM

Objective

Influenza A virus of swine (IAV-S) is widespread in the swine population and can cause significant economic loss to swine producers. IAV-S vaccines have been commonly used for control of IAV-S in the US. Unfortunately, the efficacy of whole inactivated virus (WIV) vaccines is far from satisfactory as they fail to confer optimal levels of heterologous protection, mainly due to the substantial genetic diversity of IAV-S circulating in the field. The ultimate goal of this proposed research is to develop a novel vaccine against IAV-S that would elicit the broadest level of heterologous protection.

Methods

Hemagglutinin (HA) sequences of the IAV-S originating in the U.S. were collected from the Influenza Virus Resources database. Computational methods were used to design a consensus IAV-S HA immunogen which was cloned into a novel tri-segmented Pichinde virus (PICV) vector which is used to deliver the consensus HA immunogen in pigs.

Results

A recombinant PICV expressing an HA antigen was constructed. In vitro study showed that HA protein was expressed in cells infected with the recombinant pichinde virus.

Conclusions

This is an early phase of the project. The preliminary results demonstrated that PICV can be used to deliver immunogens into pig cells.

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

Notes:

P109 - Targeting subdominant rhoptry-associated proteins of *Babesia bovis* in a subunit vaccine against bovine babesiosis

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Session: Vaccines and vaccinology, Dec. 6, 6:00 - 8:00 PM

Objective

Babesia bovis is the causative agent of bovine babesiosis, an economically important disease that threats the US cattle industry. Live attenuated vaccines against *B. bovis* are available, but not licensed in the US due to several limitations. Development of sustainable and efficient subunit vaccines to control bovine babesiosis is a priority. Previous attempts to develop vaccines to *B. bovis* based on immunodominant antigens have so far failed. Our goal is to develop a subunit vaccine including subdominant conserved and functionally relevant antigens of *B. bovis*. We hypothesize that a subunit vaccine targeting the N-terminal (NT) immunosubdominant segment of the Rhoptry Associated Protein 1 (RAP-1) and the full-length RAP-1 Related Antigen (RRA) of *B. bovis* fused with the molecular adjuvant flagellin FliC, to potentiate immunogenicity, will protect cattle against acute bovine babesiosis.

Methods

As an initial step, we expressed and purified RAP-1 NT, RAP-1/FliC, RRA, RRA/FliC, and the control immunodominant RAP-1 C-terminal (CT) proteins for antigenicity analysis. Coding regions for these antigens were cloned into the eukaryotic expression plasmid pcDNA3.4 and successfully expressed as His tag fusion recombinant proteins in human embryonic kidney (HEK) 293 cells.

Results

The target proteins were efficiently expressed and purified using immunoaffinity. Purified proteins were recognized by an anti-His tag monoclonal antibody, and will be tested with bovine sera from *B. bovis* infected and protected cattle to reveal the antigenic profile of the subdominant antigens RAP-1 NT and RRA, in comparison to the immunodominant RAP-1 CT.

Conclusions

Next, we will evaluate whether the presence of FliC as a fused segment affects recognition of the target proteins by antibodies against the native versions of the proteins. Collectively, results from this phase of the study will set the rationale for vaccine trials in cattle using FliC-fused *B. bovis* RAP-1 NT and RRA as components of a subunit vaccine to control acute bovine babesiosis.

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

Notes:

P110 - Outer membrane proteins as potential vaccine candidates against *Fusobacterium necrophorum* infections

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Session: Vaccines and vaccinology, Dec. 6, 6:00 - 8:00 PM

Objective

Fusobacterium necrophorum is a Gram-negative, obligate anaerobe. Necrotic infections caused in cattle lead to liver abscess, foot rot, and calf diphtheria resulting in a high financial impact on the feedlot industry. Antibiotic administration is the mainstay to treat these infections, but resistance is rising. Hence, a vaccine could be the best alternative prophylaxis. As bacterial attachment to the host cell is a crucial step in most Gram-negative pathogenesis, Outer Membrane Protein (OMP) adhesins is an active study area for vaccine development.

Methods

The role of the *F. necrophorum* OMPs in the attachment, pathogenicity, and immunogenicity have not been studied thoroughly. High binding affinity adhesins (17 and 22kDa OMPs), and 67kDa cell surface protein (CSP) were identified by binding assays and pull-down assays, respectively, with bovine endothelial (EJG) cells. The corresponding genes were sequenced and cloned in the expression plasmids. The recombinant proteins were purified, and polyclonal antibodies were generated against these OMPs. The efficacy of these polyclonal antibodies was studied through adhesion inhibition assay, *in-vitro.*

Results

Our results showed that combinatorial treatment using 17 and 22kDa OMP antibodies significantly decreased bacterial adhesion to the host cells. Also, individual polyclonal antibody treatment raised against 67kDa CSP exerted a significant bacterial adhesion inhibition. Overall, the combinatorial inhibitory effect on adhesion was more significant.

Conclusions

We identified OMPs with potential roles in bacterial attachment to the host cell, which could serve as candidates for vaccine development. Further *in vivo* investigation is required to confirm the role of OMPs and develop the OMP-based vaccine against fusobacterial infection.

Financial Support
Purdue University; USDA Veterinary Services

Notes:

P111 - Development of an *in ovo* compatible live attenuated influenza vaccine by targeting multiple viral proteins

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Session: Vaccines and vaccinology, Dec. 6, 6:00 - 8:00 PM

Objective

Emerging avian influenza viruses continue to pose a great concern for poultry farms worldwide, necessitating the development of more broadly protective vaccines. Live attenuated influenza vaccines offer superb efficacies but their use in poultry farms is discouraged due to safety concerns related to emergence of reassortant viruses. Vaccination of chicken embryos inside eggs (*in ovo*) induces early immunity in young chicks while eliminates the safety concerns related to the use of live vaccines on farms. However, *in ovo* vaccination using live influenza vaccines results in high embryo mortality and therefore, severely affects the egg hatchability. The aim of this study is to develop a safe and effective live vaccine candidate that can be used for vaccination of chickens *in ovo*.

Methods

In ovo-compatible vaccine candidates were developed by mutating the reverse genetics plasmids for vaccine virus to either replace the hemagglutinin (HA) cleavage site of the H7 vaccine with that of the H6 virus or abrogate the expression of polymerase acidic X (PA-X) protein. Single and double mutant viruses were then rescued through reverse genetics and assessed for their pathogenicity in 10- and 18-day-old chicken embryos.

Results

In 10-day-old embryos, the HA and PA-X mutations reduced embryo lethality by ~25% and ~30%, respectively, while the combination of both mutations reduced the embryo lethality by ~70%. Accordingly, *in ovo* vaccination of 18-day-old embryos with the double mutant virus significantly improved the hatchability compared to the unmutated virus (83.3% vs 36.7%). Addition of two other innate immune-enhancing mutations into the polymerase basic 2 and non-structural protein 1 of the double mutant virus further improved the hatchability to levels comparable to mock vaccination (96.7%). Protective efficacy of the *in ovo*-compatible vaccines is currently being tested against a heterologous influenza virus challenge.

Conclusions

Successful outcome of this study is a step forward towards development and commercialization of safe, cost-effective, and broadly effective influenza vaccines for poultry.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture; Ohio State University

Notes:

P112 - Maternally derived antibody against canine parvovirus part 1: Dam transfer rate and variability within the litter

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Session: Vaccines and vaccinology, Dec. 6, 6:00 - 8:00 PM

Objective

Nomograph analysis of breeding dam titers against canine parvovirus (CPV-2) allows a better understanding of maternally derived antibody (MDA) levels in the resulting litter, with the end goal of improved puppy immunization outcomes. Criticisms of this method include uncertainty regarding antibody transfer rate from the dam and variability of absorption of MDA by the individual pups. The nomograph is currently based on the assumption of 100% transfer at birth and includes standard deviation of 1.0± to correct for intra-litter variability. The objectives of this study were to analyze the transfer rate of anti-CPV-2 antibody from bitch to litter and identify variability of maternally derived IgG between pups within the same litter.

Methods

Hemagglutination inhibition assay (HIA) determined quantitative titers against CPV-2 in 45 gravid beagle bitches at 4 weeks pre-whelp and in 45 resulting litters at 14 days of life. To obtain a birth estimate, a reverse half-life of 12 days was applied to each puppy titer. Geometric mean titer for each litter at birth was compared with that of the dam to determine rate of transfer. Transfer rates were compared using chi square.
Titers of individual beagle pups (n = 238) were compared within litter groups (n = 45) to determine variability of MDA absorption. Sample variance was calculated in Excel (VAR.S) for each litter, with >1.0 considered highly variable.
To control for breed, pre-vaccination puppy sera submitted to CAVIDS were similarly compared. This group included 87 privately-owned pups from 27 litters and 15 breeds (average 31 days old).

Results

Mean transfer rate was 80% (range 59-97%). Chi-square test showed no significant difference in IgG transfer rate between bitches (p= 0.8901).
Within 45 beagle litters, 6 litters (13%) had sample variance >1.0. For the privately-owned pups, one litter (3.7%) showed a sample variance >1.0.

Conclusions

These findings confirm nomograph expectations of 100% transfer and minimal variability within litters. Follow-up immunity testing after vaccination series remains crucial for all pups regardless of nomograph.

Financial Support
Winston's Challenge Fund Grant

Notes:

P113 - Maternally derived antibody against canine parvovirus part 2: Degradation half-life and vaccine interference

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Session: Vaccines and vaccinology, Dec. 6, 6:00 - 8:00 PM

Objective

Maternally derived antibody (MDA) interference causes immunization failure in pups. Nomograph analysis of dam titers aims to improve immunization outcomes by estimating the duration of vaccine interference in a litter. It is crucial to characterize MDA degradation to optimize the nomograph. This study analyzed sera from a group of 101 unvaccinated 14-day-old beagle puppies over 42 days to determine the MDA degradation rate and half-life. Degradation variability between individual puppies was also examined. Finally, this study analyzed the efficacy of parvovirus vaccination at various titers to characterize MDA blockage.

Methods

Serum samples were collected at Days 14, 28, 42, and 56. For half-life calculation, a subset of 48 pups with sufficient initial titers were used. All sera were tested via canine parvovirus hemagglutination inhibition assay. Resulting geometric titers were analyzed via repeated measures, one-way ANOVA with Tukey's multiple comparisons *post hoc* correction. Significance was set at 0.05 (95% CI). All pups were vaccinated against CPV-2 at day 42.

Results

Average half-life was found to be 11.7 days with a range of 7-21 days. The average geomean titer values were plotted to obtain a trend line (slope = -0.861 titer value/day). A repeated measures, one-way ANOVA found no significance in degradation (p = 0.531) between individual pups. Lastly, response to vaccination was maximized (91%) when maternally derived antibody declined below 1:20. MDA above 1:80 completely blocked active response.

Conclusions

Confirmation of the MDA half-life and relatively consistent decline between individual pups supports that nomograph analysis can be used to accurately predict an optimal vaccination schedule which can be applied to an entire litter. Follow-up testing of pups at the end of the vaccination schedule is essential to ensure active immunization.

Financial Support
Winston's Challenge Fund Grant

Notes:

P114 - A spike protein-based subunit COVID-19 vaccine for pets: Immunogenicity and protective efficacy in cats

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Session: Vaccines and vaccinology, Dec. 6, 6:00 - 8:00 PM

Objective

Multiple vaccines have been or are being developed to curb the spread of Severe Acute Respiratory Syndrome-Coronavirus-2 (SARS-CoV-2) among humans; however, there are only a few known vaccines that are being developed for pets. There is currently no evidence that pets play a significant role in human infection; however, reverse zoonosis is possible if infected owners expose their domestic pets to the virus during acute infection. The purpose of this study was to develop a Spike protein-based subunit COVID-19 vaccine for pets.

Methods

Sixteen 8-12-week-old outbred female and male kittens (n=4/group) were randomly assigned into four treatment groups: Group 1, antigen alone; Group 2, ESSAI oil-in-water nanoemulsion adjuvant (O/W Adj); Group 3, Alhydrogel® adjuvant (Alum Adj); Group 4, PBS administered control animals. All animals were vaccinated twice at day 0 and 14, intramuscularly in a volume of 0.5 mL (Groups 1-3: 5 µg of spike protein). On days 0 and 28 serum, samples were collected to evaluate IgG, ACE-2 cell receptor blocking, neutralizing antibodies (SARS-CoV-2 wild-type and Delta variants) and whole blood for hematology studies. At day 28, animals of all groups were challenged with wild-type virus intranasally at dose 10^6 $TCID_{50}$. On day 31, tissue samples (lung, heart, and nasal turbinates) were collected with further viral RNA detection and virus titration. All tissues processed for histology.

Results

This vaccine with O/W and Alum adjuvants for pets induces strong SARS-CoV-2-specific IgG, angiotensin-converting enzyme 2 (ACE-2) blocking and neutralizing (against both wild-type and Delta variant) antibodies in vaccinated kittens. In contrast to control group, infectious virus not detected in oropharyngeal swabs and lung of vaccinated kittens after challenge.

Conclusions

This study established that a spike protein-based subunit COVID-19 vaccine with O/W and Alum adjuvant formulations immunogenic and protective against SARS-CoV-2 infection in kittens.

Financial Support
This research was supported by the Kazakh National Agrarian Research University and partially by the grant AP09259609 funded by the Science Committee of the Ministry of Education and Science of the Republic of Kazakhstan

Notes:

P115 - Subcutaneous BCG administration induces innate training in peripheral blood monocytes in pre-weaned calves

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Session: Vaccines and vaccinology, Dec. 6, 6:00 - 8:00 PM

Objective

The Bacillus Calmette Guerin vaccine is administered to prevent human and bovine tuberculosis. Studies have shown that BCG administration induces non-specific protection against other infections. This non-specific protection is attributed to the induction of innate training. This study aimed to evaluate the efficacy of BCG-induced innate training on the outcome of a respiratory virus challenge in pre-weaned calves.

Methods

Calves were vaccinated subcutaneously with 10^7 CFU BCG Danish strain (control calves received PBS). Peripheral blood was collected at two weeks and four weeks post-administration to evaluate innate cytokine production. PBMCs and CD14+ monocytes were stimulated in-vitro - E. coli LPS (1 µg/mL) or Pam3CSK4 (10 µg/mL). The production of proinflammatory cytokines IL-1b and IL-6 were measured using a commercial ELISA kit. At five weeks post-administration, the calves were challenged via aerosol inoculation of ~10^4 $TCID_{50}$ BRSV 375 and euthanized eight days post-challenge.

Results

PBMCs from BCG-treated calves showed enhanced IL-1b production after in-vitro stimulation with LPS compared to control calves two weeks post-administration. CD14+ monocytes from vaccinated calves showed increased IL-1b and IL-6 secretion at four weeks post-administration.

Conclusions

Subcutaneous BCG administration can train innate immune cells to exhibit a “memory-like” phenotype and ameliorate disease outcomes in BRSV challenged calves. Current efforts are focused on the characterization of epigenetic reprogramming in trained innate immune cells.

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

Notes:

P116 - New adjuvants for injectable poultry vaccines, presentation of two new case studies.

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Session: Vaccines and vaccinology, Dec. 6, 6:00 - 8:00 PM

Objective

Oil-based adjuvants are the most used in poultry injectable vaccines due to their ability to induce high and long term immunity. A major challenge of oily adjuvants is to induce a fast onset of immune response to improve early protection of animals. In this aim, a new water-in-oil (W/O) adjuvant (Montanide™ ISA 78 VG (ISA 78)) was developed. Furthermore, a new adjuvant based on polymeric technology was also developed. This aqueous adjuvant (Montanide™ GEL P PR (GEL P)) meets a growing need for safety, particularly for species raised for meat production.

Methods

In a first trial, 28-day-old SPF chickens were injected with bivalent ND and Avian Influenza H9N2 (AI) vaccine adjuvanted with ISA 78 or standard water in oil (W/O). The vaccine based on ISA 78 induced significantly higher antibody titers against AI and ND than standard W/O adjuvant from D7 to D21.
In a second trial, 7-day-old yellow-feather broilers were injected twice (0.3ml - SC in the neck; boost on D28 - 0.3 ml - IM on chest) with a multivalent vaccine based on GEL P or standard water-in-oil adjuvant.

Results

In the 1st trial, for each antigen, the antibody threshold ensuring animal protection was reached earlier in the ISA 78 vaccine group. At D28 post vaccination, an AI challenge was performed. At 5 days post challenge, mucosal swabs were assessed for virus presence by HI titration in embryos. A rate of protection of 100% was observed in adjuvanted vaccine groups.
In the 2nd trial, no vaccine residue was visible 21 days after each injection with GEL P, whereas oil-based vaccines left local reactions in the injection site. A challenge with three serotypes of E. coli (O2, O35, O78) was performed 21 days after boost, the GEL P adjuvanted vaccine induced a higher protection against the three serotypes.

Conclusions

These results show that GEL P is balanced in terms of safety profile, and protection against bacterial diseases. Besides, ISA 78 can induce a strong and fast immune response compared to standard oil-based adjuvants. Both adjuvants are suitable candidate adjuvants for the formulation of inactivated poultry vaccines.

Notes:

P117 - African swine fever virus CD2v protein induces β-interferon expression and apoptosis in swine peripheral blood mononuclear cells

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Session: Virology, Dec. 6, 6:00 - 8:00 PM

Objective

African swine fever (ASF) is characterized by severe destruction of lymphoid tissue and massive lymphocyte depletion due to apoptosis which is most likely due to proteins or factors released or secreted from ASF virus (ASFV) infected macrophages. As shown by our preliminary data, ASFV CD2v is present in the supernatant of CD2v expressing cells and exposure of cells to CD2v leads to induction of IFN-β and ISGs. The objectives of this study are to define the mechanism of induction of IFN-β by ASFV CD2v, and to determine the effect of CD2v on swine lymphocytes/macrophages survival.

Methods

IFN-β transcription and NF-κB-p65 nuclear translocation in CD2v-expressing or CD2v-treated cells were assessed by RT-PCR and immunofluorescence (IF), respectively. Interaction of CD2v with CD58, the natural CD2 ligand, was confirmed using co-immunoprecipitation (Co-IP) and co-localization assays. To examine the involvement of CD2v-CD58 interaction in CD2v-mediated IFN-β induction and NF-κB nuclear translocation, siRNA knock-down of CD58 was performed. Finally, the effect of CD2v treatment on swine PBMCs and macrophage apoptosis were assessed by western blot (caspase-3 and PARP1 cleavage) and TUNEL assay.

Results

CD2v expression in swine PK15 cells induces NF-κB-dependent IFN-β and ISGs transcription, and an antiviral state. Similar results were observed for CD2v protein treated swine PBMCs and macrophages, the major ASFV target cell. Co-IP and co-localization studies revealed that CD2v interacts with CD58, the natural host CD2 ligand. And, CD58 knockdown in cells or treatment of cells with an NF-κB inhibitor significantly reduced CD2v-mediated NF-κB activation and IFN-β induction. Further, antibodies directed against CD2v inhibited CD2v-induced NF-κB activation and IFN-β transcription in cells. Notably, treatment of swine PBMCs and macrophages with CD2v protein induced apoptosis.

Conclusions

ASFV CD2v activates NF-κB which induces IFN signaling and apoptosis in swine lymphocytes /macrophages thus suggesting its potential role in the lymphoid tissue damage and lymphocyte depletion observed during acute ASF.

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

Notes:

P118 - Assessment of SARS-CoV-2 strain competition in co-infected adult white-tailed deer

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Session: Virology, Dec. 6, 6:00 - 8:00 PM

Objective

The zoonotic *betacoronavirus* SARS-CoV-2 has spread rapidly around the world resulting in a global public health crisis. Early in the pandemic, the genetic lineage A of SARS-CoV-2 dominated. However, as the virus continues to circulate, variants of concern (VOCs), characterized by increased fitness and transmissibility, have emerged. The lineage B alpha VOC, (B.1.1.7 or UK variant) emerged in the fall of 2020, and has rapidly become a dominant variant. Additional beta, gamma, and delta VOCs emerged in mid to late 2020 in South Africa, Brazil, and India, respectively.
A thorough understanding of potential wildlife reservoirs is needed for developing effective control strategies for SARS-CoV-2. White-tailed deer (*Odocoileus virginianus)* fawns have been shown to be susceptible to SARS-CoV-2. To investigate the role of an emerging VOC in this host, we conducted a competition experiment whereby adult white-tailed deer (WTD) were co-infected with lineage A (USA-WA1/2020) and lineage B alpha variant (B.1.1.7-like; USA/CA_CDC_5545/2020) SARS-CoV-2 isolates.

Methods

Nasal/oropharyngeal swabs and respiratory tissues from adult WTD, which were co-infected intranasally and orally, were evaluated using next generation sequencing (NGS) and BLAST-based analysis, and the percentages of the individual lineage A and B viruses were determined at multiple time points post-infection. NGS results were confirmed using lineage A and B-specific RT-qPCR assays targeting a non-conserved region within the spike gene.

Results

The SARS-CoV-2 lineage B alpha variant quickly became the dominant virus in WTD following co-infection with lineage A and B viruses. The lineage A strain was only recovered at early time points in small quantities. Additionally, our preliminary data shows that the RT-qPCR assays were capable of differentiating both SARS-CoV-2 lineages.

Conclusions

The SARS-CoV-2 lineage B alpha variant has improved fitness compared to the lineage A virus in adult WTD. The RT-qPCR assays could potentially be used in epidemiological surveillance for VOCs in human and animal populations.

Financial Support
Center of Excellence for Emerging and Zoonotic Animal Diseases; U.S. Department of Agriculture, Animal and Plant Health Inspection Services; Center on Emerging and Zoonotic Infectious Diseases

Notes:

P119 - Ruminant susceptibility to SARS-CoV-2: Experimental infection of sheep and adult white-tailed deer

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Session: Virology, Dec. 6, 6:00 - 8:00 PM

Objective

Natural and experimental infection of multiple animal species with SARS-CoV-2 has been demonstrated, most commonly in rodents, mustelids, and companion animals, suggestive of a broad host tropism of this virus. However, studies evaluating the SARS-CoV-2 susceptibility of important agricultural species are sparse and limited to lineage A virus strains. White-tailed deer (WTD) fawns have been identified as a susceptible animal species. Here, we investigated the susceptibility of two farmed ruminant species: sheep and adult WTD.

Methods

In separate experiments, eight sheep and six WTD were intranasally and orally inoculated with a 2mL dose of $1x10^6$ $TCID_{50}$ per animal. Two sentinels were included in each experiment to evaluate transmission. Nasal, oral, and rectal swabs were analyzed for the presence of SARS-CoV-2-specific RNA (vRNA). Serum was evaluated for the presence of virus neutralizing antibodies and antibodies to the SARS-CoV-2 nucleocapsid (N) and receptor binding domain (RBD) proteins by indirect ELISAs.

Results

WTD: Inoculated deer shed vRNA and/or infectious virus through their nasal, oral and rectal cavities for up to 7 days post challenge (DPC), with vRNA detected in tissues at 4 and 18 DPC. Also, infected WTD produced SARS-CoV-2-specific neutralizing and N- and RBD-specific antibodies. Transmission to sentinels was efficient within 2 days of co-housing. Sheep: vRNA was detected in the nasal swabs of all inoculated sheep on 1 DPC; persisting in one animal until 3 DPC. Several animals developed N- and RBD–specific antibodies. One principal infected animal developed neutralizing antibodies. vRNA was also detected in tissues collected at 4 and 8 DPC. One sentinel sheep tested positive for vRNA in oral swabs two days after co-housing with the principal animals. This animal also had vRNA in tissues at necropsy on 21 DPC.

Conclusions

These studies indicate that adult WTD are highly susceptible to experimental SARS-CoV-2 infection, whereas sheep show low susceptibility. This provides evidence of a broader range of domestic and wild ruminant hosts which are susceptible to SARS-CoV-2.

Financial Support
U.S. Department of Agriculture, Animal and Plant Health Inspection Services; Center of Excellence for Emerging and Zoonotic Animal Diseases; Center on Emerging and Zoonotic Infectious Diseases

Notes:

P120 - Toward a bovine breathalyzer: Association between bronchoalveolar lavage volatile organic compounds and calf health

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Session: Virology, Dec. 6, 6:00 - 8:00 PM

Objective

Volatile organic compounds (VOCs) are released from cells and tissues in the body and can be used as a diagnostic indicator of disease. In this study, we aim to use VOCs and cytologies obtained via bronchoalveolar lavage (BAL) in bovines to determine presence of respiratory disease, with the goal of analyzing VOCs to predict respiratory disease outbreaks in a herd before they occur. This pilot study will lead the way towards use of bovine breathalyzers in herd management.

Methods

Sampling from 18 calves was performed, n= 9 healthy and n= 9 declared diseased, at two different time points approximately 10 weeks apart. The diseased calves were selected based on the presence of clinical symptoms such as cough, dropped head carriage, excessive nasal discharge, and dull mentation. A respiratory grade of mild, moderate, or severe was given to each calf based on lung auscultation, nasal discharge, presence or absence of cough, dyspnea, and thoracic ultrasound, on a scale of 0-12. BAL samples were collected and divided into two aliquots per calf at each timepoint. One aliquot was reserved for fluid cytology, and the other was sent for VOC analysis.

Results

Samples for each calf will be compared across the two timepoints. On BAL cytologies, there were three main categories the calves fell into - unremarkable, nonsuppurative inflammation, and chronic inflammation. We expect to see qualitative differences in the VOC composition between healthy and diseased calves, and improvement in the VOC composition after initially diseased calves are treated and re-sampled at the second time point.

Conclusions

The qualitative differences in VOCs between diseased and non diseased bovines will serve useful as a diagnostic tool to predict and manage herd outbreaks of respiratory disease. This pilot study serves as the basis for future use of bovine breathalyzers in herd management.

Notes:

P121 - Cloning of porcine macrophage cell lines suitable for genetic screens for host factors involved in PRRSV infection

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Session: Virology, Dec. 6, 6:00 - 8:00 PM

Objective

Porcine reproductive and respiratory syndrome virus (PRRSV) is the causative agent of PRRS, an economically devastating disease of swine that is characterized by reproductive failure in pregnant sows and respiratory problems and growth retardation in piglets. The goal of this study is to understand the initial process of PRRSV gaining access to the interior of host cells.

Methods

To accomplish this goal, we first cloned eight porcine alveolar macrophage-derived single-cell clones (PAM sc1 to sc8) that differ in susceptibility to PRRSV infection for our gain-of-function screens. We further engineered one of the single-cell clones (PAM sc3) displaying higher susceptibility to PRRSV infection, in order to stably express the Cas9 protein (PAM sc3/Cas9) for our loss-of-function screens.

Results

This work is an ongoing project involving the use of two complementary, technologically advanced genome-scale genetic screens for gain- and loss-of-function of PRRSV entry. For the gain-of-function screen, we are using a cyclical packaging rescue strategy with a retroviral cDNA library, derived from the PRRSV-susceptible porcine macrophage cell line PAM sc3, to identify one or more cellular genes that confer susceptibility to PRRSV infection on the PRRSV-nonsusceptible porcine kidney cell line PK-15. For the loss-of-function screen, we are using a multiplexed CRISPR screen strategy with a lentiviral porcine sgRNA library to identify cellular genes that play an important role in PRRSV entry into the PRRSV-susceptible porcine macrophage cell line PAM sc3/Cas9.

Conclusions

The outcomes of this study will provide a framework for a more complete understanding of how PRRSV-host cell interactions occur at the level of PRRSV entry and offer new multiple targets for the prevention of PRRSV infection.

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

Notes:

P122 - Clinical and pathological presentation of African swine fever in pigs slaughtered in central Uganda

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Session: Virology, Dec. 6, 6:00 - 8:00 PM

Objective

African swine fever (ASF) is an infectious disease of pigs caused by a DNA virus in the family *Asfarviridae*. The virulence of its clinical presentation and pathological lesions depends on the infecting virus' genotype. Although ASF is endemic in Uganda, there is not a comprehensive understanding of its clinical and pathological manifestations there. The objective of this presentation is to describe the clinical and pathological presentation of ASF suspect pigs in slaughterhouses in the Kampala metropolitan area.

Methods

This study is ongoing and at least 1200 pigs will be sampled from Wambizi, Lusanja, Budo, Katabi, Buwate, and Kyetume pig slaughterhouses over a 13-month period from May 2021 through June 2022. Data of 192 pigs sampled from May 2021 through July 2021 will be presented. Stratified random sampling coupled with systematic sampling of the pigs on the day of sampling is being used to select pigs from each slaughterhouse. Clinical and pathological scoring algorithms capture the ASF clinical signs and lesions in the pigs. Frequencies and proportions were used to summarize the data.

Results

Of the 192, 90 (46.9%) were of exotic breed, 47 (24.5%) were mixed, 53 (27.6%) were of local breed, and two (1%) were of unknown breed. Of the 192 pigs with clinical signs suggestive of ASF, 42 (21.9%) had skin discolorations and 8 (4.2%) had evidence of diarrhea. For the postmortem signs typical of ASF, 51 (26.6%) of the 192 pigs had mildly to grossly enlarged spleens and 60 (31.3%) had hemorrhagic spleens. The gastro-hepatic lymph nodes were enlarged, hemorrhagic, and/or edematous in 69 (35.9%) pigs. Petechial hemorrhages were found on 55 (28.6%) of the pigs' kidneys as well.

Conclusions

Our findings suggest that up to 30% of pigs slaughtered in central Uganda may have clinical signs and lesions suggestive of ASF. The serologic and molecular diagnostic testing as well as the sequencing that will be conducted at a later stage will give us deeper insights into the ASF situation in Uganda. These findings could inform efforts aimed at developing an ASF slaughterhouse surveillance system for Uganda.

Financial Support
U.S. Defense Threat Reduction Agency; U.S. Department of Defense, Defense Threat Reduction Agency

Notes:

P123 - Assessment of viremia associated with porcine epidemic diarrhea coronavirus using different molecular techniques

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Session: Virology, Dec. 6, 6:00 - 8:00 PM

Objective

Porcine epidemic diarrhea virus (PEDV) causes a highly contagious watery diarrhea affecting pigs. In April 2013, PEDV emerged for first time in the USA and spread very rapidly causing devastating losses. Spray dried porcine plasma (SDPP) contaminated with PEDV was implicated in the infection of PEDV in some cases. Although the presence of PEDV RNA has been described in the plasma of infected animals, it is unknown whether this detection actually corresponds to the presence of viable virus. In this study we will provide new insights into the detection of PEDV in plasma through the application of molecular techniques that allow to differentiate between viable and non-viable PEDV particles.

Methods

A total of 15 samples (5 sera and 10 SDPP) positive for PEDV by qPCR were tested for the presence of viable virus using the following methods: 1) Four overlapping fragments of the spike gene were amplified to subsequently obtain the complete sequence of the S-gene by sequencing in both directions by Sanger methodology; 2) Total RNA was directly sequenced, without using any primer or amplification step, through next generation sequencing (NGS) on an Illumina Miseq platform, obtaining the PEDV sequences by applying a virus-specific script developed by us.; 3) Each sample was analyzed by viability PCR (vPCR) using two metal compounds, platinum chloride (PtCl4) and cis-diamineplatinum dichloride (CDDP).

Results

None of the 4 fragments that constitute the S gene was amplified by PCR in any of the samples studied. Similarly, it was not possible to obtain any PEDV sequence by NGS from the sera or SDDP samples. Finally, viable virus was not detected by vPCR in any of the samples analyzed and the amplification signal was completely eliminated by PtCl4 or CDDP.

Conclusions

The results obtained support the finding that there was no viable virus in these samples. Thus, detection of viral RNA in these sera and SDDP samples was probably a consequence of the intestinal absorption of nucleic acid fragments released during the viral replication process and not a real spread of the virus in the blood or viremia.

Financial Support
National Institute of Agricultural and Food Research and Technology (INIA project E-RTA2015- 0003- C02- 01 and E-RTA2015- 0003- C02- 02)

Notes:

P124 - Vitamin A deficiency reduces innate and humoral immunity in rotavirus A-challenged sows and piglets protection

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Session: Virology, Dec. 6, 6:00 - 8:00 PM

Objective

We examined the effects of vitamin A (VA) deficiency on innate and humoral immune responses against porcine rotavirus A (RVA) infection in RVA seropositive conventional pregant sows and lactogenic immunity in their RVA infected piglets.

Methods

Conventional pregnant sows were fed VA deficient (VAD) or VA sufficient (VAS) diets from gestation day (GD) 30 and challenged with RVA OSU (G5P7) at GD88. Sow blood was collected at GD88-90, GD109-113, 3-5 days post-partum (DPP), 10-14DPP and 21DPP while milk was collected at 0 DPP and the corresponding DPP. Serum RVA-specific IgA/ IgG antibodies (Ab) and IgA/IgG antibody secreting cells (ASC) were determined using ELISA and ELISPOT, respectively. Mononuclear cells (MNCs) were isolated from blood and milk at above times and from various tissues at 21DPP. All MNCs were stained and analyzed to quantify frequencies of dendritic cells (DC) and natural killer (NK) cells using flow cytometry. Piglets were challenged at PPD5 with RVA OSU (G5P7) and RVA shedding titers determined using qRT-PCR.

Results

RVA-specific IgA Ab titers in serum were decreased in VAD at 3-5 DPP and10-14DPP, whereas RVA-specific IgG Ab titers were decreased in VAD at DPP10-14 and DPP 21-30. RVA-specific IgA ASCs were decreased at all times while RVA-specific IgG ASC were decreased at 10-14 and 21-30 DPP in VAD serum. The frequencies of activated and $MHCII^+$ conventional DCs in blood and all tissues MNCs were decreased in VAD compared with VAS. NK cell frequencies were lower in blood at 10-14 and 21-30 DPP, ileum, mammary gland, and mesenteric lymph nodes MNCs in VAD than VAS. Lastly, RVA RNA shedding titers in RV challenged nursing piglets were higher in VAD piglets from post-challenge day (PCD) 3 to 12 than in VAS piglets.

Conclusions

Our preliminary results reveal that VA deficiency reduces innate and humoral immune responses in RV challenged conventional pregnant sows as well as lactogenic protection of their piglets, as reflected in higher RVA shedding titers in VAD derived piglets.

Financial Support
U.S. National Institutes of Health

Notes:

P125 - Krüppel-like factor 4 and type 1 nuclear hormones transactivate the bovine herpes virus 1 ICP0 early promoter

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Session: Virology, Dec. 6, 6:00 - 8:00 PM

Objective

Bovine Herpesvirus 1 (BoHV-1) is a major pathogen of cattle. Infection causes serious respiratory disease and reproductive failure. BoHV-1 establishes lifelong latency in neurons, marked by periodic reactivation from latency. Understanding this cycle is key to reducing the disease burden in cattle. Stress reliably induces BoHV-1 reactivation from latency, activation of the glucocorticoid receptor (GR) and expression of cellular genes, including Kruppel-like factor 4 (KLF4). GR and related progesterone and androgen nuclear hormone receptors (NHR) cooperate with KLF4 to transactivate viral genes that drive productive infection and reactivation, including Infected Cell Protein 0 (bICP0) and bICP4. The studies presented here identify regulatory sequences for transactivation of the bICP0 early promoter (EP) by KLF4 and the NHRs.

Methods

The bICP0 EP (EP-943) was cloned into pGL3-basic, a luciferase reporter plasmid, along with truncated fragments, EP-638 and EP-328. KLF4 and related Sp1 binding sites were identified and mutated in all three promoter fragments. The promoter constructs were transfected into Neuro-2A cells, along with KLF4 and NHR expression plasmids, and then treated with the respective hormone. Promoter activity was measured by dual luciferase assay. Binding of KLF4 and the NHRs to the bICP0 E promoter was measured by chromatin immunoprecipitation.

Results

KLF4 and the NHRs cooperatively transactivated all three bICP0-EP fragments and associated with the promoter DNA. Transactivation and promoter occupancy were reduced by mutating specific KLF4 and Sp1 binding sites. Treatment with the respective hormones reduced NHR-dependent transactivation.

Conclusions

Key bICP0 EP regulatory sequences were identified by reduced activation and occupancy of the mutated bICP0 E promoter. Occupancy of the promoters did not always match changes in transactivation, suggesting promoter occupancy alone by KLF4 and the NHRs is not sufficient for transactivation. Hormone treatment reduced transactivation by all three NHRs suggests promoter activation occurred via a ligand-independent mechanism.

Financial Support
U.S. National Institutes of Health; Oklahoma State University; USDA-NIFA

Notes:

P126 - Immunological evaluation and comparison of different Cache Valley virus vaccine candidates

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Session: Virology, Dec. 6, 6:00 - 8:00 PM

Objective

Cache Valley virus (CVV) is a mosquito-borne arbovirus that is enzootic throughout the New World. Whilst CVV is known as an important agricultural pathogen, primarily associated with embryonic lethality and abortions in ruminants, it has recently been recognized for its expansion as a zoonotic pathogen. With the increased emergence of bunyaviruses with human and veterinary importance, there have been significant efforts dedicated to the development of bunyavirus vaccines. In this study, immunogenicity of a CVV vaccine candidate based on the deletion of NSs and NSm genes (CVVdelNSs/delNSm) was evaluated and compared to a vaccine candidate created through the inactivation of CVV using binary ethylenimine (BEI) with the addition of an aluminum hydroxide adjuvant (BEI-CVV) in sheep.

Methods

Immunization of 20 sheep with one of the two vaccine candidates was performed followed by two booster immunizations. Blood was taken for sera collection on multiple days throughout the study. Plaque reduction neutralization test was then used to monitor the development of neutralizing antibodies elicited by each vaccine candidate.

Results

Adverse events were not observed in sheep that received the CVVdelNSs/delNSm candidate, however, small granulomas appeared at the vaccination site of the sheep that received the BEI-CVV candidate, which is most likely due to the adjuvant used. Immune protection was observed between both vaccine candidates with no significant difference when comparing them overall.

Conclusions

This study has identified a promising vaccine candidate for CVV and possibly other bunyaviruses. The development of a vaccine for ruminants could lead to less human exposure and a platform for CVV and other emerging bunyaviruses that have already or could potentially cause future outbreaks.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture; Biotechnology and Biological Sciences Research Council; USDA APHIS NBAF Scientist Training Program

Notes:

P127 - Construction of a mouse-feline chimeric neutralizing antibody against SARS-CoV-2

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Session: Virology, Dec. 6, 6:00 - 8:00 PM

Objective

The COVID-19 pandemic caused by SARS-CoV-2 has become the biggest challenge in public health worldwide. Recent studies have reported that felid animals are susceptible to SARS-CoV-2 infection. The objective of this study is to develop a mouse-feline chimeric neutralizing antibody for potential use as diagnostic tool and therapeutic agent against SARS-CoV-2 infections in cats.

Methods

A synthetic peptide of neutralizing epitope (ILPDPSKPSKRSFI) from S2 protein was used for mice immunization. SARS-CoV-2 specific mAbs were screened using *in vitro* expression system. The chimeric mAb was constructed by fusing the variable region of mouse mAb against SARS-CoV-2 S2 to the constant region of the feline antibody. Isotype of mAb was initially determined; Subsequently, variable regions were amplified and inserted into the plasmid pFUSE-CHIG and pFUSE-CLIG, respectively. The chimeric antibody was expressed by co-transfecting 293T cells with plasmids containing heavy chain and light chain. The binding activity of chimeric antibody was verified by IFA, while the neutralizing activity was determined using pseudovirus system expressing SARS-CoV-2 S protein.

Results

A panel of mAbs against SARS-CoV-2 S2 neutralizing epitope was generated and mAb #220-C1 was selected for the construction of mouse-feline chimeric neutralizing antibody. This mAb has an isotype of IgG2a. The chimeric mAb #220-cmC1 was successfully expressed and purified. IFA result shows that the chimeric mAb can recognize the spike proteins expressed in transfected cells and can be further detected by anti-feline secondary antibodies. In addition, result from pseudovirus assay demonstrates that the chimeric mAb has comparable neutralizing activity as mouse-origin mAb.

Conclusions

We developed a mouse-feline chimeric neutralizing antibody, which provides a valuable tool for SARS-CoV-2 diagnostics and may have potential use in therapeutics, especially in infected cats. The plasmid constructs and methodology developed in this study can be adapted to construct chimeric mAbs for other feline pathogens in aid of disease control in felid animals.

Notes:

P128 - Effects of the nasal microbiota and antibiotic treatment on the infectivity of swine influenza virus in pigs.

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Session: Virology, Dec. 6, 6:00 - 8:00 PM

Objective

The aim of this project was to study the influence of nasal microbiota changes and antibiotic treatment on the infectivity of swine influenza virus in pigs.

Methods

In this study, 24 weaned piglets were used and divided in 4 groups (6 piglets/group). Piglets were quarantined for 3 days. On day 4, nasal swab and blood samples were collected and piglets from group 2 and 4 were treated with tulathromycin. After 5 days of tulathromycin treatment, nasal swab, and blood samples were collected prior to virus challenge. Group 1 and 2 piglets were mock inoculated with PBS. Group 3 and 4 piglets were inoculated with swine influenza A virus. Nasal swabs were collected at 1-, 3-, and 5-days post challenge (DPC). At 5 DPC, all pigs were euthanized. Blood, nasal swab, mediastinal lymph node (MLN), and lung samples were collected, and lungs were examined for pneumonic lesions.

Results

All nasal microbiome samples were sequenced using 16sRNA gene sequencing. Four major phyla namely Firmicutes, Proteobacteria, Bacteroidetes, and Actinobacteria were found in the nasal swabs. After 5 days of tulathromycin treatment, phylum Actinobacteria and Proteobacteria were found diminishing in their relative abundance. After 10 days post antibiotic treatment relative abundance of Actinobacteria phyla was drastically lower in all samples; however, relative abundance of proteobacteria increased. At family level, antibiotic reduced the abundance of Pasteurellaceae after 5 days of treatment, but Lactobacillaceae and Lachnospiracea showed increased abundance. Viral titers in nasal swabs and lung homogenate were not different between two virus infected groups. Group 3 piglets had lung lesion score of 27.75 ± 6.34 and were significantly different from group 4 piglets (11.34±4.87). MLN samples were analyzed for expression of many cytokine genes, but no significant differences were observed among 4 groups.

Conclusions

This study showed that nasal microbiome changed in response to use of tulathromycin in pigs and this antibiotic treatment did not change virus titers but provided protection against influenza virus infection.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture; USDA-NIFA

Notes:

V-001 - Transcriptomes of the bivalve pathogen *Vibrio coralliilyticus* and the probiont *Phaeobacter inhibens* in co-culture

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Session: Antimicrobial Alternatives

Objective

Oyster aquaculture is a growing industry, though it carries large risks due to disease outbreaks that decimate larval and juvenile stocks. The addition of the probiotic bacterium *Phaeobacter inhibens* S4 to aquaculture systems has been demonstrated to protect oyster larvae against bacterial pathogens such as *Vibrio coralliilyticus* RE22. The purpose of this study was to gain a more comprehensive understanding of the complex interaction between RE22 and S4 to identify novel pathways involved in probiont activity.

Methods

RE22 and S4 were co-cultured for five hours after which biofilm and planktonic samples were collected during the exponential growth phase. RNA was extracted and processed via RNASeq using Illumina technology. The sequencing reads were then analyzed using a DESeq2 bioinformatics pipeline for differential gene expression between samples grown in monoculture and those grown in co-culture.

Results

Differential gene expression in the pathogen RE22 in co-culture with probiont S4 revealed down regulation of genes involved in RE22 virulence including the tssI gene repressed to just 3.6% of the control level, and up regulation of genes involved in detoxification and bacterial immune response such as the arsenic efflux protein increased 52-fold the rate of the control. In the probiont S4, an ROK family transcription regulator was slightly upregulated in co-culture. This family of transcription regulators has been linked to antibiotic production in other bacteria and may serve a similar role here. This transcriptional study corroborates results from studies using deletion mutants demonstrating the role of T6SS on RE22 competition with other bacterial species, as well as the role of antibiotic production and quorum quenching on S4 probiotic activity against RE22.

Conclusions

This study provides a comprehensive description of processes involved in bacterial competition between the pathogen RE22 and the probiont S4. It also highlights new pathways that may impact the effectiveness of probiotic bacteria in mitigating diseases in aquaculture systems.

Financial Support
U.S. Department of Agriculture

Notes:

V-002 - Phage endolysins as alternative antibiotics to control clostridia in poultry

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Session: Antimicrobial Alternatives

Objective

The endolysin PlyCP41 is known to exhibit lytic activity against *Clostridium perfringens* (CP) cells *in vitro*, and therefore could potentially be used *in vivo* to achieve similar results within the gastrointestinal tract (GIT). The objectives of this study were to confirm the lytic activity of PlyCP41 using a plate lysis assay and determine the abundance of commensal CP in the crop, small intestine, and ceca of healthy broiler chickens.

Methods

Three different enzyme preparations of PlyCP41 were diluted to a starting concentration of 2 mg/ml then spot plated onto 11 ml of semisolid agar containing 1 ml of CP cells at 55 OD concentration. Each enzyme was serial-diluted then plated on a grid using 5 µl per spot in descending concentrations. The plates were incubated at 37°C for 2 h then observed for lytic activity. Next, to measure CP levels in the gut, twenty newly hatched Cobb-500 broilers were raised on built-up litter from 0-14d. At 14d, three randomly selected birds were euthanized, then their crop, small intestine, and cecal contents were removed and immediately diluted 1:10 in PBS. Serial dilutions were conducted to 10^{-6}, then 0.1 ml of each dilution was spread in duplicate onto Tryptose Sulfite Cycloserine (TSC) agar and incubated under anaerobic conditions for 24h at 37°C. Typical black colonies were counted, and CFU/g of contents was estimated. A 1 g aliquot of contents from each section was also reserved for DNA extraction and qPCR to determine CP abundance.

Results

All three enzyme preparations lysed CP cells at 100 ng of enzyme per spot, and one purified protein preparation was effective at 10 ng per spot. The crop contained the fewest CP cells, with TSC plates averaging 7 x 10^4 CFU/g and qPCR averaging a Cq value of 24.9. The small intestine harbored approximately 8 x 10^5 CFU/g (Cq of 19.9), and the ceca had a similar population of 6 x 10^5 CFU/g and an average Cq of 18.8.

Conclusions

Future studies will test these enzyme preparations *in vivo* to determine the ability of PlyCP41 to reduce CP cells in the GIT of broiler chickens.

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

Notes:

V-003 - Detection of multidrug-resistant extended-spectrum β-lactamase producing *E. coli* from bulk tank milk obtained from dairy cattle farms

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Session: Antimicrobial Resistance/Use

Objective

Third-generation cephalosporin (3GC) is a critically important beta-lactam antibiotic class commonly used to prevent and treat several diseases of dairy cattle, including mastitis in U.S. dairy farms. As a result, resistance to 3GC is on the rise in dairy farms. We hypothesize that extended-spectrum beta-lactamase (ESBL) producing *E. coli* could be present in raw milk in the farms. Therefore, this study aimed to determine the presence of ESBLs *E. coli* and other potentially co-selected resistance genes in bulk tank milk obtained from four farms.

Methods

Bulk tank milk samples (n=8) were collected from four dairy farms in Tennessee. *E. coli* were isolated and screened on plain CHROMagar *E. coli*. Up to two colonies of *E.coli* were picked from the agar and plated on CHROMagar *E. coli* supplemented with tetracycline (16mg/L), cefotaxime (4 mg/L), and nalidixic acid (32 mg/L). Cefotaxime, a 3GC, resistant *E. coli* isolates were tested for the ESBLs gene using PCR. All 3GC resistant *E. coli* isolates were also tested using PCR for genes conferring co-resistance to the other five antibiotic classes.

Results

From fourteen (14) *E. coli* isolates obtained from bulk tank milk, five of the isolates were resistant to cefotaxime, a 3GC. All cefotaxime-resistant isolates were concurrently resistant to tetracycline. All but one cefotaxime resistant *E. coli* carry the $bla_{CTX\text{-}M}$ gene, an ESBL encoding gene. All cefotaxime-resistant *E. coli* were multidrug-resistant and carried multiple resistance genes. In addition to $bla_{CTX\text{-}M}$, we detected genes associated with tetracycline (*tetA, tetB,& tetM*), sulphonamide (*sul*I), streptomycin (*strA*), and fluorophenols (*floR*) resistance.

Conclusions

Our results indicate the occurrence of ESBLs-*E. coli* in bulk tank milk with multidrug resistance profile. Thus, the consumption of raw milk and dairy products made from raw milk poses a significant milk safety risk to the public. The precise source of ESBLs-*E. coli* contamination, whether from the udder or contamination during milking, needs to be identified and controlled.

Notes:

V-004 - Prevalence and mechanisms of antibiotic resistance in *Escherichia coli* isolated from mastitic dairy cattle in Canada

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Session: Antimicrobial Resistance/Use

Objective

Bovine mastitis is the most common infectious disease in dairy cattle with major economic implications for the dairy industry worldwide. Continuous monitoring for the emergence of antimicrobial resistance (AMR) among bacterial isolates from dairy farms is vital not only for animal husbandry but also for public health.

Methods

In this study, the prevalence of AMR in 113 *Escherichia coli* isolates from cases of bovine clinical mastitis in Canada was investigated. Kirby-Bauer disk diffusion test with 18 antibiotics and microdilution method with 3 heavy metals (copper, zinc, and silver) was performed to determine the antibiotic and heavy-metal susceptibility. Resistant strains were assessed for efflux and ß-lactamase activities besides assessing biofilm formation and hemolysis. Whole-genome sequences for each of the isolates were examined to detect the presence of genes corresponding to the observed AMR and virulence factors.

Results

Phenotypic analysis revealed that 32 isolates were resistant to one or more antibiotics and 107 showed resistance against at least one heavy metal. Quinolones and silver were the most efficient against the tested isolates. Among the AMR isolates, AcrAB-TolC efflux activity and ß-lactamase enzyme activities were detected in 13 and 14 isolates, respectively. All isolates produced biofilm but with different capacities, and 33 isolates showed α-hemolysin activity. A positive correlation (Pearson r = +0.89) between efflux pump activity and quantity of biofilm was observed. Genes associated with aggregation, adhesion, cyclic di-GMP, quorum sensing were detected in the AMR isolates corroborating phenotype observations.

Conclusions

This investigation showed the prevalence of AMR in *E. coli* isolates from bovine clinical mastitis. The results also suggest the inadequacy of antimicrobials with a single mode of action to curtail AMR bacteria with multiple mechanisms of resistance and virulence factors. Therefore, it calls for combinatorial therapy for the effective management of AMR infections in dairy farms and combats its potential transmission to the food supply chain through the milk and dairy products.

Financial Support
Canada Research Chair Funding; Institute of Nutrition and Functional Foods

Notes:

V-005 - Genetic determinants of integron-encoded antimicrobial resistance from *Salmonella enterica* of cattle origin

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Session: Antimicrobial Resistance/Use

Objective

Antimicrobial resistance (AMR) is an escalating global public health concern in *S. enterica,* a zoonotic livestock pathogen. *S. enterica* AMR is especially dangerous when isolates possess virulence factors and genes that contribute to pathogenicity. Previous studies suggest that integron presence is associated with carriage of AMR genes especially conferring multidrug resistance. However, the association of virulence factor genes with integron-containing *S. enterica* is still unknown. The objective of the current study was to compare the presence of AMR and virulence factor genes within the whole genome of *S. enterica* with and without integrons.

Methods

S. enterica isolated from cattle (n=33) was sourced from veterinary diagnostic laboratories across the US. Broth microdilution was used to establish phenotypic AMR. PCR and gel electrophoresis were used to identify integrons. DNA was isolated for whole genome sequencing on an Illumina MiSeq. Denovo assembly algorithms reconstructed each genome (chromosome and/or plasmids) in SPAdes through a Geneious Prime interface. Open-source databases were used to screen each genome for antimicrobial resistance and virulence factor genes. A Fisher's exact test was used to examine statistical associations of integron presence with AMR and virulence genes.

Results

Genome analysis revealed *S. enterica* containing integrons were significantly ($p<0.05$) more likely to have resistance to beta lactams, sulfa drugs, tetracycline, and multiple phenicol drugs. Integron-containing *S. enterica* were resistant to twice as many drugs compared to *S. enterica* without integrons. The virulence factor *rck*, which allows *S. enterica* to evade complement proteins of the host innate immune system, were significantly more abundant ($p<0.05$) in integron containing *S. enterica.*

Conclusions

The results from this study can be applied to predict the pathogenicity of *S. enterica* isolates based on the presence of integron-associated AMR genes and identify antimicrobial resistant isolates that may pose a disease risk to cattle and people.

Financial Support
Colorado State University; U.S. Department of Agriculture; Bill and Melinda Gates Foundation

Notes:

V-006 - Microbial quality and phenotypic expression of extended spectrum beta – lactamases (ESBLs) producing *Escherichia Coli* and *Klebsiella* species in dressed chicken meat in Maiduguri Metropolis, Northeastern Nigeria.

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Session: Antimicrobial Resistance/Use

Objective

A cross sectional study was conducted in Maiduguri metropolis (MMC) and Jere LGAs from 2020-2021. With the aim of detecting Extended-Spectrum β-lactams (ESBLs) producing *E. coli* and *Klebsiella* species in dressed chicken meat.

Methods

A total of n=384 samples were collected (Swab and intestinal contents) from two live bird markets (LBMs) in MMC and one in Jere. Samples were transported to the laboratory, colony count, culture and isolation and biochemical tests were conducted to differentiate the isolates and antimicrobial sensitivity test was performed using Kirby Bauer method.

Results

Tashan Bama had the average level 64×10^6 dressed chicken meat contamination found in this study, *E. coli*, had the highest number, 178 (46.4%) and Klebsiella species 28 (7.3%) of the samples. Though, there was no statistically significant association between sample type, location and presence of the isolates in dressed chicken meat sampled (χ^2 =0.263; p= 0.679). The study also revealed a high number 19(86.4%) of antimicrobial resistant isolates due to *E. coli* and *Klebsiella* species, mostly resistance to β-lactams and also revealed a phenotypic expression of multi-drug resistance (n=10) patterns among those isolates in dressed chicken meat. Explaining that, they are β_lactams producing *E. coli* and *Klebsiella* species.

Conclusions

The study recommended for further molecular detection of resistance genes encoded in these isolates from dressed chicken meat in Maiduguri for better understanding of the molecular epidemiology of those isolates conferring multi-drug resistance genes. There is a need to reduce this burden at the critical control points of LBMs and chicken meat industries in Maiduguri.

Notes:

V-007 - Study of antibiotics and symbiotic effects on sperm quality using the CASA system in goats

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Session: Antimicrobial Resistance/Use

Objective

The objective of the current work was to study *in vitro* sperm quality after antibiotics and symbiotic (Combination of prebiotic-probiotic) administration and to evaluate treatment administered before insemination with the aim of reducing artificial insemination failures in goats.

Methods

This experimental study was carried out at the Animal Reproduction Biotechnology Laboratory (Blida, Algeria). Semen analysis was performed using the Computer-assisted sperm analysis system. In the first experimental approach, we used the antibiotics most commonly administered in the veterinary field for the treatment of subclinical endometritis. A total of eight antibiotics were studied. Each antibiotic tested was co-incubated with frozen goat semen brought from the Centre for Artificial Insemination and Genetic Improvement. For the second experimental approach, we incubated semen with a symbiotic (Symbiovéba). Finally, we selected two antibiotics among those used, namely colistin and cotrimoxazole, and these were co-incubated with the symbiotic and the semen, to examine possible combinations of antibiotics with symbiotics in the treatment and prevention of uterine infections (broad spectrum synergistic activity).

Results

Antibiotics have been shown to have a detrimental effect on the sperm cell, by decreasing sperm motility. The average value calculated on all antibiotics was 18% (as opposed to initial motility of 78% in the control group), with an alteration of the linear speed that would have a negative impact on fertilization. On the other hand, symbiotics had a beneficial effect on spermatozoa motility and vitality. The combination of the symbiotic and colistin proved to be very promising.

Conclusions

In conclusion, the use of symbiotics in the treatment of subclinical endometriosis at the time of goat insemination is beneficial, and requires greater attention in future research.

Notes:

V-008 - Assessment of knowledge and behavior of backyard farmers in California about animal health and antimicrobial use

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Session: Antimicrobial Resistance/Use

Objective

Backyard livestock and poultry premises have grown rapidly in California. This growth prompted concerns about disease management on backyard farms. The lack of access of veterinary oversight on backyard farms is a significant issue regarding antimicrobial misuse. This study evaluated knowledge and behavior of backyard farmers in California regarding animal health management, antimicrobial use, Veterinary Feed Directive (VFD) and CA Senate Bill (SB) 27.

Methods

The survey consisted of 38 questions about demographics, antimicrobial purchase and use, the impact of the VFD and SB27, and the veterinarian-client-patient-relationship (VCPR). Descriptive statistics summarized responses. Multivariable logistic regression evaluated the association of antimicrobials purchased and used in 12 months, and the impact of VFD and SB27 on antimicrobial use, with demographics and farm management variables.

Results

A total of 253 backyard farmers responded to the survey. They mostly raised chickens and/or small ruminants with a small herd size (< 10). Half of respondents used antimicrobials mainly for individual animal treatment. Backyard farmers who sold livestock or raised small ruminants were more likely to purchase and use antimicrobials, but those who raised duck/turkey/geese were less likely to purchase antimicrobials.

Conclusions

One fifth of respondents and one third of respondents answered that the VFD and SB27 affected their antimicrobial use, respectively. The two laws generally encouraged backyard famers to meet with veterinarians more frequently, or treat fewer animals with antimicrobials. The VFD affected antimicrobial use of respondents who were a 4-H/FFA member or used antimicrobials in feed. SB27 affected antimicrobial use of respondents who were a 4-H/FFA member, purchased antimicrobials online, made antimicrobial use decisions by themselves, raised small ruminants or had a VCPR. VFD and SB27 promoted judicious antimicrobial use to many Californian backyard farmers. A better understanding of backyard farm management and promoting the VCPR will improve proper antimicrobial use of backyard farms.

Financial Support
California Department of Food and Agriculture

Notes:

V-009 - Use of antimicrobials in the treatment of calf diarrhea: A systematic review

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Session: Antimicrobial Resistance/Use

Objective

The goals were 1) to systematically review the scientific literature on the efficacy of antimicrobial (AM) treatments for calf diarrhea, and 2) to evaluate the efficacy of AM on calf diarrhea by a meta-analysis.

Methods

A review protocol was developed based on PRISMA-P guidelines. Electronic databases (CAB Abstracts, Medline, Scopus, and Biosis) were searched in Jul-2019 and updated in Jun-2020. Eligible studies were controlled trials that evaluated the efficacy of AM treatments for diarrhea in calves ≤6 months, with at least one health or performance outcome. Titles, abstracts, and full text were screened for eligibility, and data were extracted from eligible trials. Risk of bias was assessed by "Cochrane Risk of Bias Tool for Randomized Trials 2.0". The adherence of trials to the REFLECT statement was evaluated.

Results

The literature search resulted in 2,899 records, of which 11 studies (11 trials) were included in the review. Fluoroquinolones (n = 3), β-lactams (n = 3), and nitazoxanide (n = 2) were the AM most assessed. Diarrhea severity (n = 7) and mortality (n = 6) were the most common outcomes. Heterogeneity in interventions and outcomes made it unfeasible to conduct a meta-analysis. Studies comparing AM and a negative comparator showed a statistically significant reduction in diarrhea severity (n = 1) and mortality (n = 2). However, no significant effects in diarrhea severity and mortality were reported when AM were compared with positive controls. Completeness of the REFLECT statement and risk of bias assessment revealed concerns with reporting of key trial features, as most trials incompletely reported both experimental design and results. It was noteworthy the lack of standardized disease and outcomes definitions.

Conclusions

The results of this review do not provide compelling evidence for or against the efficacy of AM for the treatment of calf diarrhea. Further research is necessary to elucidate the AM efficacy on calf diarrhea. Validated, standardized methods to evaluate clinical outcomes are needed. Quality of reporting must be improved to adhere to REFLECT statement.

Financial Support
California Department of Food and Agriculture

Notes:

V-010 - Use of antimicrobials in the prophylaxis and metaphylaxis of calf diarrhea: A scoping review (ScR)

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Session: Antimicrobial Resistance/Use

Objective

The goal of this ScR was to map and describe the scientific literature on prophylactic and metaphylactic antimicrobial (AM) approaches for calf diarrhea.

Methods

The ScR was conducted following the PRISMA-ScR guidelines. Electronic databases (CAB Abstracts, Medline, Scopus, and Biosis) were searched in Jul-2019 and updated on Jun-2020. Eligible publications were controlled trials using prophylactic and metaphylactic AM interventions for diarrhea in calves ≤6 months, which evaluated 1) fecal consistency (FC), 2) fecal pathogen shedding, or 3) both. Titles, abstracts, and full text were screened for eligibility, and data were extracted from eligible trials.

Results

From 2,899 initially identified records, 32 manuscripts reporting 51 trials were included in the ScR. Most trials were conducted in North American (49%) and European (43.1%) institutions and were performed between 2001 and 2020 (45.1%). Average sample size per treatment group was 14 calves (range = 2-259). Most trials (92.1%) identified the etiological agent of diarrhea, of which *Eimeria* sp. (48.9%), *Cryptosporidium parvum* (36.1%), and *Salmonella* sp. (10.6%) were the most reported. Across trials, 90 interventions were evaluated, of which most were antiprotozoal drugs against coccidia [n = 45; mainly lasalocid (28.8%) and triazines (28.8%)] and *C. parvum* [n = 31; mainly halofuginone (58.1%)], followed by antibiotics [n = 9; mainly tetracyclines (66.6%)]. Calves' health status at enrollment was often unreported making it unclear if trials followed a prophylactic or metaphylactic approach. Most trials (96.1%) measured FC, but only 59.1% of those informed a scoring system that was often unclearly outlined. Oocyst count and stool culture were reported in 45.1% and 11.8% of trials, respectively. It was noteworthy the lack of standardized disease definitions, outcome assessment methods, and comprehensive reporting.

Conclusions

Future studies must have high reproducibility, standardize disease and outcomes definitions, and define if AM were used as a prophylactic or metaphylactic approach for calf diarrhea.

Financial Support
California Department of Food and Agriculture

Notes:

V-011 - Phenotypic and genotypic characterization of anti-microbial resistant *Escherichia coli* isolates from retail meats

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Session: Antimicrobial Resistance/Use

Objective

Antimicrobial-resistant pathogens in retail meats must be monitored in order to determine their role as potential human transmission sources along the food chain. This report aimed to phenotypically and genotypically characterize antimicrobial resistant *Escherichia coli* from retail meats in North Carolina as part of the National Antimicrobial Resistance Monitoring (NARMS) surveillance system.

Methods

From 2018 to 2019, 178 *E. coli* isolates were isolated from meat products, including chicken (n=37), ground turkey (n=74), pork (n=31), and ground beef (n=36). By using broth microdilution, these isolates were phenotypically tested for resistance to a panel of antimicrobial agents. The Illumina next-generation sequencing platform was also used to sequence them.

Results

Our isolates showed resistance to aminoglycosides, sulfonamides, beta-lactams, tetracyclines, polypeptides, and fluoroquinolones. The highest prevalence of *E. coli* was found in samples from Turkey (41.5 %) and chicken (20.7 %), followed by ground beef (20.22 %) and pork (20.22 %). All isolates were susceptible to Meropenem. When compared to beef and pork isolates, poultry meat isolates had a higher percentage of resistance to all drugs tested. 11.2 % of the turkey isolates were multidrug-resistant (resistance to >3 classes) compared to 3.3 % of chicken, 0.011 % of pork, and 0.011 % of beef isolates. We found 160 sequence types (STs), with ST10 being the most prevalent. A resistant clone ST131 was observed twice and it was identified as an important ST in multidrug-resistant *E.coli* in the United States. Seven Uropathogenic *E. coli* (UPEC) strains were found after further subtyping of these isolates. Our isolates were classified into seven phylogroups using the Clermont typing tool, with B1 (35.75 %) and A (25 %) being the most dominant, followed by B2 (9 %), D (4.8 %), F (3.78 %), E (2.16 %), and C (1.85 %).

Conclusions

In this study, we determined the antimicrobial resistance and virulence profile of *E.coli* isolated from retail meats in North Carolina which highlight the necessity for surveillance.

Notes:

V-012 - Evaluation of internal biosecurity practices combined with sow vaccination to wean influenza negative piglets

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Session: Biosecurity & Infection Control

Objective

Influenza A virus (IAV) in pigs causes respiratory disease which can result in decreased growth and increased susceptibility to secondary infections. Vaccination has been the main tool for controlling IAV infections in breeding herds but has not been sufficient to wean IAV negative pigs. A study that evaluated the impact of enhanced biosecurity measures showed a significant reduction in IAV infections, but it was not enough to reduce IAV prevalence at weaning. The objective of the present study was to evaluate the impact of combining sow vaccination and internal biosecurity practices to reduce the IAV prevalence at weaning.

Methods

Six IAV positive breeding herds were selected for the study. Five farms were assigned to the treatment group, which consisted of internal biosecurity protocols with sow vaccination. One farm was assigned as control, in which there was no changes in management practices. The internal biosecurity measures consisted of not using cross-fostering after 3 days of life or nurse sows, changing of gloves before handling piglets and daily disinfection of tools used in farrowing rooms. Ninety udder skin wipes were collected from lactating sows during 3 weeks prior to implementing the protocols and the sampling was repeated six weeks after having implemented the mass IAV vaccination. Samples were tested using an IAV rRT-PCR.

Results

Three of the five farms in the treatment group tested IAV negative in all 3 sampling points post-intervention. One of the treatment farms had a significant decrease in IAV prevalence post intervention but IAV could still be detected in low levels in the last sampling event. Treatment in one farm did not change IAV prevalence at weaning. As expected, IAV status in the control farm was not altered.

Conclusions

This study provides proof of concept on the use of a protocol that combines sow vaccination and enhanced internal biosecurity practices to wean IAV negative pigs. This protocol can serve as a guide to pork producers that have the goal of controlling, and potentially eliminating, IAV infections in their breeding herds.

Notes:

V-013 - Evaluation of parity, personnel and cross-fostering in influenza infections during the pre-weaning period

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Session: Biosecurity & Infection Control

Objective

Influenza A virus (IAV) is an important respiratory pathogen that impacts growth efficiency and can lead to mortality in pigs due to secondary infections. We evaluated whether adopting internal biosecurity practices during the lactation period and parity are associated with IAV prevalence at weaning.

Methods

360 litters distributed across 3 farms were enrolled into the study. Farrowing rooms were allocated to either "control" or "treatment" groups. Litters within the control rooms were processed and handled with no protocol restrictions. Litters in the treatment rooms were not cross-fostered after processing, and any handling of the pigs was done by changing gloves between litters. Litters were sampled at 1, 8, 13 and 18 days of age to assess IAV status using udder skin wipes. Samples were also collected from worker's hands after piglet handling using a cotton gauze to evaluate the degree of IAV contamination. Samples were tested using an IAV rRT-PCR. Statistical differences between groups and parities were assessed using a multivariate statistical model.

Results

Litters from the treatment group showed an overall lower IAV prevalence of 29% (209/720) compared to the 43% of the control group (318/720). Differences were statistically significant at day 8 and 13 of age. At day 18, both control and treatment groups had similar IAV prevalence. No differences in IAV prevalence were seen between young parity sows (37.5%) and older parity sows (35.7%) at any sampling point. Samples collected from farm worker's hands had 58% positivity to IAV.

Conclusions

Our results indicate that specific management practices directed at minimizing spread of IAV in pigs can slow down IAV transmission within farrowing rooms but by themselves do not appear to be sufficient to prevent infection at weaning. We found no evidence that sow parity was associated with IAV litter prevalence during the lactation period. Lastly, the high prevalence of IAV in the samples collected from farm worker's hands and tools is most likely facilitating the indirect transmission of IAV within farrowing rooms while workers handle piglets.

Financial Support
Boehringer Ingelheim Animal Health

Notes:

V-014 - Constructing an integrated biosecurity-biosafety assessment tool for the dairy farm environment

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Session: Biosecurity & Infection Control

Objective

Biosecurity and biosafety can be applied on dairy farms to prevent diseases in animals and people. This study aims to construct an integrated biosecurity-biosafety assessment tool for use on Colorado Front Range dairies with emphasis on infectious diseases. Analysis of producer knowledge, attitudes, and practices (KAP) on biosecurity and biosafety and feedback on an existing tool facilitated construction of a valid measure for biosecurity.

Methods

A KAP questionnaire was constructed based on biosecurity and biosafety literature and producer input. It was used on 2 organic and 4 conventional Colorado Front Range dairies to assess worker and manager biosecurity and biosafety KAP. Topics included animal and human infectious diseases, zoonoses, COVID-19, obstacles to disease prevention, sources of information, PPE, vaccination, visitor and sick leave policies, social distancing, training, and cleaning/disinfecting. Farm managers completed the BioCheck.UGent tool and provided feedback on its questions and overall utility. Based on KAP questionnaire results and BioCheck.UGent feedback, an integrated biosecurity-biosafety dairy assessment tool was developed.

Results

KAP questionnaire construction revealed shared animal-human infection prevention principles (e.g., animal density and social distancing). Questionnaire construction revealed areas where efforts can be streamlined, including foreign animal disease and pandemic preparedness. Results highlight KAP differences between organic and conventional settings and dairy occupations and clarify perceived importance of biosecurity topics. Results guided development of topics and concepts forming the integrated tool.

Conclusions

Construction of an integrated biosecurity-biosafety tool based on producer KAP and feedback will maximize utility to producers. The tool will enable assessment of infectious diseases or other biological hazards and guide efficient, cost-effective practices to protect livestock and people. It will lay the groundwork for development of similar tools for use in a variety of domestic and international production settings.

Financial Support
High Plains Intermountain Center for Agricultural Health and Safety (HICAHS); Colorado State University College of Veterinary Medicine and Biomedical Sciences College Research Council (CRC)

Notes:

V-015 - Stability of Senecavirus A in animal feed ingredients and infection following consumption of contaminated feed

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Session: Biosecurity & Infection Control

Objective

Animal feed and feed ingredients have recently been investigated as sources of pathogen introduction into farms and as a potential source of infection post-consumption. Survival of several viruses for a prolonged period has been demonstrated in feed. Here we aimed to determine the rate of decay of Senecavirus A (SVA) in swine feed ingredients as a function of time and temperature and established half-life estimates for the virus. Additionally, the ability of SVA to infect swine via ingestion of contaminated feed was investigated in 3-week old, weaned pigs.

Methods

Five grams of dried distillers' grains with solubles (DDGS), soybean meal (SBM), lysine and vitamin D were spiked with a constant amount of SVA (10^5 $TCID_{50}$) and incubated at 4°C, 15°C and 30°C for up to 91 days. The Virus viability and the presence of viral RNA were assessed in samples collected over time through viral titration and rRT-PCR assays. The animal study was conducted by providing complete feed spiked with three concentrations of SVA (10^5, 10^6 and 10^7 per 200 g of feed) to 3 week old piglets, and allowing the animals to naturally consume the contaminated feed. This procedure was repeated for 3 consecutive days. Collection of serum and rectal swabs were performed at days 0, 3, 7, and 14 post feeding. Tonsils were collected at day 14 post feeding. SVA infection was assessed through viral neutralization and rRT-PCR.

Results

At the three different temperatures investigated, dried distillers' grains with solubles (DDGS) and soybean meal (SBM) provided the most stable matrix for SVA, resulting in half-lives of 25.6 and 9.8 days, respectively. At 30°C, SVA was completely inactivated in all feed ingredients and in the control sample, which did not contain a feed matrix. Although virus infectivity was lost, viral RNA remained stable and at consistent levels throughout the experimental period. Infection of pigs through consumption of contaminated feed was confirmed by virus neutralization assay and the detection of SVA in serum, fecal swabs and tonsils by rRT-PCR, with evidence of replication in tonsils and virus shedding in feces.

Conclusions

Our findings demonstrate that feed matrices are able to extend the survival of SVA, protecting the virus from decay. Additionally, we demonstrated that consumption of contaminated feed can lead to productive SVA infection.

Financial Support
Swine Health Information Center

Notes:

V-016 - Colorado dairy farmer knowledge, attitudes and practices for livestock and human infectious disease prevention

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Session: Biosecurity & Infection Control

Objective

Biosecurity and biosafety principles can be applied on dairy farms to prevent infectious diseases in animals and people. This study aims to construct and use a knowledge, attitudes, and practices (KAP) questionnaire for the Colorado dairy farm environment to understand producer KAP on prevention of animal diseases, zoonoses and infectious diseases transmitted person-person.

Methods

A KAP questionnaire was constructed based on published farm biosecurity and biosafety comprehensive literature as well as producer input. Topics included vaccinations, perceived risks of animal diseases, zoonoses, and COVID-19, trusted sources of information for disease prevention, perceived efficacy of preventive practices including personal protective equipment (PPE), and attitudes toward obstacles of disease prevention. English and Spanish questionnaire versions were used on four conventional and two organic Colorado Front Range dairies to assess worker (n=35), manager (n=12), and owner (n=2) biosecurity and biosafety KAP. Question were constructed in multiple choice, Likert scale, and short answer format.

Results

KAP questionnaire construction revealed common animal-human infection prevention principles (e.g., animal density and social distancing) and areas where efforts can be streamlined, including foreign animal disease and pandemic preparedness. Results highlight KAP differences between organic and conventional settings and occupations and clarify perceived importance of biosecurity topics. Descriptive data will be presented with preliminary analysis of trends.

Conclusions

Constructing and using a comprehensive KAP questionnaire is an important step in incorporating an integrated framework to disease prevention. Questionnaire construction and results obtained in this pilot study shed light on Colorado Front Range dairy infectious disease prevention KAP and help guide development of integrated assessment tools aiming to effectively prevent animal and human infectious diseases on a variety of domestic and international animal production settings.

Financial Support
High Plains Intermountain Center for Agricultural Health and Safety (HICAHS); Colorado State University College of Veterinary Medicine and Biomedical Sciences College Research Council (CRC)

Notes:

V-017 - Does the language of information delivery influence biosecurity compliance? Insights from experimental simulations

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Session: Biosecurity & Infection Control

Objective

Biosecurity reduces the economic impacts of infectious diseases in US animal agriculture. Yet, more than 1/5 of animal production and processing workers speak a language other than English at home. Language may influence risk preference and vulnerability to biases and thus, affect communication of biosecurity strategies.
Therefore, we hypothesized that language may affect biosecurity compliance contingent upon three additional covariates: (1) the risk of acquiring an infection, (2) the delivery method of the infection risk, and (3) the certainty of the infection risk information.

Methods

We designed an experimental game to test the effect of language on compliance with a line of separation (LOS) biosecurity tactic in a swine production facility, where participants were tasked with completing tasks inside and outside of the facility. Data were collected using games translated into the three most spoken languages in the US: English (EN), Spanish (SP), and Chinese (CN). Participants made binary decisions about whether to use the LOS biosecurity tactic based on the risk information provided. Kruskal-Wallis tests and mixed-effect logistic models were used to test the effects of languages and the covariates on using the LOS tactic.

Results

Biosecurity compliance rates of SP participants showed a significant increase compared to their EN counterparts ($p<0.001$). However, SP participants were more risk-tolerant to high-risk messaging and were more risk-averse to low-risk ones.
There are significant differences in how numeric risk information is perceived between EN and SP ($p<0.001$) and SP and CN participants ($p<0.05$) which argues against using numbers to communicate risk information regarding biosecurity because results suggest a high likelihood of perception differences across language groups.

Conclusions

When confronted with situational biosecurity decisions, risk preferences change when information is presented using different languages. Effective biosecurity communication need to understand these differences and not assume that simply translating risk messages will result in effective communication.

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

Notes:

V-018 - Development and validation of serological assays to detect the spillover of SARS-CoV-2 in livestock

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Session: Diagnostic Testing

Objective

The COVID-19 pandemic caused by the severe acute respiratory syndrome-corona virus-2 (SARS-CoV-2) remains the most significant public health crisis in the modern era. The unprecedented global spread of SARS-CoV-2 could pose a significant risk of exposure to livestock. Natural human to the animal transmission of SARS-CoV-2 was documented in several animal species, including dogs, cats, and minks. Spillover of SARS-CoV-2 into livestock could lead to virus adaptation and efficient transmission among livestock. Implementation of SARS-CoV-2 virus detection by PCR to assess potential exposure is not a practical approach as the animals exposed to SARS-CoV-2 will only be virus-positive for a few days, thus making the window for virus testing concise. Infection of animals with a virus typically results in antibody response, and the serological signature of virus infection lasts much longer after animal tests negative for the virus. Therefore, a rational approach to assess the exposure of livestock to SARS-CoV-2 is to carryout sero-surveillance.

Methods

We developed SARS-CoV-2 receptor-binding domain (RBD) indirect ELISA (iELISA) assays to detect antibodies in cattle, pigs, and chickens. We raised species-specific positive control antibodies for assay development by hyper immunization of animals with recombinant RBD protein. In addition, we have also developed a Luciferase Immunoprecipitation System (LIPS) assay to screen SARS-CoV-2 antibodies in other domestic animals such as sheep, goats, and captive white-tailed deer.

Results

We determined the cut-off of iELISA assays with the pre-pandemic sera. The LIPS is a liquid phase assay, so it maintains native antigen conformation and is highly specific and sensitive. LIPS assay does not require secondary antibodies and, therefore, can be implemented across multiple livestock and agricultural species. The assays were validated with a live virus neutralization assay conducted in a biosafety level-3 laboratory.

Conclusions

These tools will aid in rapid decision-making strategies to safeguard the agriculture supply chain, health and security of livestock.

Financial Support
U.S. Department of Agriculture

Notes:

V-019 - Development of epizootic hemorrhagic disease agar gel immunodiffusuion reagents for bovine and ovine export testing

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Session: Diagnostic Testing

Objective

Epizootic Hemorrhagic Disease (EHD) is an Orbivirus that causes disease primarily of cervids but can infect domestic cattle and sheep. Cattle and small ruminants and their products often require testing prior to export for EHD antibodies.When the Agar Gel Immunodiffusuion (AGID) manufacturer for EHD went out of business, NVSL was tasked to develop an EHD reagent to serve this market.

Methods

The EHD serotype 2 strain was chosen for AGID development, and EDHV2 was propagated in C6/36 cells. The virus was concentrated using a 10KD MW cutoff ultrafiltration membrane. The concentrated EHD virus was used as the antigen with a purified EHD antiserum from hyperimmunized cattle.
To determine if the EHD AGID reagent was fit for the purpose, testing of the reagent consisted of three elements. First, it was tested against a serum panel provided by the Wisconsin Veterinary Diagnostic Laboratory that was selected to be EHD positive and Bluetongue (BT) negative. Second, the EHD AGID reagent was used to test EHD serotype 2 experimentally infected elk at pre challenge and 8 weeks post challenge. Third, the EHD AGID reagent was used to test samples from various exotic ruminant species from a confirmed EHD-6 outbreak at the Minnesota Zoo.

Results

The EHD AGID reagent was shown to be reactive to all eight (8) serotypes of EHD and five (5) endemic serotypes of BT in North America.
The EHD AGID reagent demonstrated agreement with WVDL results: 97.5% on the positive samples and 100% on the negative samples.
The EHD AGID test also compared favorably to the EHD VN test based on the experimentally infected elk samples at 19/22 (86.3%) of the sample results. The three discrepant samples were low level positive on the EHD VN assay.
For the MN zoo samples, 39/41 (95.1%) agreed across the AGID and VN assays, including over 9 different species tested.

Conclusions

The NVSL EHD AGID assay demonstrated that it is robust and compared fit for the purpose of export testing samples for entry into other countries and works across multiple ruminant species.

Notes:

V-020 - Targeted next-generation sequencing for comprehensive testing for vector-borne pathogens in canines

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Session: Diagnostic Testing

Objective

The gold standard for detecting vector-borne pathogens is quantitative PCR (qPCR). However, this requires individual testing for each pathogen and many tests to obtain an accurate diagnosis. The purpose of this study was to develop and validate a targeted next-generation sequencing (NGS) assay for vector-borne pathogens.

Methods

Test feasibility and analytical specificity were evaluated with type strains or validated positive clinical samples from dogs. We compared the analytical sensitivity of the method to the Ct values obtained by qPCR testing. Diagnostic sensitivity and specificity were assessed with a set of known positive and negative clinical samples, based on qPCR testing. Positive and negative percent agreements and Cohen's kappa were calculated. For each sample, pathogen target regions were amplified via PCR, and DNA libraries were prepared with the Ion AmpliSeq Library Kit Plus, loaded onto chips using the Ion Chef, and sequenced with the Ion Torrent S5. Data were assembled using SPAdes and mapped to a reference file containing sequences from the pathogens. Geneious software was used to process the raw reads, and the BLAST analysis was performed to confirm the results.

Results

The primer sets used for amplification were specific for the intended targets, based on sequence analysis of the amplified products, and the method detected 17 different pathogens. Analytical sensitivity was equivalent to a qPCR Ct value of approximately 35-36. Cohen's kappa was 0.804, which indicates almost perfect agreement between the qPCR assay and the targeted NGS assay. The positive percent agreement was 92% and the missed qPCR positives were due to failure to detect pathogens in samples with high Ct values. The negative percent agreement was 88%, and targeted NGS was able to detect multiple pathogens in a sample with a single test, including samples missed by qPCR.

Conclusions

Using a targeted method reduces costs associated with NGS sequencing and allows for a 2-3 day turn-around time, making this a viable method for detecting vector-borne pathogens in canine whole blood samples.

Notes:

V-021 - Anatomical distribution of *Brucella ovis* in naturally infected rams and correlation to diagnostic test results

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Session: Diagnostic Testing

Objective

The primary objective is to develop a serum bank of well-characterized, representative, serum from *Brucella ovis* (*B. ovis*) infected sheep. A secondary objective is to compare culture, semen PCR, and serological testing of individual animals.

Methods

Animals were included in the study based on a positive serological test with PCR positive semen or multiple positive serological tests with clinical lesions indicative of *Brucella ovis*. Animals were sedated, electroejaculated, euthanized, followed by blood and tissue collection. Blood was processed for serum banking and tested using a cELISA. Necropsy samples were cultured for Brucella and confirmed with AMOS PCR.

Results

To date, 30 animals from 2 different locations (MO and UT) have been obtained. *B. ovis* was cultured from 25/30 from at least 1 of 20 anatomical locations sampled. Serological testing from blood collected at euthanasia resulted in 28/30 animals testing positive with 1 suspect on cELISA. The suspect and 1 of the negative serological tested animals matched culture of no isolation. 1 of the serological negative animals had *B. ovis* isolated from 2 locations. Serological testing had an apparent sensitivity and specificity of 96% and 80%, respectively. Out of the 20 anatomical locations, the semen (21) and seminal vesicles (20) were the most common locations for isolation of *B. ovis* followed by the epididymis (17). The other 16 locations ranged from 1 to 10 isolations. No *B. ovis* was isolated from the popliteal LN. Sufficient semen was collected from 23/30 animals for PCR testing. PCR detected *B. ovis* DNA in 22/23 samples. The PCR negative animal was also culture negative; however, 4 culture negative animals had high Ct values.

Conclusions

Development of an improved serum-based *B. ovis* test requires a sufficient number of infected animals to validate the new test. To aid validation of these tests, a *B. ovis* serum bank is being developed. Tissues associated with the reproductive tract were the most common sites of *B. ovis* colonization and should be targeted for sampling. There was good correlation between infection and the cELISA.

Notes:

V-022 - A defined antigen skin test for the diagnosis of bovine tuberculosis

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Session: Diagnostic Testing

Objective

Bovine tuberculosis (bTB) is a chronic inflammatory disease of zoonotic and economic importance caused by members of the *Mycobacterium tuberculosis* complex (MTBC). The tuberculin skin test has been the primary ante-mortem diagnostic test for bTB since the 1890s, but has several limitations including difficulty in manufacture and quality control, as well as interference with vaccination programs with Bacille Calmette–Guérin (BCG) strains by sensitizing immunized animals. Here, we evaluate the performance characteristics of a novel peptide based defined antigen skin test (DST).

Methods

We recently developed a defined antigen skin test (DST) using synthetic peptides representing ESAT-6, CFP-10 and Rv3615c, antigens that are present in pathogenic members of MTBC but absent in BCG. As part of field validation of the DST in endemic country settings, we here evaluated the performance characteristics of 10ug (DST10) and 30ug (DST30) per peptide constituent, alongside the tuberculin antigens (PPD-A and B), interferon-gamma release assay (IGRA) and ELISA, in a total of 257 animals from 14 farms in Ethiopia. The results were analyzed on Graph Pad Prism and on R programming.

Results

Skin test results for 118 of the 257 animals (0.46; 95% CI: 0.40-0.62) were fully concordant across all antigens tested. A Walter-Hui latent class model was fitted to infer true prevalence (

Conclusions

The results show that the DSTs may help fill the urgent need for fit-for-purpose diagnostic assays that can differentiate infected and vaccinated animals (DIVA) for the implementation of future (vaccination based) control programs alongside conventional test and slaughter approaches.

Financial Support
Bill and Melinda Gates Foundation

Notes:

V-023 - A novel diagnostic modality for identifying equine herpesvirus type-1

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Session: Diagnostic Testing

Objective

Equine herpesvirus type 1 (EHV-1) is ubiquitous affecting horses of every breed and discipline. Mild respiratory disease is typical, however infection is also associated with late term abortion, neonatal foal death, and neurologic disease, which can occur as devastating outbreaks. Current detection methods, such as PCR and virus isolation, can take multiple days before results become available. A novel DNA sequencing technology, MinION nanopore sequencer, offers a new tool to rapidly detect and differentiate EHV-1 (i.e., neurotropic and non-neurotropic strains) as a stall- side diagnostic and in field applications. Extraction of EHV-1 DNA from nasal swabs is often low yield. Loop-mediated isothermal amplification (LAMP) allows for simple and rapid viral amplification and does not require a thermocycler nor nucleic acid purification, thus it can be done quickly with minimal equipment and will be less effected by inhibitors found in clinical samples. The purpose of our study is to determine the efficacy of LAMP and DNA sequencing using the MinION nanopore sequencer for diagnosing equine herpesvirus type-1 in equine nasal swabs.

Methods

Nasal swabs were collected from six horses with clinical signs of EHV-1 and that tested positive with qPCR; three of each variant (A2254 and G2254). For negative controls, nasal swabs samples from six horses that did not have clinical signs of any respiratory or neurologic disease and were negative for EHV-1 via qPCR. EHV-1 vaccine was the positive control. DNA extractions were performed followed by LAMP. Library preparation, sequencing and bioinformatics were performed. Data was analyzed for presence of EHV-1, variant, number and length of reads, and Ct values.

Results

EHV-1 was recovered via sequencing in 6/6 EHV-1 positive nasal swabs, the majority of which were A2254 (4/6) and the remainder were G2254 variant. EHV-1 was not found in the negative samples.

Conclusions

LAMP is an effective way to amplify EHV-1 DNA in clinical equine nasal swabs for nanopore sequencing. Further investigation is needed for the possibility of infection with both variants.

Financial Support
American Quarter Horse Foundation; VMCVM Veterinary Memorial Fund

Notes:

V-024 - Implementation of surgical abomasal cannulation for smartphone measurement of abomasal pH in beef calves

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Session: Diagnostic Testing

Objective

To evaluate the use of human percutaneous gastrotomy (PEG) tube for surgical cannulation of the abomasum in beef calves, and validation of a point-of-care smartphone-based pH meter for abomasal fluid analysis in beef calves.

Methods

PEG tubes were implanted under injectable anesthesia in six beef-breed calves. The abomasum was pexied to the body wall and the PEG tube was inserted via a stab incision and fixed in position with a purse-string suture.
Abomasal fluid was collected via the PEG tube. Determination of fluid pH was performed immediately after sample collection. Both smartphone-based pH meter and conventional benchtop pH meter were used for each sample. Results were compared by commercial statistical software.

Results

Four of six PEG tubes remained in place throughout the entirety of the study. Two smaller size (24 fr vs 18 fr) PEG tubes dislodged from the abomasum. Remaining PEG tubes were patent throughout the entire 17-day study period and 296 collections. All calves remained healthy at the conclusion of the study.
Regression analysis of the smart-phone pH meter compared to the standard pH meter indicated a relationship of $Y = 1.003X - 0.03243$. R^2 was 0.9864. Bland-Altman analysis indicated a bias of -0.01734 ± 0.1609 and 95% limits of agreement were -0.3329 to 0.2979.

Conclusions

Calves tolerated implanted PEG tubes well, and both standard and smartphone pH devices demonstrated high agreement. Future research should evaluate appropriate size of a PEG tube to decrease the displacement risk in calves.

Financial Support
COE Summer Scholars Program, College of Veterinary Medicine, University of Tennessee

Notes:

V-025 - *Salmonella* in animal feeds: A scoping review

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Session: Epidemiology

Objective

The objective was to describe the published literature on *Salmonella* in animal feeds using scoping review methodology. An additional objective of this presentation is to describe two novel approaches: artificial intelligence to assist with eligibility screening, and evidence gap maps to portray results.

Methods

The review included a structured search for eligible studies, duplicate reviewer screening for eligibility with machine learning to prioritize and order citations for the reviewers, and duplicate reviewer data characterization. Results were summarized descriptively as well as using evidence gap maps.

Results

There were 547 relevant studies: 15 studies conducted in the fields in which animal feeds are grown, 106 in the manufacturing sector, 11 during transportation, 15 at retail, and 226 on-farm, with the sector not described in 204 studies. Common study purposes were to estimate the prevalence of *Salmonella* in animal feeds (372 studies) and to identify serovars (195). The serovars found in animal feeds included serovars associated with human illness, animal illness, and identified in livestock and poultry meat. There were 120 intervention studies and 83 risk factor studies. Within intervention and risk factor studies, there may be a sufficient volume of research to conduct systematic reviews in the areas of heat interventions, fermentation and ensiling, organic acids, season, and geographic region. Deficiencies were identified in the completeness of reporting of key features in the relevant studies. The use of artificial intelligence to facilitate screening presented a considerable time savings and the evidence gap maps allowed presentation of results in a 3-dimensional format.

Conclusions

The results provide a summary of the available literature related to *Salmonella* in animal feeds which could be used to facilitate further evidence synthesis or to prioritize research. New approaches, such as artificial intelligence and evidence gap maps can increase efficiencies and enhance clarity of results when conducting scoping reviews.

Financial Support
Pew Charitable Trusts

Notes:

V-026 - Equine parvovirus-hepatitis transmission

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Session: Epidemiology

Objective

Equine parvovirus-hepatitis (EqPV-H) has recently been identified as the cause of Theiler's disease, also known as severe acute hepatic necrosis or equine serum hepatitis. To prevent or reduce incidence of disease, it is important to understand how the virus is transmitted between horses. Iatrogenic transmission via administration of allogeneic equine origin biologic products is a well-established mode of transmission. However, since many cases occur without such treatment history, a natural mode of transmission must also exist. A seasonal presentation of clinical cases in the late summer through fall suggests possible insect vectoring.

Methods

To evaluate vertical transmission in a breeding herd, we used serial herd serum PCR. To evaluate potential insect vectoring, we inoculated naïve horses by horse fly bite from flies that had fed on highly viremic horses (n = 3) and to evaluate horizontal transmission, naïve horses were inoculated orally (n=13) and then nasally 8 weeks later, or vice versa, with equine serum containing $1x10^6$ genome equivalents of EqPV-H. Infection status was monitored by serum PCR. We also assessed viral shedding by PCR of nasal, oral, and fecal swabs from infected horses (n = 6).

Results

No vertical transmission was observed from 15 EqPV-H viremic dams. No horse got infected via horse fly bites. For the horizonal trasnmission, 12 out of the 13 horses became EqPV-H positive. Virus was found to be shed via nasal, oral, and fecal secretions in all 6 infected horses for at least 10 weeks after inoculation.

Conclusions

This work demonstrates that horizontal transmission is possible, either via ingestion and/or inhalation. Although biting fly transmission was not demonstrated in this small study, it remains a likely route of transmission given the seasonality of clinical cases.

Financial Support
U.S. Department of Agriculture; Boehringer Ingelheim Animal Health; Harry M. Zweig Memorial Fund for Equine Research; U.S. National Institute of Allergy and Infectious Diseases

Notes:

V-028 - Africa swine fever: Prevalence, farm characteristics, farmer's insight and attitude towards reporting of African swine fever in Cameroon.

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Session: Epidemiology

Objective

The African swine fever virus (ASFV) has been circulating and ravaging the pig industry in Cameroon for decades with yearly outbreaks from April to August due to poor management practices employed by the pig farmers and other middlemen involved in the pig industry. With the absence of vaccines and antiviral drugs against this virus, biosecurity has been the most effective tool when properly applied to control the spread and eventual eradication of the virus. Many outbreak investigations have been effected in Cameroon with prevalence from 15% to 43%. The absence of pre-outbreak studies necessitated the undertaking of the present study to reveal the status of the animals before an eventual outbreak.

Methods

A cross-sectional study was conducted from January to March 2020 with 277 samples collected for pre-outbreak status determination by PCR using ASFV specific primers. A questionnaire to understand the characteristics and Biosecurity measures of the farm, the farmers' awareness and attitude towards the reporting of ASF was used to get first-hand information from the farmers and the data was analysed with excel.

Results

The study revealed a prevalence of 9.75% by PCR. A look into the farm characteristics, awareness, and attitude of the farmers towards ASF through data collected during a survey shows that: 34% of the farms were Backyard cemented piggeries with the majority having less than 10 pigs (54%), 91% of the farmers knew though 70% were ignorant of the clinical signs, 74% treat the sick pigs (with no mortality) with antibiotics while 79% will not treat but sell the pigs presenting clinical signs similar to ASF with increase Mortality. Sixty-three per cent had reported a case of ASF in the past and do believe reporting was useful and had no negative consequences on other farmers or third parties.

Conclusions

We found out that poor implementation of biosecurity measures is hugely contributing to the enzootic nature of the virus in the country with a major challenge being the attitude of the pig farmers which is greatly favouring the spread of the virus.

Notes:

V-029 - Investigation of geospatial risk factors on infectious bronchitis virus (IBV) antibody titer levels in Midwestern poultry farms

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Session: Epidemiology

Objective

The avian infectious bronchitis virus (IBV) is a highly contagious disease that impacts the global poultry industry by increasing mortality rates. The pathogenesis and site-level risk factors of IBV have been well documented inside the farm environment, but the environmental-level risks surrounding the farm remains unclear. The objective of this study was to investigate the impact of geospatial risk factors on mean site-level IBV antibody titers during a 3-year period.

Methods

Site-level IBV antibody titer data (n = 1,118) between Jan 2018 to Dec 2020 were obtained from broiler farms (n=130). Geospatial data including land cover, terrain slope, and farm's distance to main roads, urban areas, slaughter plants, and waterways were obtained from publicly available sources. High titer levels were defined as >240. Confounders such as age and season were also captured. All data were analyzed in STATA 15.1 using a mixed effect logistic regression model using farm as random effect. Statistical significance was defined as $P \leq 0.05$.

Results

The median antibody titer value across all farms was 261 (IQR=136-414) and the median herd age was 3.7 weeks (IQR=3.6-4). The final regression model showed increased odds of higher IBV titers for 2020 compared to 2018 (OR=1.62, P=0.014). There was a trend for increased odds of high titers for farms located more than 10 miles from the nearest slaughter plant compared to farms located less than 2 miles (OR=1.16, P=0.086). An interaction between season and landcover was found where farms situated in forested areas had reduced odds of high titers in the summer compared to spring (OR=0.08, P=0.0187) and fall months (OR=0.09, P=0.0239). In addition, farms located in cultivated farmland had a reduced odds of high titer levels during winter compared to spring months (OR=0.58, P=0.0278).

Conclusions

This study showed a seasonal shift in titer levels, in consensus with previous literature. The results also indicated that titer levels may be influenced by landcover composition.

Financial Support
USDA-NIFA

Notes:

V-030 - Examining the potential transmission of SARS-CoV-2 by insect vectors

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Session: Epidemiology

Objective

SARS-CoV-2 is a recently emerged, highly contagious coronavirus and the cause of the current COVID-19 pandemic. Arthropods transmit numerous viral, parasitic, and bacterial diseases; however, the potential role of arthropods in SARS-CoV-2 transmission is not fully understood. Public health guidelines for SARS-CoV-2 state that arthropods play no role in its transmission, despite an absence of supporting experimental scientific data. Therefore, we examined the susceptibility of biting insect species to SARS-CoV-2 infection and the role of house flies in mechanical transmission of SARS-CoV-2.

Methods

We first tested cell lines derived from biting midges (*Culicoides sonorensis*-W8a) and mosquitoes (*Aedes aegypti*-C6/36; *Culex quinquefasciatus*-HSU; *Culex tarsalis*-CxTrR2) for susceptibility to SARS-CoV-2 infection. Then, biting midges and two *Culex* species of mosquitoes were orally fed with SARS-CoV-2-spiked blood meal and held for 10 days post blood meal. The arthropods were then examined for SARS-CoV-2 RNA and infectious virus. In addition, we performed two independent studies to examine mechanical transmission of SARS-CoV-2 by house flies, *Musca domestica*. In the first study, house flies were tested for SARS-CoV-2 RNA and infectious virus after exposure to a SARS-CoV-2-spiked food source. In the second study, environmental samples were tested for SARS-CoV-2 RNA and infectious virus after contact with SARS-CoV-2-exposed flies.

Results

Our results indicate that the arthropod cells and the biting insects did not support SARS-CoV-2 replication. Interestingly, house flies were able to acquire and harbor infectious SARS-CoV-2 for up to 24 hours post-exposure, and were able to mechanically transfer SARS-CoV-2 genomic RNA to the surrounding environment.

Conclusions

Overall, our arthropod studies support the public health statement that insects do not play a significant role in SARS-CoV-2 transmission. However, further studies are warranted to determine if contamination and transmission of house flies with SARS-CoV-2 occurs naturally, and the potential public health implications of such events.

Financial Support
U.S. Department of Agriculture; U.S. Department of Homeland Security

Notes:

V-031 - Estimation of the distribution of bushpigs in Madagascar and its implication for African swine fever risk

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Session: Epidemiology

Objective

Bushpigs (*Potamochoerus larvatus*) may act as a source of infectious diseases for pigs. An important example is African Swine Fever (ASF), which is not only an economical constraint for endemic countries, but also a cause of severe international trade restrictions and a serious threat to disease-free high-export pork countries, such as United States. In Madagascar, it is hypothesized that bushpigs might compromise the success of ASF eradication programs by sporadically transmitting the virus to domestic pigs. However, the distribution of this species in Madagascar is largely unknown, which makes it difficult to identify areas at high risk of inter-species transmission where surveillance and mitigation strategies could be implemented. In this study, we estimated the distribution of bushpigs in Madagascar using an ecological niche model (ENM) to identify areas where ASF risk-based surveillance could be targeted.

Methods

We retrieved 206 bushpig sightings in Madagascar during 1990-2016 and 22 climatic, agricultural, geographic and socioeconomic variables related to the presence of bushpigs. We used this data to run a presence-background maximum entropy ENM.

Results

We obtained a highly accurate (AUC = 0.83) map of the bushpigs distribution and identified three areas with a high probability of presence in the east, north and west of the country. The main contributors to the model were the vegetation index (51.3%), forest density (17.6%) and average annual precipitation (12.6%). In addition, we identified an area in the central part of the country with high density of domestic pigs and high probability of presence of bushpigs.

Conclusions

Our results may be useful for designing targeted surveillance and research studies to more accurately assess ASF transmission between domestic pigs and bushpigs and they contribute to improve the understanding and control of ASF. In addition, they could be helpful for other zoonotic diseases shared between these species in Madagascar (i.e. cysticercosis or hepatitis E). Our approach could also be extrapolated to other species of wild suids and other countries.

Financial Support
USDA-NIFA

Notes:

V-033 - Rodents act as reservoir hosts to excrete multiple species and serogroups of *Leptospira* concurrently

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Session: Epidemiology

Objective

Rodents are an important reservoir host of pathogenic leptospires in the US Virgin Islands. Our previous studies showed, by whole genome sequencing (WGS) of recovered cultures (n=60), that rodents are carriers of *L. borgpetersenii* (n= 48) and *L. kirschneri* (n=3) and that nine rodents were carriers of a mixed population of pathogenic *Leptospira* species. The aim of this study was to obtain clonal isolates from the mixed populations of pathogenic *Leptospira* species cultured from the nine infected rodents.

Methods

To recover clonal isolates, positive cultures of mixed species of pathogenic leptospires from rodent kidneys (designated LR1, LR5, LR37, LR57, LR60, LR61, LR68, LR70 and LR72) were propagated in HAN growth media at 29 and 37°C, and T80/40/LH growth media at 29°C. Multiple approaches were used to enrich for individual species including agglutination with reference antisera specific for serogroups Icterohaemorrhagiae and Ballum, subculture after agglutination in HAN at 29 and 37°C, and plating for individual colonies on HAN agar. Purity of individual colonies was validated by WGS and serotyping.

Results

At least one clonal isolate was recovered from all nine mixed cultures: 5/9 mixed cultures had one isolate obtained, while 4/9 mixed cultures had two clonal isolates obtained: *L. borgpetersenii* serogroup Ballum was recovered from LR1, LR5 and LR37 and *L. kirschneri* serogroup Icterohaemorrhagiae was recovered from LR60 and LR72. Individual clonal isolates of both *L. borgpetersenii* serogroup Ballum and *L. kirschneri* serogroup Icterohaemorrhagiae were recovered in four rodent samples (LR57, LR61, LR68 and LR70).

Conclusions

This study demonstrates that rodents can carry and excrete multiple species and serogroups of pathogenic leptospires concurrently. A combination of selective approaches and plating on HAN agar at 37°C enabled the separation and recovery of different species that can be characterized to further understand how mixed infections contribute to the epidemiology of leptospirosis and their role in the transmission of infection to humans and domestic animals.

Notes:

V-035 - Assessment of the RT-QuIC assay for detection of chronic wasting disease in white-tailed deer: A Bayesian approach

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Objective

Chronic wasting disease (CWD) is a transmissible prion disease of the *cervidae* family, with concerning zoonotic potential. Diagnostic methods for the routine detection of CWD in cervids include ELISA and IHC tests, primarily performed on postmortem tissues consisting of the medial retropharyngeal lymph node (RPLN) and obex. Although ELISA and IHC tests are considered the diagnostic gold standards for CWD, differences in CWD transmission and pathogenesis observed across populations can limit performance, thus impacting test sensitivity (Se) and specificity (Sp). Given this uncertainty, Bayesian approaches are useful methods to evaluate the accuracy of diagnostic tests without relying on putative "gold standards". Here, we assessed the accuracy of real-time quaking-induced conversion (RT-QuIC), an increasingly used prion amplification assay to diagnose CWD, on tonsil (TLN), parotid (PLN) and submandibular lymph nodes (SMLN), and ELISA/IHC on RPLN of white-tailed deer (WTD) sampled from Minnesota under field conditions using a Bayesian approach.

Methods

Dichotomous RT-QuIC results from wild (n=61) and farmed (n=46) WTD, and ELISA/IHC results obtained from the CWD reference laboratory in CSU–Fort Collins were analyzed with two-dependent-test one-population models.

Results

Results suggested that RT-QuIC performed on TLN and SMLN in the wild WTD population had similar Se (median range: 92.2-95.1) to the ELISA/IHC on RPLN (median range: 91.1-92.3). Slightly lower (4-7%) values of Se were observed when estimates were obtained from the farmed animal and PLN models. RT-QuIC Sp median estimates from all models were high (median range: 94.5–98.5%) and similar to those of ELISA/IHC (median range: 95.7-97.6%).

Conclusions

This study highlights the high Se and Sp observed with the use of RT-QuIC on TLN and SMLN and suggests avoiding the use of PLN for CWD diagnosis. Our results are the first report on the performance of RT-QuIC and ELISA/IHC at the population level and under field conditions, which represents an important step forward with respect to diagnostic tests for the management of CWD.

Financial Support
Minnesota State Legislature through Rapid Agricultural Response Fund (RARF); Environment and Natural Resources Trust Fund through the Legislative-Citizen Commission on Minnesota Resources; University of Minnesota Office of the Vice President for Research

Notes:

V-036 - Sublethal microcystin LR exposure predisposes channel catfish to lethal *Aeromonas hydrophila* infection.

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Session: Epidemiology

Objective

Cyanobacteria are the dominant algae of channel catfish aquaculture. These blooms have intensified with increasing fish density. This is concerning because Cyanobacteria can produce toxins that affect fish. Microcystins are among the most common of these toxins. They are cyclic oligopeptides that cause substantial liver damage. Given the importance of the liver in the immune system, we hypothesize sublethal microcystin exposure predisposes channel catfish to infectious diseases.

Methods

To study this, we used microcystin Leucine-Arginine (LR) (MC-LR). Fish were given a single intracoelomic dose of 500ng/g bw MC-LR and histopathology and serum chemistry were compared to saline-injected controls over a 6-day period. In ex-vivo studies, channel catfish leukocytes were exposed to 0, 10, 100 or 1000 ng/ml of MC-LR for 6 hours and evaluated for phagocytic ability. In the third part, the survival of fish that were injected with 500 ng/g bw MC-LR was compared to fish injected with saline and after exposure to an LD20 dose of a virulent strain of *Aeromonas hydrophila* (vAh).

Results

The MC-LR exposed fish appeared normal but stopped eating. Serum AST and ALT levels were significantly elevated from 6 hours through 96 hours post-exposure and histology confirmed diffuse hepatic injury in the treated fish and substantial recovery by 6 days post-exposure. In leukocyte studies, MC-LR exposure decreased the number of cells that endocytosed dextran 40, and dextran 80 and that phagocytosed the bacterial pathogen *Edwardsiella ictaluri*. In the *Aeromonas hydrophila*- MC-LR challenge study, fish that received microcystin experienced 67.3% mortality after vAh exposure compared to 22.4 % mortality in fish that were given saline injections and vAh exposure ($P<0.001$ in Kaplan–Meier survival analysis). There were no losses in the MC-LR-only or the saline-only injected fish.

Conclusions

These data demonstrate that sublethal MC-LR exposure can make channel catfish more susceptible to a common bacterial pathogen and suggests that managing cyanobacterial blooms may reduce infectious disease outbreaks in aquaculture.

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

Notes:

V-037 - Prediction of metritis cure in non-antibiotic treated dairy cows

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Session: Epidemiology

Objective

The study objectives were to identify cow-level and environmental factors associated with the spontaneous metritis cure and to evaluate and predict spontaneous metritis cure.

Methods

Data from cows in three studies performed in Florida (FL), Texas (TX), and California (CA) comparing the efficacy of ceftiofur and self-cure for metritis were used. Cows randomized to ceftiofur or remain untreated. To evaluate metritis spontaneous cure, only untreated cows (CON) were included in the present study (n = 438). Cure was defined by vaginal discharge became mucoid and not fetid up to 14 days after metritis diagnosis. Days in milk at metritis diagnosis (DIM D0), season, month of metritis diagnosis, lactation number, calving score, dystocia, retained fetal membranes, body condition score at d 5 DIM, vulvovaginal laceration score, rectal temperature at metritis diagnosis, fever at diagnosis, milk production difference from the day before and the day of metritis diagnosis (MD), and milk increase (slope from d 1 up to 5, 7 and 9 DIM (MI)) were offered to univariate logistic regression models. Variables with $P < 0.10$ were included in the multivariable logistic regression.

Results

Cows developing metritis after the first week postpartum had 1.24 times higher odds of spontaneous cure of metritis than cows having metritis in the first week postpartum ($P < 0.01$). Each degree Celsius above 39.3°C (mean rectal temperature at metritis diagnosis) was associated with 0.63 lower odds of spontaneous metritis cure ($P = 0.02$). Also, each kilogram of milk production above 0.91kg (mean of MD) was associated with 1.05 greater odds of metritis spontaneous cure ($P < 0.01$). The multivariate ROC curve revealed an area under the curve (AUC), accuracy, precision, and F1 score of 0.68, 0.66, 0.65, and 0.78 respectively.

Conclusions

The current study suggests that cows developing metritis after 7 DIM, with an increase in milk production from the day before to the day of metritis diagnosis and rectal temperature ≤ 39.3°C have increased odds of spontaneous cure of metritis with an accuracy of 66%.

Financial Support
U.S. Food and Drug Administration

Notes:

V-038 - Anthrax outbreaks among domestic ruminants in Arua District, Uganda, 2016-2018

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Session: Epidemiology

Objective

In 2017, Uganda Ministry of Health established 8 probable human anthrax cases (mostly cutaneous type) in Arua District (CFR=12.5%), associated with unvaccinated domestic ruminants that died of anthrax (burden unknown). In 2018, we investigated to assess the magnitude of anthrax infection in domestic ruminants, identify exposures, and recommend control measures.

Methods

We defined suspected case as sudden death with unclotted blood from body orifices in a domestic ruminant from January 1, 2016 to July 2018 in Arua District. A probable case was suspected case positive to rapid diagnostic test, consistent with *B. anthracis.* A confirmed case was suspected case positive by PCR. To identify case-animals, we reviewed the district veterinary anthrax records and conducted active community animal-case finding and updated the line list. We looked out for anthrax affected areas and collected specimens from carcasses. We conducted a case-control study to compare exposures among case-kraals and control-kraals, frequency matched by village with a ratio of 1:1.

Results

We identified 140 case-kraals and line listed 1600 animal cases by 22nd July, 2018. 967 (60%) of the animals had died at grazing area; 633 (40%) in kraals. Rigbo Subcounty (AR/1000 = 37), was the most affected. 5/6 animal carcasses tested positive by RDT and Gram positive rods identified by microscopy were consistent with *B. anthracis.* In a case control study, skinning infected dead animal carcasses on the pastureland (OR=7.5; CI = 3.9 - 14.3); and grazing animals near the river bank where previous suspected anthrax animals died (OR=2.3; CI=1.2 - 4.4) were the main exposures. Both communal grazing near the River bank and remains of the animal carcasses were observed in the grazing field.

Conclusions

Skinning infected dead animals (carcasses) on the grazing area was associated with the outbreak. We recommended immediate anthrax vaccination of domestic ruminants at risk followed by annual vaccinations and sensitization of communities; animal and medical health workers about anthrax control and prevention.

Notes:

V-039 - Plant-derived compounds modulate *Salmonella* Enteritidis proteome critical for colonization in chickens.

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Session: Food Safety

Objective

Salmonella Enteritidis (SE) is a foodborne pathogen of global concern. Chickens act as a reservoir host for SE, wherein the pathogen colonizes the ceca leading to carcass contamination and subsequent human infections. Several multi-drug resistant SE strains have been isolated from poultry fuelling the research for developing antibiotic alternatives for controlling SE in chickens. Trans-cinnamaldehyde (TC), Eugenol (EG) and carvacrol (CAR) are plant-derived compounds that have been reported to exert significant anti-*Salmonella* efficacy in chickens. However, the underlying molecular mechanisms of their action are still unclear. This study investigated the effect of TC, EG and CAR on the proteome profile of SE, especially the expression of proteins critical for colonization in chickens.

Methods

Sub-inhibitory concentrations (SICs; compound concentrations below the MIC that do not affect bacterial growth) of TC, EG and CAR against SE-31 strain were determined by growth curve assay. For proteome profiling, SE-31 was cultured either in the presence or absence of SICs of phytocompounds for 24 h at 37^{o}C followed by protein extraction, quantification, and LC-MS/MS analysis. Differentially expressed proteins between samples were analyzed using Student's t-test on Scaffold 5 ($P<0.05$).

Results

The sub-inhibitory concentration of TC, EG and CAR was 0.01%, 0.01%, and 0.008%, respectively. Approximately 1440 proteins were identified of which ~ 170 proteins were down-regulated and ~100 proteins were upregulated by the phytocompounds. All phytocompounds down-regulated critical virulence proteins contributing to PSI-1 type III secretion system (SipA, SipB, SipC), motility (SefB), cellular metabolism (GlpB, MetK, SpeF), and polymyxin resistance (ArnA) ($P<0.05$). The expression of proteins involved in stress response or cellular transport (GrolL, TolC, AcrA) was upregulated by phytocompounds ($P<0.05$).

Conclusions

Overall, these results delineate the prospective mechanism of action of TC, EG and CAR against SE and provide a basis for their combinatorial application.

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

Notes:

V-040 - Genetic fingerprints and virulence profiles of *E. coli* O157:H7 from humans and cows

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Session: Food Safety

Objective

E. coli O157:H7 strains cause severe stomach pain, bloody diarrhea, vomiting, fever, and kidney failure in humans. These illnesses affect about 2.5 million people in the world annually. Consumption of undercooked contaminated food is the main risk factor. Cattle is the major reservoir host. The virulence profiles present in the strains determine the pathogenesis. This study evaluated the genetic fingerprints and virulence profiles of O157:H7 strains isolated from humans and cows from USA and Argentina.

Methods

The isolates (n= 13) were provided by Pennsylvania State University. They were isolated from 11 U.S. States (n=11) and Argentina (n=2) from six body sites of humans (n=5) and cows (n=8), namely feces (n=7), hide (n=1), kidney (n=2), stomach (n=1), urine (n=1), and uterus (n=1). We genotyped the isolates by ERIC-PCR. The presence of virulence genes (i.e. stx1, stx2, and eae) was screened by PCR. A 2% gel electrophoresis resolved the PCR products. I constructed phylogenetic tree using PyElph 1.3 software for genetic fingerprinting of the isolates.

Results

The 13 isolates were clustered into seven genetic groups. Most isolates from bovine kidney (n=2) were related. Some isolates from digestive tract of cows were related with those isolates from stool of humans irrespective of the geographical origin. Some isolates from uterus and hide of cows were related with those from feces of cows and stool of humans. Despite geographically distant, an isolate from stool of human from Argentina was closely related to some isolates of cows and humans in USA. For virulence, *stax*2 was detected in most isolates (8/13) followed *eae* (7/13) and stx1 (3/13).

Conclusions

Although the sample size is small, we conclude that some of the isolates from different geographical locations, host species, and body parts are closely related genetically. Most of these isolates harbor *stx*2 toxin followed *eae* and *stx*1. Molecular basis of host infection and pathogenesis of the isolates from a wider geographical ecology, host ranges, and body parts that are within the same and different genetic clusters requires further study.

Financial Support
Long Island University

Notes:

V-041 - Mouse specific pathogen free microbiota to reduce *Campylobacter jejuni* colonization in chickens

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Session: Food Safety

Objective

Campylobacter jejuni is one of the worldwide prevalent foodborne bacterial pathogens mainly transmitted from poultry. However, few mechanisms are available on why *C. jejuni* colonizes chickens. In this study, we aimed to investigate the mechanism of transplanting microbiota on *C. jejun*i chicken colonization.

Methods

Mouse specific pathogen free (SPF) microbiota was cultured on Brain Heart Infusion agar (BHI) and collected as SPF-Aerobe and SPF-Anaerobe. Birds raised on floor pens were colonized with 108 CFU/bird SPF-Aerbe and SPF-Anaero at d 0 and infected with 109 CFU/bird *C. jejuni* chicken isolate AR101 at d 12. Birds were sacrificed at day 28 to enumerate *C. jejuni* cecal colonization on selective Campylobacter plates. *C. jejun*i AR101 was co-cultured with SPF-Anaero and chicken anaerobic microbiota for examining the impact of microbiota on the pathogen in vitro growth at an inoculation ratio of 1:100, and 1:1000.

Results

As a result, we found that the SPF-Aerobe and SPF-Anaero microbiota reduced 91 % and 96 % of *C. jejuni* colonization in chicken ceca at day 28 ($1.9x10^5$ and 8.5x104 vs. 2.4x106 CFU/bird, respectively) compared to infected alone birds. Notably, transplanted SPF-aerobe and SPF-Anaero increased Bacteroidetes (51.89 and 47.60% vs. 12.33%, respectively) compared to infected control birds. SPF-Anaero reduced *C. jejuni* growth by 99% (1.4 x105, and 0 CFU/ml) for both 1:100, and 1:1000 inoculations, while Ch-Anaero reduced C. jejuni growth by 97% and 99% (5.6 x105 and 0 CFU/ml) for the inoculations. Interestingly, SPF-Anaero and Ch-Anaero were comparable on inhibiting C. jejuni growth.

Conclusions

These results suggest that *C. jejuni* directly modulates the chicken gut environment and gut microbiome to restrict its ability to prevent foreign colonization by *C. jejuni*.

Financial Support
Arkansas Biosciences Institute

Notes:

V-042 - Serological survey of antibody immunity against *Salmonella* in a commercial breeder farm using inactivated vaccine

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Session: Food Safety

Objective

The objective of the present study was to assess whether a breeder farm using an inactivated vaccine against *S.* Enteriditis and Thyphimurium produced a progeny having MD antibodies.

Methods

The survey was conducted in a commercial breeding farm which hens were vaccinated by using the inactivated vaccine AVISAN® SECURE (Laboratorios HIPRA, Spain) and following the manufacturer's instructions. Flocks of hens of 21, 25, 35, 48 and 54 weeks old were recruited and blood samples were collected from 25 hens per flock. Moreover, yolks were collected from 25 eggs produced by the recruited breeder flocks on the day of the bleeding. Finally, blood samples were collected from 25 day-old chicks hatched from eggs collected on the day of the bleeding and produced by the recruited breeder flocks. The level of IgY antibodies against the O-serogroup B and D of *Salmonella* in the samples was determined using the quantitative ELISA kits Salm Gp B/D (Biochek, Spain) and flocktype® Salmonella Ab (Qiagen, Spain).

Results

Results showed positivity to antibodies in 96-100% of the inspected birds; notably, even the oldest flock of approximately 54 weeks showed such results. The yolks and day-old chicks showed positivity in 100% and 84-100% of the inspected samples, respectively; these percentages were drastically reduced when the same samples were analysed by the ELISA kit Salm Gp B/D. The antibodies of day-old chicks were 26-41% or 37-62% the levels of their respective hen flocks

Conclusions

The present observational study suggested that hen flocks vaccinated with AVISAN® SECURE may generate a long-lasting antibody response against the O-serogroup B and D of *Salmonella* and this may be transmitted to their progeny.

Notes:

V-044 - Innate immune cell mTOR responsiveness to LPS challenge in postpartum dairy cows following amino acid infusion

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Session: Immunology

Objective

The nutrient-sensing mammalian target of rapamycin (mTOR) pathway is as a key orchestrator integrating metabolic cues in immune cell activation. We have previously shown that nutrient availability altered mTOR responsiveness to an inflammatory stimulus in bovine monocytes *ex vivo*; however, responsiveness has not been described *in vivo*. Our objective was to assess mTOR responsiveness during an *in vivo* lipopolysaccharide (LPS) challenge in postpartum dairy cows previously supplemented or not with intravenous (IV) amino acids (AA). We hypothesized that increasing systemic availability of AA prior to immune activation would improve energy status and increase mTOR pathway responsiveness of mononuclear cells (MNC) during this time of nutrient deficit.

Methods

Postpartum cows (n=14, 4 ± 1 DIM) were IV infused for 4 d in a matched-pair randomized controlled design and received 0.9% NaCl (control) or IV AA (IVAA) to supply 1 g/kg BW of AA. After infusion ended, cows were IV infused with LPS (62.5 ng/kg BW over 1 h) and blood was sampled at 0, 1, 2, 8, and 24 h relative to the start of LPS infusion to assess AA and energy status. Ratios of mean fluorescence intensity for phosphorylated (p) to total protein of AKT and mTORC1 substrates S6RP and 4EBP1 were analyzed in MNC by flow cytometry.

Results

Baseline circulating AA were increased ($P<0.01$) but glucose and insulin decreased ($P<0.01$) in IVAA. Ratios of p4EBP1:4EBP1 increased ($P<0.01$) and pS6RP:S6RP ($P<0.01$) decreased following LPS infusion, but pAKT:AKT did not change ($P=0.12$) during challenge. Ratios of phosphorylated to total protein did not differ ($P\geq0.17$) between groups during challenge.

Conclusions

We demonstrated that the mTOR pathway in bovine MNC is responsive to LPS stimulation *in vivo*, extending previous *ex vivo* findings; however, the mTOR pathway was not responsive to increased AA availability during the precarious postpartum time in dairy cows in contrast to our hypothesis. These *in vivo* results may differ from our previous *ex vivo* studies due to the timing of nutrient availability because AA supplementation was ended before challenge.

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

Notes:

V-045 - Demise of a myth: Macrophages are not a safe-haven for mycobacterial pathogens.

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Session: Immunology

Objective

The objective of the study was to demonstrate CD8 cytotoxic T cells kill intracellular mycobacteria by the perforin, Granzyme B, granulysin pathway.

Methods

Monocyte derived macrophages (moMacs) were placed in tissue culture plates and infected with *Mbv* BCG and overlayed with unstimulated PBMC or PBMC stimulated with BCG or a BCG *relA* mutant (BCG/relA) using an ex vivo culture platform for generating cytotoxic T cells (CTL). Two methods were used to analyze cytotoxicity: visual inspection of tissue culture plates; use of a quantitative PCR method to distinguish live from dead bacteria. Flow cytometry was used to demonstrate CTL killing of infected moMac.

Results

Visual inspection of infected moMacs overlayed with unstimulated and PBMC stimulated with BCG or BCG/relA revealed infected moMacs lost adherence following incubation with PBMC stimulated BCG or BCG/relA. Infected moMacs remained adherent when incubated with unstimulated PBMC. Analysis of killing of bacteria revealed PBMC stimulated with BCG or BCG/relA killed infected target cells through the perforin, Granzyme B, and granulysin pathway.

Conclusions

Extensive studies conducted to characterize the mechanism used by mycobacterial pathogens to evade immune elimination. Although studies provided important information, studies were conducted without concurrent studies in the presence and absence of PBMC stimulated with mycobacterial pathogens. Analysis of the interaction of antigen stimulated PBMC with infected moMacs has demonstrated macrophages are not a safe-haven. Studies conducted with PBMC stimulated with BCG or BCG/relA demonstrated killing is mediated by CD8 CTL. Results presented here duplicate studies with *M. a. paratuberculosis*. Further studies are needed to determine how mycobacterial pathogens are able to evade immune elimination.

Financial Support
Washington State University

Notes:

V-046 - Converse trends with adaptive and innate immune responses following intravenous BCG inoculation in pigs

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Session: Immunology

Objective

Percutaneous (PC) administration of Bacillus Calmette-Guerin (BCG) is the only approved route of vaccination in humans and has been used in cattle. However, intravenous (IV) BCG vaccination enhanced protection against tuberculosis above PC vaccination routes in rhesus macaques. BCG administration in humans can induce innate memory as well as adaptive memory. Various routes of BCG inoculation in pigs were assessed for induction of innate memory and adaptive immune responses to confirm BCG exposure. An inverse relationship between induction of innate memory and adaptive memory was noted.

Methods

Four-wk-old pigs were inoculated by subcutaneous (SQ), intramuscular (IM), or IV route with BCG or saline (noBCG – control pigs). A sample size of 8-10 pigs/treatment for each study was maintained. To assess adaptive immune responses, at 5-8 weeks post-inoculation (wpi), ex vivo peripheral blood mononuclear cell (PBMC) IFNg responses to PPD bovine (PPDb) were measured by ELISA. Innate memory was assessed at 2-8 wpi by culturing monocytes with LPS and measuring TNF production. At 6 wpi, inoculation site (SQ group) and liver (SQ and IV groups) samples were collected for BCG culture.

Results

PBMC IFNg production following ex vivo PPDb stimulation was increased in SQ and IM groups (5.1 and 3.6-fold, respectively), but not in IV inoculated pigs. Conversely, TNF production from monocytes isolated from IV inoculated pigs and stimulated with LPS was heightened over SQ and IM groups (3.9 fold). However, BCG was recovered from the site of SQ inoculation and liver of IV but not SQ pigs.

Conclusions

The lack of peripheral adaptive immune responsiveness following IV but not SQ or IM inoculation along with the converse enhancement of innate immune responses suggests that how or where an antigen is presented to the immune system plays a critical role in the type of immune response generated. In addition, the recovery of BCG from organs of animals with enhanced innate responses calls into question whether constant exposure to BCG or true cellular changes are associated with innate memory.

Notes:

V-047 - The role of exosomes in Marek's disease virus lymphomagenesis and immunity

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Session: Immunology

Objective

Marek's disease (MD) is a T-cell lymphoma of chickens caused by Marek's disease virus (MDV). Losses due to MD are controlled via the use of live, apathogenic vaccines, however the mechanisms mediating systemic lifelong protection are not fully understood. Chickens vaccinated *in ovo* or at hatch are protected from tumor formation, but not superinfection with oncogenic MDV field strains. The purpose of our research is to identify the contributions of serum exosomes to lymphomagenesis, tumor progression, immune suppression and conversely, systemic anti-tumor immunity. Our hypotheses are that (1) serum exosomes expressed during MDV latency contribute to tumorigenesis and systemic immune suppression and (2) serum exosomes produced during vaccine virus replication elicit lifelong systemic anti-viral and anti-tumor responses.

Methods

In previous work, we found that serum exosomes from tumor-bearing birds contained miRNAs targeting lymphocyte activation, suggesting a possible mechanism of immune suppression. In serum exosomes from vaccinated and protected chickens (VEX), we found miRNAs targeting cell proliferation, and mRNAs spanning the entire MDV genome. This year, we conducted experiments to determine the cells taking up VEX, if the mRNAs delivered by these exosomes are indeed expressed as proteins, and performed RNA-seq on VEX from unvaccinated and vaccinated chickens held in isolation.

Results

We report that macrophages patterned to become dendritic cells actively took up exosomes, suggesting that these mRNAs may be expressed and presented as antigens in the context of MHC-I. To further address this, we are examining the proteomes of these cells after overnight exposure to VEX. In terms of our RNA-Seq data, we found that vaccine miRNAs begin to accumulate in exosomes at 14 and 21 dph.

Conclusions

Our data suggest that serum exosomes in MD-vaccinated chickens mediate systemic antiviral and anti-tumor immunity via the delivery of viral antigen mRNAs to antigen-presenting cells throughout the body, and targeting pathways active in MDV latently-infected T-cells, respectively.

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

Notes:

V-048 - Impact of respiratory viral infections on the microbiome in avian and mammalian animal models

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Session: Modeling

Objective

The respiratory and intestinal microbiome has a direct impact on host cells or an indirect impact on the immune system during viral infections. Previous studies have shown that bacterial communities that reside in the respiratory or intestinal tract affect respiratory viral infections, such as the enhancement of influenza virus transmission or host responses during SARS-CoV-2 infection. Outbreaks of avian influenza virus (AIV) can have detrimental effects in poultry flocks resulting in mortality and indirect costs within poultry food production. Diversely, SARS-CoV-2 has resulted in millions of human deaths and declining economies around the world. Therefore, the aim of this work was to characterize the respiratory and intestinal microbiome of an avian and mammalian animal model throughout the duration of a respiratory viral infection.

Methods

We explored the microbial changes within the oropharyngeal and fecal microbiome of broiler chickens infected with an H9N2 subtype AIV and the lung and cecum microbiome of K18-hACE2 mice infected with a SARS-CoV-2 virus.

Results

Broiler chickens infected with AIV showed diversity changes within the intestinal microbiome throughout infection, particularly, the composition was similar among infected and not infected feces at 21 days post-infection but comparatively different among earlier days post-infection. Similarly, the intestinal microbiome using K18-hACE2 mice infected with SARS-CoV-2 showed decreased Shannon and Inv Simpson diversity index correlating with infection dosage and a difference of Bray-Curtis dissimilarity distances among control and infected mice. However, the lung microbiome of SARS-CoV-2 infected mice showed limited diversity changes but a shift from Bacteroidetes to increased Firmicutes and Proteobacteria.

Conclusions

We identified changes in the microbiome of two different animal models throughout infection with distinct respiratory viral pathogens. These studies add to a better understanding of the complexities associated with the host microbiome during respiratory infections.

Financial Support
U.S. National Institute of Allergy and Infectious Diseases

Notes:

V-049 - Generation of a bovine 2-D primary enteroid monolayer cell culture model to investigate host-pathogen interactions

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Session: Modeling

Objective

Interactions between the gastrointestinal (GI) enterocytes and immune cells located below the mucosal surface are critical in maintaining GI homeostasis and initiating immune responses to pathogens. Generation of *ex vivo* 3D-enteroid cell culture models have greatly our ability to understand the cell-cell interactions occurring at the intestinal interface. However, this model results in the luminal aspect being inaccessible without microinjection or disruption of the 3D structure thus impeding the investigation of interactions between enterocytes and host immune cells. Improved monolayer models are needed to capture a more relevant picture of *in vivo* dynamics. The objective of this study was to establish standardized methodology for generating 2D bovine intestinal monolayers derived from primary 3D ileal enteroids that can be used to investigate host cell-pathogen interactions with distinct apical and basolateral interfaces.

Methods

Ileal crypts from healthy adult cattle were used to generate 3D enteroids with downstream cryopreservation and subsequent 2D monolayer generation on transwell inserts. Monolayers were characterized by measuring barrier function and polarization via transepithelial electrical resistance (TEER) throughout culture, assessing expression of actin and E-cadherin with immunofluorescent staining, and determining the response to TNF-α stimulation by measuring IL-8 secretion.

Results

Monolayers exhibited physiologically relevant TEER measurements throughout culture and displayed tight cellular organization after 5 days in culture as evidenced by immunofluorescent staining. Furthermore, basolateral IL-8 secretion by the monolayers was upregulated with TNF-α stimulation, displaying their capacity for cytokine-mediated responses.

Conclusions

This culture system offers a well-defined protocol for efficient, reliable, and reproducible generation of a bovine primary cell enteroid monolayer that mimics *in vivo* infection, permitting investigation of pathogen interactions on the apical surface of enterocytes and of communication dynamics with underlying innate immune cells.

Financial Support
U.S. Department of Agriculture; USDA-NIFA AFRI

Notes:

V-050 - Predictive modeling of pH in an aquaponics system to inform system maintenance

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Session: Modeling

Objective

One of the most important water quality parameters in aquaponics is pH. This parameter is often unstable and must be continually monitored and adjusted. A risk with this triage approach is that overcompensation with the corrective compounds or too sudden of a shift in pH could threaten system health. Our research proposes that data generated through daily system monitoring could be utilized to build a model to predict pH shifts.

Methods

We conducted both traditional and Bayesian linear regression using water quality data from two separate aquaponics systems. The data from one system was used to train the model, while the other was used for testing model accuracy. This order was then reversed to examine the impact of the choice of training data set on model performance. The top two models obtained from each regression method were compared and the model with the lowest root mean square error (RMSE) and largest Nash Sutcliffe Efficiency (NSE) score was selected. The sensitivity and specificity of this model to pH safe range violations were calculated. This model was then incorporated into an online web application to allow aquaponics farmers to perform basic data analysis and predict pH fluctuations in their own systems.

Results

We found that a standard regression model with the pH values measured one and two days prior to the target date could predict the pH with both arrangements of training and testing data. The selected model had a NSE score of 0.8 and a RMSE of 0.181. The sensitivity (0.78) and specificity (0.99) of the predictions of range violations showed the model is better at correctly classifying values in the safe range than values outside. Specifically, the model is more likely to yield false negatives than false positives.

Conclusions

Our study demonstrates that pH in an aquaponics system can be modeled with reasonable accuracy using linear regression. Improvements to this model could be made if continuous water quality monitoring could be implemented in these systems to increase the amount of data and frequency of measurement.

Notes:

V-051 - Dynamics of bovine tuberculosis outbreaks: A study of interdependence between seasonal host movement and pathogen abundance

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Session: Modeling

Objective

Bovine Tuberculosis (bTB) is a zoonotic disease carrying significant risk for ecosystem services, public health, and agricultural industries. In wildlife populations, it may reach epidemic proportions due to seasonal animal movement that facilitates indirect transmission, i.e., contacts between susceptible hosts and shed pathogens. Conversely, physical changes associated with infection, e.g., weakness and loss of appetite, may restrict the host's movement behavior and where the pathogens are shed relative to conspecifics. A mechanistic understanding interdependence between host behavior and disease dynamics is therefore critical to the optimization of surveillance targets for bTB in many ecological systems.

Methods

Our model describes a closed ungulate population in which individuals are randomly distributed and express seasonal site-fidelity (home ranging behavior). Movement is directionally biased and stochastic step length varies as a function of disease state, thus allowing individuals to transition between sedentary and exploratory space use patterns over time. Pathogens shed by infected hosts are assumed to decay at seasonal rates (smaller rates in spring/summer and higher in fall/winter).

Results

Our results indicate a strong link between temporal home range distribution and disease incidence. The relationship we found between host movement and the risk of onward transmission is generalizable and can help predict rapid changes in contact structure and the likelihood of an epidemic. By highlighting the cascading effects of short-term movement responses, we further demonstrated the importance of considering transient behavior in the development of surveillance and management strategies, and underlined the large-scale, long-term consequences of overlooking this element.

Conclusions

Our study identifies dynamic feedback between movement and disease ecology that are critical to the enhancement of real-time outbreak intervention and scenario planning.

Financial Support
University of Georgia; Intelligence Community Postdoctoral Research Fellowship Program

Notes:

V-052 - Linking experimental games with agent based models to quantify agricultural outbreak dynamics

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Session: Modeling

Objective

The spread of disease among U.S. livestock supply chains results in economic losses in excess of a billion dollars annually. Investing in preventative biosecurity can reduce the likelihood of pandemics and new disease emergence. Here we show how human behavior and decision-making regarding biosecurity investment and adoption may impact systemwide disease spread. Our approach uses experimental games and agent based models to quantify behavioral risk related to biosecurity investment.

Methods

Our experimental games emulate a pork production supply chain during an outbreak. Participants can invest simulated resources to increase their disease protection. Various information regarding disease risk, neighboring biosecurity, and outbreak status are conveyed to test their effect on disease risk management. We quantify how these behavioral effects will impact supply chain dynamics and the spread of disease with an agent based model using real-world mechanics, like movement of animals and feed between farms.

Results

Using experimental game data we identified prominent behavioral profiles and tested how risk communication and information uncertainty affected decision-making. Then with our agent based model we tested how different distributions of risk profiles observed in the game affect outbreak severity and volatility. We found as simulated farmers' tolerance to disease risk increases, unpredictability of disease spread expands, and the outbreak becomes more difficult to control. This observed outbreak variability persists even if half of the simulated farmers were to transition to risk averse behaviors in response. This illustrates how preparedness is crucial in stemming the spread of disease.

Conclusions

Our research shows that we should account for human behavior to model disease spread. Experimental games can be applied for testing behavioral effects and internalization of risk communication strategies in order to promote more risk-averse and disease resilient systems. This change in risk and behavior can be studied with agent based models to simulate the effect on herd health.

Financial Support
USDA National Institute of Food and Agriculture, under award number 2015-69004-23273.

Notes:

V-053 - Assessing the animal and public health hazard of urban wild boars using an agent-based model approach

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Session: Modeling

Objective

Wild boar (WB, Sus scrofa) populations are globally increasing, accompanied by growing synurbization in European cities such as Barcelona (Spain), enhancing the major epidemiological role as pathogen host. Synurbization increases WB aggregation, contact rates and close interactions with humans, with the consequent risk of pathogen transmission, generating a new human-WB interface and creating epidemiological public health concern. This study aims to inform animal and public health risk assessments and support risk-based decision-making.

Methods

Using the BCNWB-prototype, a validated agent-based model of the social-ecological factors driving the use of the urban ecosystem by synurbic WB and the related human-WB interactions in Barcelona, this study develops an epidemiological expansion, the BCNWB-EPI model, in order to test epidemiological scenarios of three pathogens, two of them of zoonotic potential (i.e., hepatitis E virus, HEV, and antimicrobial-resistant Campylobacter, AR-CB) and one affecting suids (i.e., African swine fever virus, ASFV), at the WB-human interface in the (peri)urban area of Barcelona.

Results

Citizen exposure was similar for HEV (0.79%) and AR-CB (0.80%) and agreed with the World Health Organization (WHO) estimates of global exposure for these pathogens. The similar human exposure despite the difference in pathogen prevalence previously found in WB (HEV, 20%; AR-CB, 60%), suggests a major role of feces in the transmission of pathogens to humans in urban areas, resulting in a non-negligible public health risk. The entire WB population was exposed to ASFV, through carcasses (87.6%) or direct contact (12.6%), 51-71 days after the first case. The outbreak lasted 71-124 days, reducing 95% of the initial population, similar to previous reports of ASFV outbreaks in other European countries.

Conclusions

The spatially-explicit epidemiological predictions generated by the model are valuable to evaluate the animal and public health risks posed by HEV, AR-CB and ASFV in Barcelona and could be easily adapted to other diseases at the WB-livestock-human interface.

Notes:

V-054 - Genetic diversity of *Anaplasma marginale* in Tennessee beef cattle herds

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Session: Omics

Objective

Beef cattle are among the top 3 agricultural commodities in Tennessee. Bovine anaplasmosis threatens sustainable beef production in this state and other beef producing states. Estimated to cost the U.S. cattle industry $300 million annually, anaplasmosis can cause reduced feed intake/weight gain, drop in milk yield, reduced calving success, and death in severe cases. Numerous strains of *Anaplasma marginale* strains have been identified which can differ in transmissibility, virulence, antigenicity, and drug susceptibility; however, the genetic diversity of *A. marginale* in Tennessee is unknown. Therefore, the objective of this study was to evaluate the genetic diversity of *A. marginale* in Tennessee.

Methods

Blood samples were collected from beef cattle throughout Tennessee. Samples were initially evaluated for *A. marginale* infection using a quantitative PCR assay targeting a portion of the conserved major surface protein 5 gene (*msp5*). From up to 5 *msp5*-positive samples per herd, the variable region of Msp1a was PCR amplified, cloned and sequenced for *A. marginale* Msp1a genotype identification.

Results

Of 1,093 sampled cattle from a total of 18 herds, 210 (19.2%) cattle were positive for *A. marginale* infection. From the 11 positive herds, 48 cattle were selected for *A. marginale* Msp1a genotype determination. From the selected samples, 72 different *A. marginale* genotypes, comprised of different combinations and configurations of 28 tandemly arranged short repeat sequences were identified. Of these Msp1a genotypes, 62 have not previously been described in the U.S.

Conclusions

With more than 45,000 beef cattle operations, beef production in Tennessee is a significant portion of the state's agricultural economy. Understanding the genetic diversity of pathogens such as *A. marginale* that threaten this industry is pivotal to supporting cattle health and profitable beef production. This study is the first to describe extensive *A. marginale* genetic diversity in Tennessee. Understanding *A. marginale* strain diversity is important for the development of broadly effective disease management strategies.

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

Notes:

V-055 - Leptospira pangenome analysis reveals recombination events in the core and accessory genes

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Session: Omics

Objective

Leptospirosis, a neglected bacterial zoonosis caused by various *Leptospira* species, is reemerging due to ecological changes. In this study, we investigated the association of genomic variation events, including gene gains/losses and homologous recombination in *Leptospira*, in shaping infection patterns, geographical specificity, and serological and epidemiological characteristics. We performed bacterial pangenome and recombination analyses on 519 *L. interrogans* whole genome sequences (WGS), covering isolates from 40 countries/regions and 34 distinct serovars.

Methods

For a comprehensive understanding of the genomic compositions, we used a suite of bioinformatics software programs for hierarchical clustering (hierBAPS) and pangenome construction (Roary). The relationships among these *L. interrogans* isolates were then delineated by clustering their genomes based on their gene presence/absence profiles in the pangenome using the principal component analysis (PCA). In addition, we explored the recombination regions using the software Gubbins.

Results

Among the 30,851 genes identified in the pangenome, only 1,059 were identified as the core genes (shared by 99% of the isolates). Clustering based on gene presence/absence profiles was closely associated with serological classifications and their geographical origins. These clusters consisted of 8 lineages separated based on the hierarchical clustering of the core genome's nucleotide similarities, suggesting serological and geographical specificity. Moreover, we identified 9,411 recombination regions across the dataset found both on the core and accessory genes.

Conclusions

Our results provide a comprehensive description of the structural and compositional variations within *L. interrogans* genomes. They imply that in *Leptospira spp.* genomic plasticity is not only taking place in the core and accessory genes and emphasizes the need to explore core genome variations to gain a complete understanding of evolutionary mechanisms.

Notes:

V-056 - Analysis of genome size and mobile genetic elements in *Streptococcus suis* isolates from sick and healthy pigs

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Session: Omics

Objective

Streptococcus suis is ubiquitous in swine yet only a small percentage of pigs become clinically ill. Reduced genome size has been associated with highly virulent strains of *S. suis* and other bacterial pathogens. Additionally, mobile genetic elements (MGEs) have been found to carry virulence factor (VF) genes. The objective of this study was to investigate the genome size in *S. suis* isolates recovered from systemic (SYS) and non-systemic (NON-SYS) sites of sick and healthy pigs and to characterize MGEs within these isolates and the genes they carry.

Methods

Whole genome sequencing data of 273 isolates recovered from 65 sick pigs (47 SYS and 136 NON-SYS isolates) and 47 healthy pigs (90 NON-SYS isolates) were analyzed in this study. The sequences were assembled *de novo*, annotated and screened for MGEs namely, integrative and conjugative elements (ICEs) and phages *in silico*. The MGEs were then screened for antimicrobial resistance (AMR) and VF genes.

Results

There was a trend of smaller genome size in SYS isolates relative to NON-SYS isolates in most serotypes and in particular, serotype 2 isolates (Kruskal Wallis (KW), $p = 0.02$). Conversely, larger genomes were observed with serotype 9 SYS isolates relative to NON-SYS isolates (KW, $p = 0.02$). Further, 44% of genes prevalent only in serotype 9 SYS isolates were encoded in MGEs. In addition to the phage-related and ICE-related essential genes, a putative VF gene (*purA*) was also detected in the MGEs. In total, 345 putative ICE-related elements and 646 putative phages were detected across all of the isolates. Identical MGEs were detected in isolates from SYS and NON-SYS sites, and across multiple farms and serotypes. AMR genes were identified in 55 ICE-related elements and 17 putative phages, with genes *tetO* and *ermB* as the most prevalent.

Conclusions

Our results suggests that genomic trends may differ among *S. suis* serotypes and factors such as MGEs can play important roles in their diversification. In addition, the identification of AMR and VF genes in MGEs indicate the potential of *S. suis* as threats to the swine industry.

Financial Support
Ontario Pork; Ontario Ministry of Agriculture, Food, and Rural Affairs Research (OMAFRA); Swine Innovation Porc; Canada First Research Excellence Fund; Food From Thought

Notes:

V-057 - Characterization of the respiratory virome of Irish beef weanlings associated with bovine respiratory disease

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Session: Omics

Objective

Bovine respiratory disease (BRD), also known as shipping fever, remains a leading cause of morbidity and mortality of cattle placed in feedlots, despite decades of development and application of vaccines and antimicrobials. The aim of this study was to use untargeted metagenomic sequencing to compare the respiratory virome in BRD and healthy suckler breed beef weanlings on the day of BRD detection.

Methods

Animals were vaccinated, 24 h post-arrival in a feedlot, against BRSV, PI-3, *Mannheimia haemolytica* A1 and BoHV-1. Sterile swabs were inserted approximately 12 cm into the nasopharynx and gently rotated. This was conducted for 22 animals with BRD and 22 matched healthy control animals. Swabs were snap frozen in liquid N_2 and stored at -80ºC until required. Bead beating with RNase and DNase treatment was performed on PBS released from swabs. DNA and RNA was extracted and purified using the Qiagen MinElute Virus Spin kit and ds cDNA was generated and purified. The Rapid PCR Barcoding Kit (SQK-RPB004) and NEBNext® Ultra™ II Q5® Master Mix were used to generate sequencing libraries which were pooled and sequenced on an R9.4.1 flowcell (12 libraries per flowcell) on a MinION Mk1C. A positive control library and a negative control was included on every sequence run.

Results

Numerous viruses were detected but bovine coronavirus (BCoV) and bovine rhinitis A (BRAV) virus had by far the largest sequence read counts and had greater prevalence and higher read counts in BRD animals compared to healthy controls. There were 75 viruses (including bacteriophages) identified with a read count of ≥20 in the healthy cohort and 115 viruses identified, also including bacteriophages, with a read count of ≥20 in the BRD cohort.

Conclusions

Nanopore sequencing successfully characterized viral agents found within healthy and BRD infected animals. The Irish beef BRD-associated virome is diverse and complex however, BCoV and BRAV are prevalent amongst the BRD cohort. To the best of our knowledge, this is the first report of untargeted nanopore sequencing to characterise the Irish beef BRD-associated virome.

Financial Support
US-Ireland (Department of Agriculture, Food and the Marine (DAFM)) Tri- Partite grant (project number 2018US-IRL200)

Notes:

V-058 - A multi-omics model to identify host-microbiome interactions and pathogen dynamic impacts on *Streptococcus suis* disease

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Session: Omics

Objective

Streptococcus suis is a swine pathogen and a zoonotic agent affecting people in close contact with the swine. The purpose of this study was to develop a multi-omics model that integrates metagenome, whole genome sequencing, and host genome wide association studies data in order to assess the host-microbiome and pathogen dynamic interactions related to *S. suis* infection.

Methods

A total of 65 pigs from 9 farms were classified based on the presence of *S. suis* in the systemic sites into confirmed (21), probable (24), and healthy (20) group. Microbiota composition was assessed using the V3-V4 hypervariable region of the 16S rRNA gene and a Dirichlet-multinomial mixture model was used to study the tonsillar community. Using a GWAS approach, 41, 397 SNVs were analyzed using a Bayesian sparse linear mixed model to identify SNPs with high effects. Using a WGS approach, the relative frequency of *dltA* gene was identified and integrated into the model to evaluate the pathogen role. A LASSO-based model was developed using the three NGS data to predict associated factors related to *S. suis* disease.

Results

The probable group was significantly different from the healthy (p=0.01) and the confirmed group (p=0.016). *Streptococcus* abundance was not different between the probable, confirmed and healthy groups. A DMM model partitioned the tonsil microbiota into two distinct community types each with a unique taxonomic profile. There was an association between *S. suis* serotype 2 and community type 1 (p=0.03). Our model revealed 277 SNPs that influence the variations in the tonsil microbiome composition (R^2>0.05). One SNP in chromosome 2 was suggestively associated with the disease clinical signs.

Conclusions

While the overall abundance of *Streptococcus* ASVs was not different among the diagnosis groups, the unique profile of community type 1 and the observed correlation with *S. suis* serotype 2 could shed light on the interaction between the tonsil microbiota and their effect on *S. suis* disease. In addition, the variants identified by our model could highlight the role of the host genome in shaping the tonsillar microbiota and in *Streptococcus suis* infection.

Financial Support
Ontario Pork; Ontario Ministry of Agriculture, Food, and Rural Affairs Research (OMAFRA); Canada First Research Excellence Fund

Notes:

V-059 - *Mbovpan:* a *Mycobacterium bovis* whole-genome sequencing pangenomic framework

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Session: Omics

Objective

Bovine tuberculosis is a livestock disease caused by the transmission of the bacterial pathogen Mycobacterium bovis, which leads to wide ranging impacts in the agriculture and economics sectors, as well as in animal and human health. Next Generation Sequencing allows for better differentiation across samples, and its growing use in outbreak investigations highlight the need for user-friendly, computationally efficient data analysis tools. Current bioinformatic approaches focus on detecting Single Nucleotide Polymorphisms (SNPs), but other variations such as inference of the pangenome (description of the full complement of genes) can also be helpful. The objective of this study is to develop the bioinformatic pipeline Mbovpan (M. bovis pangenomics) to conduct comparative analysis and determine specific gene signatures of M. bovis isolates.

Methods

SNP calling was conducted utilizing tools such as BWA, Picard, and Freebayes. Pangenome inference was determined first by trimming the input reads and then by applying a de novo assembly in SPAdes. Assembly quality was analyzed by calculating assembly metrics through the tool QUAST. Scaffolds were annotated using the tool Prokka. Lastly, Roary was used to construct clusters of orthologous genes. Mbovpan was developed using Nextflow, which enables us to develop data-driven computational pipelines.

Results

From a dataset of 232 M. bovis publicly available genomes extracted from infected cattle and badgers from an endemic area in the United Kingdom (Woodchester Park), 3784 genes (35% of the identified genes) were present amongst all isolates, while 374 genes (4%) were variably present. A total of 3986 inferred genes (57%) were unknown in function, while 176 accessory genes were inferred to be pe/ppe genes, which have been shown to have wide-ranging roles in virulence and immune modulation in Mycobacterial species.

Conclusions

Mbovpan will enable researchers to study thousands of *M. bovis* isolates efficiently, while extending future *M. bovis* outbreak investigation analysis.

Financial Support
University of Georgia; Interdisciplinary Disease Ecology Across Scales PhD Program, National Science Foundation

Notes:

V-060 - Validation of a SNP panel for selection for ascites resistance in broilers

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Session: Omics

Objective

We have been pursuing the underlying genetics of ascites in broilers. We used whole genome resequencing (WGR) in our ascites experimental research line (REL) to identify 28 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 used WGR in commercial lines to determine whether the same regions are associated with ascites. We used Marker Assisted Selection (MAS) to evaluate two regions for contributions to ascites using our REL.

Results

We have WGR data for 48 genomes at an average depth of 5-10x from the two commercial lines, representing both genders and phenotypes. We analyzed this new WGR data for correlation to the 28 regions from our previous data in our research lines. This work suggests that the genes affecting ascites incidence are often gender specific, highly variable with line, and highly specific to the genetic background. We used MAS in our REL line to generate a breeding flock that is homozygous for the non-reference genotypes for both CPQ and LRRTM4. Four cohorts of progeny from this F2 MAS flock were evaluated for ascites phenotype and production traits. Two cohorts were evaluated under two different hypobaric challenge protocols, and two cohorts were evaluated for changes in production traits. Ascites incidence was reduced by 25-40% and there were no significant losses in production traits.

Conclusions

The MAS work shows that we can change the incidence of ascites without significant production loss. WGR is a highly powerful system for fine mapping complex traits in domestic species. 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

Notes:

V-061 - Single cell RNAseq of equine mesenchymal stromal cells from donor-matched tissues reveals functional heterogeneity

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Session: Omics

Objective

In the broad field of regenerative medicine, efficacy of mesenchymal stromal cell (MSC) treatments is highly dependent on the intrinsic heterogeneity of MSC, both within individual preparations isolated from a single tissue source, and between preparations of cells isolated from different tissue sources. Our lab and others have used candidate-based approaches to compare specific features of MSC derived from different sources, however, the global heterogeneity of MSC, and how this contributes to treatment outcomes, is not well described.

Methods

In this study, we used high-throughput single cell RNA sequencing (scRNA-seq) to characterize the global heterogeneity of donor-matched equine MSC isolated from bone marrow (BM), adipose tissue (AT), and peripheral blood (PB). Based on transcriptional differences detected with scRNA-seq, we performed functional experiments to examine immune regulatory and motility functions in distinct MSC populations.

Results

We observed both inter- and intra-source heterogeneity across the three sources of equine MSCs Functional experiments demonstrated that transcriptional differences correspond with phenotypic variance in cellular motility and immune regulatory function. Specifically, we found that differences in C-X-C motif chemokine ligand 6 (*CXCL6*) expression in clonal MSC lines derived from the same tissue source correlated with the chemoattractive capacity of PB-derived MSC towards neutrophils, which are an important first line of immune defense against bacterial skin infections.

Conclusions

These findings enhance our understanding of MSC heterogeneity and will lead to improvements in the therapeutic potential of MSCs, accelerating their transition from bench to bedside.

Financial Support
U.S. Department of Agriculture; Harry M. Zweig Memorial Fund for Equine Research; Department of Microbiology, Icahn School of Medicine at Mount Sinai

Notes:

V-062 - Interacting effect of weaning and prenatal stressors on the hypothalamic molecular pathways in the pig

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Session: Omics

Objective

The developing pig brain can be affected by the immune response of the sow to porcine reproductive and respiratory syndrome virus (PRRSV) infection during gestation. The long-lasting effect of PRRSV immune activation on the brain molecular pathways can interact with other stressors, disrupting offspring behavior and health. The impact of immune activation on the molecular pathways of the hypothalamus, a brain region that interconnects the nervous and endocrine systems and participates in homeostasis, response to stress, and between-sex differences, is incompletely understood. The present study investigated the effects of maternal PRRSV infection and weaning stress on the gene pathways of the pig hypothalamus.

Methods

The experimental design encompassed 48 female and male pigs representing two inflammatory exposure statuses (maternal PRRSV exposure or control) and two postnatal stress levels (nursed and weaned). The hypothalamus transcriptome was profiled at 22 days of age using RNA-sequencing technology and the effects of PRRSV exposure, weaning, and sex on genes and pathways were tested.

Results

The expression of 250 genes was impacted by PRRSV, weaning, and sex effect at false discovery rate adjusted P-value < 0.1. Genes PIGY upstream reading frame and glycoprotein hormone, alpha polypeptide were under-expressed in weaned relative to nursed pigs exposed to PRRSV. These genes have been associated with neurological and behavioral disorders elicited by inflammation during development and can impact feeding behavior and reproductive performance. The expression of genes in the gonadotropin-releasing hormone pathway was dysregulated in response to PRRSV, weaning, and sex effects.

Conclusions

The molecular pathways disrupted by the inflammatory signals and weaning stress are consistent with the role of the hypothalamus on behavior, hormone secretion, and processing of stimuli information. Our results confirm the prolonged effect of PRRSV-elicited inflammation and suggest therapeutic targets that could be incorporated into management practices. This study is supported by USDA NIFA AFRI, grant number 2018-67015-27413.

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

Notes:

V-063 - Some like it hot, some like it cold: Proteomics of *Leptospira borgpetersenii* serovar Hardjo strains at different temperatures

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Session: Omics

Objective

Leptospirosis is a global zoonotic disease affecting most mammals including humans, companion animals, and all major livestock species. Leptospires colonize the tubules of host kidneys and are subsequently shed in the urine. While vaccines for leptospirosis exist, they suffer from a lack of cross protection between serogroups, and instead, bacterins focus on multiple serovar inclusion, relying on classically utilized laboratory strains cultured at 29°C. Recently, work has shown substantial transcriptomic variation between strains of identical species and serovar, particularly between temperatures, comparing 29°C to the newly achieved culture temperature 37°C. In this work investigating strain to strain variation, substantial differences in protein abundance profiles were identified between different strains of *Leptospira borgpetersenii* serovar Hardjo; the classically evaluated HB203 strain, and the currently circulating TC129 and TC273 strains.

Methods

L. borgpetersenii serovar Hardjo strain HB203 has been used as a classic challenge strain in cattle for the last three decades. Strains TC129, and TC273 were recently isolated from an abattoir in central Iowa. All strains were cultured at 29°C and 37°C and proteomics was performed on cultured leptospiral preparations. Supportive blots of select surface membrane proteins were also performed.

Results

Proteomic patterns revealed differences not only between strains, but additionally within strains cultured between temperatures. While 388 and 385 significantly differentially expressed (DE) proteins were identified between HB203 and TC129 and TC273 at 29°C respectively, only 66 and 4 DE proteins were identified between HB203 and TC129 and TC273 at 37°C. Between temperatures HB203 had 519 significantly DE proteins, TC129 had 347 DE proteins, and TC273 had 569 DE proteins.

Conclusions

There is increasing evidence to suggest bacterin vaccine designs would benefit from considering the strain and temperature specific behavior of pathogens in vaccine composition.

Notes:

V-064 - Knowledge, attitudes, and practices of veterinary professionals towards ticks and tick-borne diseases in Illinois

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Session: Parasitology

Objective

A lack of standardized surveillance or reporting of tick-borne diseases in Southern Illinois creates uncertainty for veterinarians regarding the tick-borne diseases within in their practice geography or which tick-borne diseases may be encroaching on their area from neighboring territories. The objective of this study was to gauge the knowledge, attitudes, and practices of veterinary professionals in Southern and Central Illinois, with a goal of designing targeted educational and outreach programs to address knowledge gaps.

Methods

An online knowledge, attitudes, and practices survey was distributed to veterinary professionals in Southern and Central Illinois. Poisson regression analyses were conducted to determine factors associated with knowledge scores and with the estimated number of tick-borne disease cases diagnosed.

Results

Knowledge scores were significantly higher for those veterinary practitioners with recent trainings on tick-borne disease. The number of cases of tick-borne disease diagnosed was higher among those reporting concern about tick-borne disease, and among those who routinely test for tick-borne diseases. The types of diseases diagnosed were heavily influenced by the diagnostic method used.

Conclusions

This study paints a cohesive picture of human factors associated with diagnosing veterinary disease and of tick-borne disease prevalence in Southern and Central Illinois. In particular, these results highlight the importance of veterinary continuing education on ticks and tick-borne disease. Building capacity for training veterinarians in parasitology using partnerships between academia and industry may strengthen the knowledge and understanding of ticks and tick-borne pathogens in the veterinary community.

Financial Support
U.S. Centers for Disease Control and Prevention

Notes:

V-065 - Phosphoethanolamine methyltransferases inhibitors with broad-spectrum anthelmintic effect for livestock nematodes

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Session: Parasitology

Objective

In the United States and world-over, nematode infections are among the most economically important factors affecting livestock, costing 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 anthelmintic drugs. Our objective in this project is to identify compounds with inhibitory effect against essential phospholipid biosynthetic enzymes in various nematode species, and to test their broad-spectrum anthelmintic efficacy in vitro and in vivo. Our long-term goal is to identify lead compounds for developing efficacious broad-spectrum anthelmintic drugs for treating nematode infections in livestock and circumvent drug resistance.

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 PMTs from different nematode species possess bona fide PMT catalytic activities for synthesis of the essential phospholipid, phosphatidylcholine. Using an in vitro PMT enzymatic assay, we screened compound libraries and identified several PMTs inhibitors that are nontoxic to mammalian cells at their effective concentrations. We tested the candidate PMT inhibitors and identified those with in vitro and in vivo anthelmintic efficacy against both drug-susceptible and drug-resistant nematodes. Studies are underway to test the efficacy of candidate compounds against natural mixed nematode infections in livestock.

Conclusions

Our project has unveiled lead-compounds for developing a new generation of novel and efficacious broad-spectrum anthelmintic drugs for treating nematode infections in livestock.

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

Notes:

V-066 - Cryptic dispersal of cattle fever ticks occurs in southern Texas via infested cattle and wildlife

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Session: Parasitology

Objective

The objective of our study is to protect the US cattle industry by preventing the re-introduction of bovine babesiosis, a lethal disease of cattle that is endemic in Mexico. Bovine babesiosis is caused by the single-celled parasites *Babesia bovis* and *B. bigemina*. These parasites are transmitted only by cattle fever ticks (*Rhipicephalus microplus* and *R. annulatus*); therefore, disease management is achieved by controlling these tick vectors. The US aggressively controls tick infestations in Texas with acaricides to prevent the spread of this disease from Mexico.

Methods

We have developed genetic tools to support tick eradication efforts, including DNA fingerprinting markers for tracking the spread of ticks and qPCR assays to screen for mutations that convey resistance to acaricides. We used these tools to genotype >5,000 tick samples collected by collaborators at APHIS and USDA-ARS. We are also evaluating genetic variation in eight anti-tick vaccine targets using NGS amplicon sequencing, because the current vaccine for cattle is not always effective against *R. microplus* in North America.

Results

Our applied genetic tools have led to key findings, including 1) ticks frequently use wildlife (white-tailed deer and nilgai antelope) as alternative hosts, which then leads to infestation of cattle, 2) ticks are transported long distances within Texas on infested cattle, and 3) acaricide resistance mutations from Mexico are spilling over to the US. Of the vaccine targets that we analyzed a signaling pathway effector, subolesin, was the most conserved gene.

Conclusions

A high level of cryptic tick movement occurs in southern Texas. The spread of acaricide resistance during the past decade poses a serious threat to this important management tool. As acaricides become less effective, anti-tick vaccines for cattle may become a valuable tool for disrupting the lifecycle of ticks. Given the large number of mutations that we found in other candidate vaccine targets, it will be important to choose conserved genes such as subolesin for future vaccine development.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture; U.S. Department of Agriculture, Animal and Plant Health Inspection Services

Notes:

V-067 - Studies of a new modality of deworming: Bt crystyal proteins

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Session: Parasitology

Objective

Gastrointestinal nematodes (GINs) are critical parasites that cause significant morbidity in humans and livestock/companion animals and sometimes mortality in livestock and companion animals worldwide. For decades, infections by GINs have been treated with small-molecule anthelmintic drugs, the heavy use of which has often selected for drug-resistant GINs. A novel paraprobiotic therapy, called IBaCC, has been developed from the *Bacillus thuringiensis* anthelmintic protein Cry5B. This paraprobiotic IBaCC, or Inactivated Bacterium with Cytosolic Crystal, takes advantage of dead bacteria to safely deliver recombinant Cry5B protein and potentially other nematode-active Cry proteins. Oral delivery of Cry5B IBaCC has been shown to be effective against a significant number of relevant GIN infections in livestock, including sheep, pigs, and horses. Here we describe our efforts to use molecular biology and protein engineering to improve Cry5B efficacy against multiple GIN species *in vitro and in vivo*.

Methods

Cry5B was subjected to limited pepsin digestion and proteomics. Bioinformatics and alanine scanning were used to identify residues that can be mutated with potential to increase bioactivity in Cry5B and other nematode active Cry proteins. Alterations in amino sequence were engineered into the Cry5B gene, expressed in *Bacilli,* and then tested *in vitro* and active variants in vivo against GIN infections in small animals.

Results

We have identified residues susceptible to pepsin digestion as well as 20+ residues that can be mutated to potentially increase GI stability and bioactivity in Cry5B and other Cry proteins. Mutations in all these sites have been made and are being tested for increased activity in vitro against a number of parasitic GINs (e.g., hookworms) and active variants are being tested in vivo in small animal models of GIN infection. These studies are on-going and up to date results will be presented.

Conclusions

We have identified novel methods to further evolve protein sequences of anthelmintic Cry proteins to increase their activity against GINS with the potential to significantly to increase GI stability and bioactiivity reducing the cost of goods and volume of delivery toward the development of an improved Cry-protein based anthelmintic.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture; U.S. National Institute of Allergy and Infectious Diseases

Notes:

V-068 - *Theileria haneyi* is resistant to imidocarb diproprionate and reduces its efficacy against *T. equi* in co-infected horses

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Session: Parasitology

Objective

In the United States, control of *Theileria equi,* the major causative agent of equine theileriosis, is largely reliant on treatment of infected horses with imidocarb dipropionate (ID). However, a second causative agent of equine theileriosis, *T. haneyi*, was recently discovered in horses at the U.S.-Mexico border, and it is currently unknown if ID is effective against *T. haneyi.* Furthermore, horses co-infected with *T. equi* and *T. haneyi* have been detected, and it is unknown if imidocarb diproprionate maintains effectiveness against *T. equi* in the presence of *T. haneyi* co-infection. The purpose of this study was to address these questions using *in vivo* infection and treatment of horses.

Methods

To address these questions, we used the following three groups of horses: 1) Five *T. haneyi* infected horses; 2) Three *T. haneyi*-*T. equi* infected horses; and 3) Three *T. equi*-*T. haneyi* infected horses. Horses were infected via intravenous inoculation with infected erythrocytes, followed by close clinical monitoring to track rectal temperature, packed cell volume, and peripheral blood parasitemia. Infection was confirmed via PCR and serology. Once horses reached the persistent stage of infection, they underwent two ID treatment cycles, spaced 2-4 months apart. Clearance was first evaluated using nPCR for each *Theileria* sp. on peripheral blood samples. For definitive confirmation of infection status, horses in groups two and three underwent splenectomy post-treatment.

Results

ID failed to clear *T. haneyi* in all three groups of horses, and failed to clear *T. equi* in two of three horses in group two. Following splenectomy, *T. equi*-nPCR-positive horses in group two developed severe clinical signs and were euthanized. Remaining horses exhibited moderate signs consistent with *T. haneyi*.

Conclusions

Our results demonstrate that ID lacks efficacy against *T. haneyi*, and *T. haneyi*-*T. equi* co-infection may interfere with ID clearance of *T. equi*.

Financial Support
USDA-ARS CRIS# #2090-320000-034-00D; Washington State University, College of Veterinary Medicine Equine Infectious Disease Research Program

Notes:

V-069 - Identification of *Babesia* gametocyte chemoattractive molecules

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Session: Parasitology

Objective

Babesia bovis and *B. bigemina* are protozoan parasites transmitted by tick vectors that cause significant economic losses. It is estimated that these two parasites cause annual losses of more than $10 billion U.S. dollars worldwide. The life cycle of *Babesia* is complex and requires infection of mammalian and tick hosts for parasite transmission. Parasites undergo multiple developmental stages that are closely coordinated with tick life cycle events. Within the tick midgut, *Babesia* transform into gametes.

Methods

We have developed a protocol to induce *Babesia* gametogenesis in *in vitro* culture. Using supernatants from induced cultures, we have observed species specific migration of gametocyte subpopulations in response to soluble chemoattractant factors. This is the first time that an Apicomplexan protozoan parasite gamete soluble chemoattractant factor with signaling properties to attract gametes prior to fusion has been observed.

Results

Understanding how to disrupt extracellular communication between parasite gametes within the tick vector would provide a novel target for transmission blocking vaccine development. Due to our ability to induce gametogenesis in *in vitro Babesia* culture, we are uniquely positioned to study mechanisms of *Babesia* chemoattraction prior to gamete fusion.

Conclusions

Overall Hypothesis: *Babesia* female gametes release soluble chemoattractant factors that influences the direction of male gamete migration consummating in gamete interaction. To test the hypothesis three specific aims will be performed as follows: 1) Demonstrate species specific *Babesia* gamete soluble chemoattractant factor(s), 2) Identify soluble chemoattractant factor(s), and 3) Confirm migration response to synthesized chemoattractant factor(s).

Financial Support
Agriculture and Food Research Initiative [Grant no. 2020-67030-31476/project accession no. 1021184] from the USDA National Institute of Food and Agriculture.; Agriculture and Food Research Initiative

Notes:

V-070 - Pharmacokinetics of bumped kinase inhibitor-1748 in horses: Investigational therapeutic for EPM

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Session: Parasitology

Objective

Bumped Kinase Inhibitors (BKI), such as BKI-1748, have proven efficacious in apicomplexan infections, and are now under investigation as therapeutics for equine protozoal myeloencephalitis (EPM) caused by *Sarcocystis neurona*. BKI-1748 inhibits *S. neurona* proliferation by 50% at 39 nanomolar. Here we report the pharmacokinetics (PK) of BKI-1748 in horses.

Methods

The oral dose was based on allometric scaling and in vitro to in vivo extrapolation (IVIVE) approaches. For oral studies, BKI-1748 was suspended in 7% Tween-80/3% ethanol/90% PBS at 20 mg/mL concentration, and dosed at 4 mg/kg by stomach gavage tubes to four, two-year-old horses. Blood and CSF were sampled frequently after administration for BKI-1748 levels. For intravenous (IV) studies, BKI-1748 was dissolved in 90% polyethylene glycol-400/10% dimethylsulfoxide at 10 mg/mL and dosed at 1 mg/kg via IV injection to two, two-year-old horses. Blood was sampled frequently for BKI-1748 levels. BKI-1748 quantitation was by LC-MS/MS with a limit of detection of 0.2 μM in the first (oral) experiment and 0.01 μM in the second experiment (IV).

Results

After oral administration of BKI-1748, only one of four horses had marginally detectable BKI-1748 in blood and none had detectable values in the CSF. Our allometric scaling and IVIVE approaches predicted a maximum concentration >10 μM. To help understand the discrepancy, BKI-1748 was administered IV at 1 mg/kg. BKI-1748 levels in horse plasma began at ~20 μM with an initial distribution phase followed by a terminal phase. The observed terminal half-life of 1 hr was much shorter than the predicted half-life of 4-8 hrs.

Conclusions

These results demonstrate that BKI-1748 has a rapid terminal half-life of ~1 hr in horses and poor oral bioavailability with the current formulation. Given the promise of this compound for treating EPM in horses, we are now experimenting with other vehicles and routes of administration to improve exposure after oral dosing of horses. Given BKI-1748's rapid half-life, we are also trying alternative CDPK1 inhibitors that may have improved oral bioavailability and exposure.

Notes:

V-071 - Chenodeoxycholic acid promotes susceptibility to campylobacteriosis in *Il10*$^{-/-}$ mice

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Session: Pathogen-Host Interaction

Objective

Campylobacter jejuni is one of the prevalent foodborne (mainly poultry) pathogens with limited mechanism understanding. Cholic acid (CA) is the major bile acid in *C. jejuni*-resistant mice, while chenodeoxycholic acid (CDCA) is the main bile acid in susceptible chickens. Based on this knowledge, we reasoned that gut bile acid compositions influenced the susceptibility to *C. jejuni* infection in conventional animals. The objective of this study was to evaluate the role of CDCA on campylobacteriosis.

Methods

To examine this hypothesis, conventionally raised *Il10*$^{-/-}$ mice at 8-12 weeks of age were fed with basal or 0.15% CDCA diet for 2 weeks. The mice were then orally gavaged with 10^9 CFU/mouse *C. jejuni* human clinical isolate 81-176 and euthanized at day 8 post infection. Tissues and/or content from ileum, ceca, colon, mesenteric nod, spleen were collected for *C. jejuni* enumeration and/or bile acid quantification by metabolomics of HPLC/MS-MS. Colonic tissue was also collected for gene expression and histopathology evaluation.

Results

Notably, at day 8 post infection, mice fed CDCA diet (CDCA-mice) showed morbid signs of bloody diarrhea, dehydration, coat ruffling, hunched posture, reluctance to move and recumbency. Colonic histopathology score was increased in the CDCA-mice compared to that of the infected mice fed basal diet. Consistent with the colitis results, the proinflammatory gene expression of *Il1β*, *Il17a*, *Cxcl1*, and *Cxcl2* was dramatically increased in the colon tissue of the CDCA group. *C. jejuni* significantly colonized the colon lumen of the CDCA-mice compared to undetectable in that of the infected mice fed basal diet. *C. jejuni* also invaded deep inside the colon tissue of the CDCA-mice using fluorescence in situ hybridization assay. Metabolomics analysis revealed that the major bile in ileal content of the CDCA-mice was comprised of CDCA with reduced CA.

Conclusions

In conclusion, dietary CDCA promotes *C. jejuni*-induced intestinal inflammation in *Il10*$^{-/-}$ mice, possibly through modulating the bile compositions which may determine *C. jejuni* susceptibility in conventional animals.

Financial Support
Arkansas Biosciences Institute; USDA National Institute of Food and Agriculture (NIFA) Hatch project 1012366, NIFA Hatch/Multi State project 1018699, NIFA SAS 2019-69012-29905, NIFA project 2020-67016-31346 to Xiaolun Sun.; Poultry Federation Scholarship to Ying Fu

Notes:

V-072 - Abiotic effects of temperature and biotic effects of pathogen exposure on host performance and susceptibility.

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Session: Pathogen-Host Interaction

Objective

Ecological and evolutionary changes are rapidly occurring due to a warming climate. We are seeing shifts in species ranges, changes in population demography, and altered species interactions. The later becomes of particular importance when focusing on host-pathogen interactions since climate change may either increase or decrease disease transmission. Our research focuses on the effects of a changing climate on host development and the interaction between a host and its pathogen.

Methods

This research is being conducted in an easily manipulated insect host-pathogen system -- the fall armyworm (*Spodoptera frugiperda*), an agricultural pest, and its species-specific virus. Fall armyworms are agricultural pests with widespread populations and, like other outbreaking insects, fall armyworm population dynamics can be pathogen regulated. To quantify the effects of changing temperatures on this host and the host-pathogen interactions, we established host populations that originated from different areas of the continental United States. To examine host performance, we constructed a thermal performance curve by exposing each host population to a variety of temperatures. Then, to examine host susceptibility, we conducted a series of experiments where the hosts were exposed to a known quantities of the lethal pathogen.

Results

Our data show that temperature affects host development and survival with a clear optimum temperature for each of the host lines. Our experiments also demonstrate how susceptibility to the virus depends upon the degree of exposure.

Conclusions

While the fall armyworm is a particularly devastating pest, the conclusions from this research are applicable to numerous other silvicultural and agricultural pest species whose dynamics are controlled by pathogen infection and are also likely to be impacted by changing temperatures. This research will also improve our ability to determine how best to use these pathogens as bioinsecticides. In future research, we will use these established host lines to examine how abiotic factors affect disease transmission via host-pathogen coevolution.

Financial Support
U.S. Department of Agriculture

Notes:

V-073 - Transcriptomic analyses of a ranavirus implicate the viral interference with host interferon response

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Session: Pathogen-Host Interaction

Objective

Ranaviruses (family *Iridoviridae*) are large dsDNA viruses (~105kb) that infect cold-blood animals including reptiles, amphibians and fish, and may significantly affect aquacultural production. About 100 functional coding genes (or open reading frames, ORFs) have been annotated in different ranavirus reference genomes. However, the function of most of these genes is still unknown. The goal of this study was to characterize ranaviral coding genes by transcriptomic profiling *in vivo* across tissues in the amphibian *Xenopus laevis* infected by Frog Virus 3 (FV3).

Methods

Outbred pathogen-free adult frogs were randomly allotted into mock controls and infected groups. Infected groups were i.p. injected with two strains of frog virus 3. At 3 days post infection, the tissues were sampled from the animals and used for the virus-targeting RNA-seq. Function of viral genes and interaction with host genes were characterized *in silico* and by transcriptomic analysis.

Results

The differential expression of FV3's ORFs was analyzed in tissue-, virus- and temporal class-dependent manners. The mapped reads in samples from FV3-infected intestine, liver, spleen, lung and kidney covered the full genome at ~10x depth on both FV3's positive and negative strands. Whereas, the mapped reads from the infected thymus, skin and muscles only covered partial genomic regions. Notably, our analysis revealed putative ORFs encoding hypothetical proteins containing viral mimicking domains that are conserved in host interferon regulatory factors (IRFs) and IFN receptors.

Conclusions

To our knowledge this is the first in vivo genome-wide ranaviral gene profiling in amphibians, showing a diverse expression pattern depending on tissue types and FV3 strains. Furthermore, our findings suggest that FV3 have evolved previously unknown molecular mimics that can interfere with host's interferon responses. This study contributes to better understanding of FV3 gene function and to developing antiviral strategies beneficial for aquaculture.

Financial Support
U.S. National Science Foundation; U.S. Department of Agriculture, National Institute for Food and Agriculture; U.S. National Institutes of Health

Notes:

V-074 - Comparative analysis of transcriptome profiles between directly infected and bystander alveolar macrophages collected from pigs infected with PRRSV

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Session: Pathogen-Host Interaction

Objective

Porcine alveolar macrophage (PAM) is one of the primary cellular targets for PRRSV, but less than 2% of PAMs are infected with the virus during the acute stage of infection. The objective of this study was to comparatively analyze transcriptomic response of infected- and bystander-PAMs that were collected from pigs during acute infection with PRRSV.

Methods

Four-weeks old PRRSV negative pigs divided into two groups. Pigs in group 1 were injected with RPMI-1640 to serve as negative controls, whereas pigs in group 2 were inoculated with $10^{6.0}$ TCID50/mL of a PRRSV strain expressing-green fluorescent protein (FL12-GFP). At 7 days post infection (dpi), lung lavage from each pig was harvested and GFP^+ (i.e., PRRSV infected) and GFP^- (i.e., bystander) cells were sorted for RNA-sequencing. Samples of the lung were collected and fixed in 10% neutral buffered formalin for *in-situ* hybridization (ISH) staining.

Results

Approximately 4.2% of RNA reads from GFP^+ and 0.06% reads from GFP^- PAMs mapped to the PRRSV genome, indicating that PRRSV-infected PAMs were effectively separated from bystanders. The inflammatory cytokines, interferon-stimulated genes, and antiviral genes were highly upregulated in GFP^+ as compared to GFP^- PAMs. Importantly, negative immune regulators including NF-κB inhibitors (NFKBIA, NFKBID, NFKBIZ, and TNFAIP3), and T-cell exhaustion markers (PD-L1, PD-L2, IDO1, and TGFB2) were highly upregulated in GFP^+ as compared to GFP^- PAMs. By using ISH, RNA transcripts of TNF and NF-κB inhibitors were detected in PRRSV-infected PAMs cultured *ex vivo* and lung sections of PRRSV-infected pigs during the acute stage of infection.

Conclusions

Collectively, the results indicate that cell directly infected with PRRSV upregulates the negative regulators of inflammation to escape the host immune system.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture; U.S. Department of Agriculture, Animal Health Formula Funds

Notes:

V-075 - Specific secondary bile acids control chicken necrotic enteritis

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Session: Pathogen-Host Interaction

Objective

Necrotic enteritis (NE), mainly induced by pathogens of *Clostridium perfringens* and coccidia, causes huge economic losses in the poultry industry with limited intervention options. The phaseout of antimicrobial growth promoters is one of the main driving factors for the reemergence of NE. This study was to investigate the role of bile acids on NE development.

Methods

Day-old chicks were assigned to 6 groups: noninfected, NE, NE with four dietary bile of 0.32% chicken bile, 0.15% commercial ox bile, 0.15% lithocholic acid (LCA), or 0.15% deoxycholic acid (DCA). The birds were sequentially infected with *Eimeria maxima* at d 18 and *C. perfringens* at d 23 and 24. Birds were euthanized at d 25 and ileal tissue was collected for histopathology and mRNA accumulation analysis. Ileum content samples were collected for bile acid quantification using LC-MS/MS.

Results

Notably, birds infected with the pathogens developed clinical NE signs. The NE birds suffered severe ileitis with villus blunting, crypt hyperplasia, epithelial line disintegration, and massive immune cell infiltration, while DCA and LCA prevented the ileitis histopathology. NE induced severe body weight gain (BWG) loss, while only DCA prevented NE-induced BWG loss. Notably, DCA reduced NE-induced inflammatory response and *C. perfringens* colonization compared to NE birds. Consistently, NE reduced total bile acids in ileal digesta, while dietary DCA and commercial bile restored it.

Conclusions

Together, this study showed that DCA and LCA reduced NE histopathology, suggesting that secondary bile acids, but not total bile acid levels, play an essential role on controlling the enteritis.

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

Notes:

V-076 - Monitoring longitudinal immunological responses to bluetongue virus 17 in experimentally infected sheep

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Session: Pathogen-Host Interaction

Objective

Bluetongue Virus (BTV) is an economically important pathogen of ruminant species with worldwide prevalence. While many BTV infections are asymptomatic, animals with symptomatic presentation deteriorate quickly with the sickest succumbing to disease within one week. Animals that survive the infection often require months to recover. The immune response to BTV infection is thought to play a role in controlling the disease. Key to understanding BTV disease is profiling host immunological cellular and cytokine responses. Studies to characterize immune responses in ruminants have been limited by a lack of species-specific reagents and assay technology. A recent advancement in molecular technology permits development of probes to detect RNA transcripts rather than protein targets. These probes, when incorporated with flow cytometry (RNA flow cytometry) and combined with traditional flow cytometry, maximize identification of cellular and cytokine targets at the single cell level. Our recent studies assess longitudinal immunological responses to experimental BTV infection in sheep. We aim to identify host factors necessary for disease resolution.

Methods

We infected a cohort of sheep with BTV-17 and longitudinally monitored each for clinical disease, viremia and specific immunological parameters (B-, T- cells, monocytes) by RT-qPCR, RNA flow cytometry, traditional flow cytometry and/or fluorescent based antibody arrays.

Results

BTV-inoculated sheep exhibited clinical signs characteristic of BTV disease. Circulating virus was observed at 8 days post inoculation (dpi) and remained detectable for the remainder of the study (24 dpi). A distinct pan-leukopenia was observed between 8-14 dpi that rebounded to mock-inoculated control levels at 17 dpi. In addition, we observed increased expression of pro-inflammatory cytokines after 8 dpi.

Conclusions

Taken together, we have established a model of BTV infection in sheep, and have successfully monitored the longitudinal vertebrate host immunological response and viral infection progression using a combination of traditional methods and cutting-edge technology.

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

Notes:

V-077 - A pioneering approach to tick control: Anti-tick toxins delivered via a tick transmissible pathogen

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Session: Pathogen-Host Interaction

Objective

Due to global climate change, tick populations are increasing and expanding their geographic range in the United States. Tick-borne diseases cause substantial economic burden for the cattle industry. Ticks are obligate, hematophagous ectoparasitic arthropods that transmit a variety of animal pathogens, including *Babesia bovis*. It is estimated that bovine babesiosis causes 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 has been the only effective method available. However, the discovery of acaricide-resistant tick populations in Mexico raises concerns regarding their expansion into *Rhipicephalus*-free areas with a corresponding increase in the risk of transmitting *Babesia* spp. to U.S. livestock. Bio-insecticides, such as protein toxins derived from spiders, may have the potential to control tick vectors if an appropriate delivery system were available. We hypothesize that transfected attenuated *B. bovis* expressing an anti-tick protein toxin reduces the infestation of *R. microplus*.

Methods

In this study, we tested if transfected parasites navigate through the tick by using whole gene replacement of locus BBOV_II005480 to append a promoter and a gene for GFP-BSD. A calf was infected with transfected parasites and ticks applied to synchronize tick parasite acquisition with an ascending parasitemia. The formation of transfected parasites in tick hemolymph was evaluated. Egg masses were collected, and the hatched larvae applied to a naïve animal.

Results

Rhipicephalus microplus acquired transfected *B. bovis* from the calf and transmitted the transfected parasites to a naïve animal. We detected fluorescent parasites in the blood of the acquisition fed calf, in tick hemolymph, and in the transmission fed calf.

Conclusions

We have demonstrated the ability to transfect *B. bovis* with a plasmid cassette that will allow specific expression of an anti-tick toxin in the hemolymph milieu of the tick vector, with the expectation that it will effectively interrupt the life cycle of *Rhipicephalus* ticks.

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

Notes:

V-078 - Vimentin as a cellular protein involved in equine arteritis virus attachment and entry

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Session: Pathogen-Host Interaction

Objective

Equine arteritis virus (EAV) has the unique ability to establish long-term persistent infection in the reproductive tract of stallions. Recent studies showed that long-term persistence is associated with a specific allele of the *CXCL16* gene (*CXCL16S*), which serves as the viral receptor. However, EAV has a broad host-cell tropism and infects a variety of cells that do not express EqCXCL16S. Our objective is to identify other cellular proteins that interact with EAV and facilitate viral entry.

Methods

The virus overlay protein binding assay in combination with Far-Western blot was performed on EAV-susceptible equine endothelial cells (EECs) *in vitro*. Following identification of a protein candidate to which EAV binds, LC-MS/MS analysis was performed to identify the protein (vimentin). HEK-293T cells were transfected with equine vimentin to induce overexpression and evaluate its effect on susceptibility to EAV.

Results

The virus overlay protein binding assay in combination with Far-Western blot from EAV susceptible equine endothelial cells (EECs) has identified about 57 kDa protein as a possible EAV-binding protein. Further investigation revealed that the unidentified 57 kDa protein is also present in the membrane fraction of the protein lysates. LC-MS/MS analysis identified the protein as vimentin. Overexpression of equine vimentin (EqVim) in HEK-293T cells increased the susceptibility of the HEK-293T cells to EAV infection. Interestingly, our study also showed a direct interaction between equine vimentin and EAV.

Conclusions

Collectively, our data provides strong evidence that EAV binds to the host cell protein, equine vimentin, which is actively involved in EAV attachment and entry, suggesting that EAV interacts with multiple host cell proteins in complex host-virus interactions.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture; Louisiana State University School of Veterinary Medicine start-up funds

Notes:

V-079 - Cloning bile salt hydrolase to increase deconjugate bile acids for reducing *Clostridium perfringens*

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Session: Preventive Medicine

Objective

Clostridium perfringens and *coccidia*-induced necrotic enteritis (NE) cause a huge economic loss in the poultry industry. Dietary supplementation of deoxycholic acid (DCA) reduces chicken NE, but the accumulation of bile acids in the gut is the less effective form of conjugated tauro-DCA (TDCA). We hypothesized that bile salt hydrolase (Bsh) would increase the deconjugation of TDCA to more effectively prevent NE.

Methods

In this study, we aimed to clone and express Bsh for future NE reduction experiments. TSA plates supplemented with 0.2% TDCA and 0.03% $CaCl_2$ were used to examine the Bsh expression. The *bsh* genes were PCR-amplified, cloned into plasmids pET-28a (pET-Bsh) and pDR111 (pDR-Bsh), and Sanger-sequenced. After transformed pET-Bsh plasmid into *E. coli* DH5a and BL21 for amplification and protein expression, respectively, the His-tag purified Bsh was used to evaluate the enzyme activity by Dot-Blot, SDS-PAGE, and DCA precipitation on the TSA plates.

Results

As a result, *Bifidobacterium longum* anaerobically grew colonies surrounded with opaque precipitation zones on the TSA plates with TDCA and CaCl2 compared to no opaque zone around *E. coli* colonies. The result suggested that the *B. longum* expressed Bsh. After cloned, transformed, and expressed pET-Bsh in BL21, Bsh protein was isolated and subjected to SDS-PAGE. Coomassie Blue staining showed strong bands around 32 kDa in accordance with the size of *bsh* of 954 bp. Bsh was verified by Dot Blot assay showing darker black dot from the expressed samples compared to the negative control samples. Bsh enzyme activity was examined on the TSA plates showing opaque crystallized DCA in the path of Bsh solution compared to transparent path from the elution buffer only. Furthermore, *C. perfringens* growth was reduced in TSB with TDCA and Bsh compared to TSB with TDCA alone. Recently, we have cloned and transformed pDR-Bsh into *Bacillus subtilis* for future chicken NE experiments.

Conclusions

In conclusion, *B. longum* Bsh deconjugating TDCA was cloned into pDR-Bsh and transformed into *B. subtilis* for future chicken experiments to reduce chicken NE.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture; Arkansas Biosciences Institute; NIFA Hatch/ Multi State project and NIFA SAS

Notes:

V-080 - Effect of long-distance transportation on surplus dairy calf health

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Session: Epidemiology

Objective

Following arrival at calf raising facilities, calves experience high rates of disease; however, it is unclear if duration of transportation affects health. The objective of this study was to determine if transport duration affects calf health in the 2 wk after arrival at a veal facility.

Methods

All surplus dairy calves born on 5 dairy farms in Ontario were eligible for enrollment (n = 119). Farms were visited daily before transport to enroll calves and perform health exams evaluating fecal and respiratory scores. On the day of transport, calves from 2 to 22 d of age were randomly assigned to 6, 12, or 16 h of transportation in a gooseneck trailer. Daily health exams were performed for 14 d after arrival to the calf raising facility. Poisson regression models were built to assess the impact of transport duration on the number of days with abnormal fecal and respiratory scores.

Results

The number of days with an abnormal fecal score was greater for calves transported for 16 compared to 6 h (IRR = 1.30, 95% CI = 1.04 – 1.62, $P = 0.02$) but not 12 compared to 6 hours (IRR = 1.48, 95% CI = 0.91 – 1.45, $P = 0.24$). As age increased, calves tended to have fewer days of abnormal fecal scores (IRR = 0.98, 95% CI = 0.96 – 1.00, $P = 0.07$). The number of days with an abnormal respiratory score did not vary between 12 h (IRR = 0.90, 95% CI = 0.66 – 1.23, $P = 0.52$) or 16 h of transportation (IRR = 0.99, 95% CI = 0.73 – 1.36 $P = 0.96$) compared to 6 h. Calves transported in the spring (IRR = 0.30, 95% CI = 0.21 – 0.45, $P < 0.0001$) and fall (IRR = 0.63, 95% CI = 0.47 – 0.84, $P = 0.01$) and those with good transfer of passive immunity (serum total protein = 5.6 – 6.2 g/dL) (IRR = 0.38, 95% CI = 0.22 – 0.66, $P < 0.001$) experienced fewer days with an abnormal respiratory score compared to calves transported in the winter and those with failed transfer of passive immunity, respectively.

Conclusions

These preliminary results provide evidence that health after arrival at calf raising facilities are affected by calf age and management on the source dairy farm and may be negatively affected by longer transportation.

Notes:

V-081 - Improving dairy cow health monitoring and management using automated sensors

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Session: Preventive Medicine

Objective

Our objectives were to: (1) Characterize behavioral, physiological, and productivity parameters recorded by sensors during health and disease in dairy cows; (2) Demonstrate automated real-time data integration and 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 d in milk. Health status was monitored daily through clinical examination. Wearable sensors monitored physical activity, resting, body temperature, rumination, and eating time. Non-wearable sensors monitored milk volume and components, milk conductivity, and body weight. Environmental conditions were also recorded. Previous health and reproductive events, historical production records, and pen stocking density were retrieved from farm records. A fully automated data integration software tool was developed to create a pipeline of data from multiple sensor and non-sensor data streams for fully automated real-time data analytics. All sensor and non-sensor data were used for development and testing of machine learning algorithms for prediction of cow health status.

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 an example, the most relevant changes ($P<0.05$) for cows with displaced abomasum were reduced eating time (-22%), rumination (-34%), and activity (-15%), whereas for cows with metritis the most relevant changes ($P<0.05$) were reduced eating time (-14%), activity (-17%), and resting time (-13%; all $P<0.05$) for day -5 vs the day of diagnosis. The sensitivity and specificity for predicting cow health status on a testing dataset were 88% and 88%, 43% and 96%, and 70% and 67% for XGBoost, Multi-Layer Perceptron, and Recurrent Neural Networks, respectively. We demonstrated an on-farm fully automated real-time data integration tool for subsequent machine learning data analytics.

Conclusions

Substantial variation in parameters recorded by sensors in cows with HD could be used to automate health monitoring. Individual HD have signature patterns for sensor parameters, which may improve HD prediction and enable prediction of specific HD affecting cows. Automated real-time integration of sensor and non-sensor data in machine learning algorithms may result in reasonable prediction of cow health status although substantial variation may be observed depending on the type of machine learning method used.

Notes:

V-082 - Using Phage Display to produce anti-*Clostridium perfringens* monoclonal antibodies

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Session: Preventive Medicine

Objective

Clostridium perfringens is a bacterial pathogen causing severe chicken necrotic enteritis and human enteritis. Treatment of the pathogen is limited and ineffective. In this study, we aimed to generate monoclonal antibodies (mAb) to prevent and treat *C. perfringens*-induced diseases.

Methods

C. perfringens sporulation protein (Cp-spor-super) was subcutaneously injected three times to BL6 wild type (WT) mice to generate anti-*C. perfringens* B cells. Plasma B cells in the spleen were collected to generate RNA and cDNA after 14 days of the last booster immunization. Antibody variable region of heavy chain (Vh) and variable region of light kappa chain (Vl) were amplified. Single-chain variable fragments (scFv) were assembled by overlapping PCR of Vh and Vl. The scFv were then cloned into the phagemid pComb3xSS through digestion with *SfiI* and ligation with T4 ligase. Phage Display using antigen Cp-spor-super in a plate was performed to bio-panning the phages expressing anti-*C. perfringens* scFv. Dot-blot was conducted to detect the efficiency of eluted phages strongly binding to Cp-spor-super.

Results

The results showed that the PCR sizes of Vl and Vh segments were around 350 to 400bp, respectively. scFv product was around 800bp after overlapping PCR of Vl and Vh. After digestion and ligation, the successful construction of recombinant plasmid (pComb3xSS-scFv) was confirmed by double digestion with the NruI and SacI and gel electrophoresis. Two bands of 3318bp and 800bp were visualized to represent the linearized pComb3xSS and scFv, respectively. The scFv phage were generated after transforming pComb3xSS-scFv into *E. coli* TG1 and co-infected with M13O7 helper phage. The scFv phage was selected through bio-panning in a plate coating with Cp-spor-super. After 3 cycles of bio-panning, the eluted scFv phage showed strong black dot against Cp-spor-super with Dot-blot assay.

Conclusions

In conclusion, we have produced anti-*C. perfringens* mAb and are identifying and characterizing the individual mAb for preventing and treating *C. perfringens*-induced enteritis.

Financial Support
Arkansas Biosciences Institute; USDA National Institute of Food and Agriculture (NIFA) Hatch project 1012366, NIFA Hatch/Multi State project 1018699, NIFA SAS 2019-69012-29905, NIFA project 2020-67016-31346 to Xiaolun Sun.; Poultry Federation Scholarship to Ying Fu

Notes:

V-083 - Protective efficacy of an orf virus-vector encoding the hemagglutinin and the nucleoprotein of influenza A virus in swine

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Session: Vaccinology

Objective

Swine influenza is a highly contagious respiratory disease of pigs caused by influenza A viruses (IAV-S). IAV-S causes significant economic losses to the swine industry and poses constant challenges to public health due to its zoonotic potential. Effective influenza A virus (IAV-S) vaccines capable of providing robust protection to IAV-S in swine are lacking. Thus, there is a need to develop an effective vaccine against IAV-S which would not only benefit swine health but will also reduce the chances of zoonotic spillover of IAV-S to humans. Here, we explored the potential of orf virus-based vectors expressing the hemagglutinin (HA) or both the HA and the nucleoprotein (NP) genes of influenza A virus (IAV-S) in eliciting protection against IAV-S in pigs.

Methods

We developed two recombinant orf viruses, expressing the hemagglutinin (HA) gene (OV-HA) or both the HA and the nucleoprotein (NP) genes of IAV-S (OV-HA-NP). OV-HA was developed by inserting HA gene in ORFV121 locus of orf virus and OV-HA-NP was developed by inserting HA and NP gene in ORFV121 and ORFV127 locus respectively. The immunogenicity and protective efficacy of these two recombinant viruses were evaluated in pigs by immunizing pigs with OV-HA or OV-HA-NP and then challenging with a virulent H1N1 strain of swine influenza.

Results

Both OV-HA and OV-HA-NP recombinants elicited robust virus-neutralizing antibody response in pigs. Notably, although both recombinant viruses elicited IAV-S-specific T-cell responses, the frequency of IAV-S specific proliferating T cells secreting IFN-γ upon re-stimulation was higher in OV-HA-NP-immunized animals than in the OV-HA group. Importantly, IgG1/IgG2 isotype ELISAs revealed that immunization with OV-HA induced Th2-biased immune responses, whereas immunization with OV-HA-NP virus resulted in a Th1-biased immune response. While pigs immunized with either OV-HA or OV-HA-NP were protected when compared to non-immunized controls, immunization with OV-HA-NP resulted in better protective efficacy as evidenced by reduced virus shedding in nasal secretions and reduced viral load in the lung.

Conclusions

We show that both orf recombinant viruses (OV-HA and OV-HA-NP) elicited IAV-S-specific humoral and cell-mediated immune responses in pigs. The addition of the NP and co-expression of this protein with HA, another major influenza protective antigen, resulted in higher T cell responses which presumably led to better protection in OV-HA-NP immunized animals, as evidenced by lower levels of virus shedding and viral load in lungs. Overall, This study demonstrates the potential of ORFV-based vector for control of swine influenza virus in swine.

Financial Support
U.S. Department of Agriculture

Notes:

V-084 - Development of a novel live attenuated influenza A virus vaccine encoding the IgA-inducing protein

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Session: Vaccinology

Objective

Live attenuated influenza virus (LAIV) vaccines elicit a combination of systemic and mucosal immunity by mimicking a natural infection. To further enhance protective mucosal responses, we incorporated the gene encoding the IgA-inducing protein (IGIP) into the LAIV genomes of the cold-adapted A/Leningrad/134/17/57 (H2N2) strain (caLen) and the experimental attenuated backbone A/turkey/Ohio/313053/04 (H3N2) (OH/04att).

Methods

The gene encoding the swine IGIP mature peptide was cloned into the hemagglutinin (HA) segment of A/California/04/09 (pdmH1N1)(Ca/04). Viruses carrying the IGIP-H1 and NA of Ca/04 in the context of caLen and OH/04att were obtained by reverse genetics. The growth properties and stability were analyzed in vitro, and their safety and efficacy were evaluated in DBA/2J mice in a prime-boost regime 3 weeks apart. Three weeks after boost, mice were challenge with a lethal dose of Ca/04 and clinical signs and viral loads were evaluated. Serum, nasal washes (NW) and bronchoalveolar lavages (BALF) were collected pre- and post-challenge to analyze humoral and mucosal responses.

Results

Incorporation of IGIP into the caLen led to a virus that grew poorly in prototypical substrates. In contrast, IGIP in the OH/04att (IGIP-H1att) virus grew to titers comparable to the isogenic backbone H1att (H1N1) without IGIP. IGIP-H1att- and H1caLen-vaccinated mice were protected against lethal challenge with a homologous virus. The IGIP-H1att vaccine generated robust serum HAI responses in naïve mice against the homologous virus, equal or better than those obtained with the H1caLen vaccine. Analyses of IgG and IgA responses using a protein microarray revealed qualitative differences in humoral and mucosal responses between vaccine groups. Overall, serum and bronchoalveolar lavage samples from the IGIP-H1att group showed trends towards increased stimulation of IgG and IgA responses compared to H1caLen samples.

Conclusions

Introduction of genes encoding immunomodulatory functions into a candidate LAIV can serve as natural adjuvants to improve vaccine safety and efficacy against Influenza.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture; U.S. National Institute of Allergy and Infectious Diseases

Notes:

V-085 - Vaccine-induced antibody response and its correlation with protection against challenge with HPAI H7N3 in chickens

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Session: Vaccinology

Objective

Influenza A viruses of the H7 subtype are a threat to the poultry industry and of pandemic concern. In the Americas, episodes of highly pathogenic avian influenza (HPAI) H7N3 have been reported in Chile, Canada, and Mexico. Current vaccines against HPAI H7N3 are unable to confer adequate protection, underscoring the need to investigate the correlates of protection against H7 viruses. Our goal was to compare the antibody responses elicited by live attenuated or inactivated H7N3 avian influenza (AI) viruses and to correlate with protection against lethal challenge.

Methods

A live attenuated influenza virus (LAIV) was generated by reverse genetics using sequence information of a H7N3 strain isolated in Mexico in 2016 (Mex-2016). Using a prime-boost strategy (2 weeks apart), 2-week-old chickens were vaccinated with either 10^6 EID_{50}/chicken of LAIV and/or 512 HAU/chicken of BPL-inactivated virus supplemented with Montanide ISA71 VG. Two weeks after boost, birds were challenged with $10^{6.5}$ EID_{50}/chicken of the Mex-2016 HPAI virus. Antibody titers were determined by ELISA and HI assay before and after boost. Virus shedding was assessed by RT-qPCR from tracheal and cloacal swabs collected on days 3 and 5 post-challenge. Survival and clinical signs were recorded for 2 weeks post-challenge.

Results

Anti-NP and -HA antibodies were mostly detected in birds primed simultaneously with live and inactivated virus. Titers increased after the boost for the live/inactivated group and became detectable for all the birds primed with live and boosted with inactivated virus. Virus shedding after challenge was significantly decreased in chickens inoculated with two doses of live/inactivated or a single dose of inactivated virus. Administration of one or two doses of inactivated virus increased survival (100%) compared to the administration of live virus only (60%). Pearson correlation analysis showed that as the antibody titers increase, virus shedding and mortality decrease.

Conclusions

These results show that antibody titers inversely correlate with challenge virus shedding and protection against mortality.

Financial Support
U.S. National Institute of Allergy and Infectious Diseases; Caswell S. Eidson Endowment Funds, University of Georgia; Georgia Research Alliance

Notes:

V-086 - Efficacy of commercial BRSV vaccines in the prevention of respiratory disease in calves a meta-analysis

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Session: Vaccinology

Objective

Infection with bovine respiratory syncytial virus (BRSV) is associated with respiratory disease in young calves under 6 months of age. Producers and veterinarians commonly use vaccination as the main strategy to reduce the incidence of clinical disease; however, the evidence of BRSV vaccination efficacy has been inconsistent in the literature. The objective of this meta-analysis was to evaluate and analyze results from controlled trials on the effectiveness of vaccinating calves with commercially available BRSV vaccines for reduction of BRSV-associated morbidity and mortality in experimental challenge models.

Methods

Studies that reported the effectiveness of commercially available BRSV vaccines in experimental BRSV challenge models were included in the analysis. Meta-analyses using risk differences as effect size were performed to assess the overall effect of vaccination on both morbidity and mortality; sub-analyses to evaluate the effect of killed (KV) or modified-live (MLV) vaccines were also performed. Bias was assessed using funnel plots.

Results

Fourteen experimental BRSV challenge studies were included in the meta-analyses and results demonstrated that dairy and beef calves vaccinated with BRSV-containing vaccines had significantly lower morbidity and mortality risks compared with non-vaccinated control calves. The type of vaccine (MLV vs. KV) did not have a significant effect on BRSV-associated morbidity and mortality outcomes.

Conclusions

Based on these results, BRSV vaccination of young calves is effective at reducing clinical signs and mortality following experimental BRSV infection.

Notes:

V-087 - Compatibility of different combinations of newcastle and infectious bronchitis vaccines in layer-type chickens

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Session: Vaccinology

Objective

The use of Infectious Bronchitis (IB) classical and variants vaccines combined with Newcastle (ND) vaccine is a normal practice in many countries. The objective of this trial was to determine a possible IB seroconversion interference when combine a commercially available Newcastle (ND) and infectious bronchitis (IB) classical Massachusetts vaccine (Hipraviar® Clon/H120, Hipra) with commercially available variant IB QX and 793/b vaccines.

Methods

One hundred (100) female layer-type chickens with maternal antibodies were divided into 5 groups with 20 chickens in each group. Group 1 - Hipraviar® Clon/H120, Group 2 - Hipraviar® Clon/H120 and Qx vaccine, Group 3 - Hipraviar® Clon/H120 and IB 793B, Group 4 - Hipraviar® Clon/H120, IB QX and IB 793B and Group 5 was non-vaccinated. Blood samples were collected from chickens at 4 weeks old (WO), 6 WO and 8 WO. Sera were collected and tested for IBV antibodies by commercially available ELISA test kit IDEXX® IBV AB Test Kit. The vaccine was given at 4 WO by eye drop Intra ocular (IO), one dose per bird.

Results

The IB seroconversion using IDEXX® IBV AB Test Kit was, in Group 1: 4 WO - 40 ± 69^{A} - 6 WO - $1620\pm1,37^{a}$ - 8 WO - $1390\pm1,25^{a}$, in Group 2: 4 WO - 65±87 - 6 WO - $2230\pm1,25^{a}$ - 8 WO - $2810\pm1,59^{b}$, in Group 3: 4 WO - 117±76 - 6 WO - $3270\pm2,13^{b}$ - 8 WO - $3470\pm1,99^{b}$, in Group 4: 4 WO - 101±131 - 6 WO - $2310\pm1,27^{a}$ - 8 WO - $3700\pm2,36^{b}$ and in Group 5: 4 WO - 219±233 - 6 WO - 44 ± 42^{c} - 8 WO - Not done. [A] Mean and standard deviation (SD) [a,b,c] .The different superscript indicates a statistically significant difference ($p<0.05$). The Groups 2, 3 and 4 showed no statistically significant difference in the seroconversion compared to group 1 (Hipraviar® Clon/H120). The use of IB variant vaccines QX, 793/b and the both with the combination Hipraviar® Clon/H120 did not interfere negatively in the seroconversion for IB at 8 WO.

Conclusions

All the vaccinated groups showed substantial IB seroconversion and the combination of IB QX and 793/b variant vaccines and Hipraviar® Clon/ H120 exhibited good compatibility and did not have a negative impact on IB seroconversion.

Notes:

V-088 - Distribution of swine leukocyte antigen (SLA) haplotypes in European farmed pigs

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Session: Vaccinology

Objective

Globally, pigs represent economically important farm animals and furthermore have become a preferred preclinical large animal model for biomedical studies, transplantation, and regenerative medicine research. The need for swine leukocyte antigen (SLA) typing is increasing with the expanded use of pigs as models for human diseases and organ-transplantation experiments, their use in infection studies, and for design of veterinary vaccines.

Methods

In this study, we characterized SLA class I (*SLA-1, SLA-2, SLA-3*) and class II (*DRB1, DQB1, DQA*) genes of 549 farmed pigs representing six pig lines of global commercial interest (Landrace, Yorkshire, Hampshire, Duroc, Large White, Pietrain) by PCR-based low-resolution (Lr) haplotyping. Criteria and nomenclature used for SLA class I (SLA-I) and class II (SLA-II) haplotyping were proposed by the ISAG/IUIS SLA Nomenclature Committee. Low-resolution SLA-I and SLA-II haplotypes were assigned based on the comparison with already known breed or farm-specific allele group combinations.

Results

In total, 50 SLA-I and 37 SLA-II haplotypes were identified in the studied cohort. The most common SLA-I haplotypes Lr-04.0 (SLA-1*04XX-SLA-3*04XX(04:04)-SLA-2*04XX) and Lr-32.0 (SLA-1*07XX-SLA-3*04XX(04:04)-SLA-2*02XX) occurred at frequencies of 11.02 and 8.20%, respectively. For SLA-II, the most prevalent haplotypes Lr-0.15b (DRB1*04XX(04:05/04:06)-DQB1*02XX(02:02)-DQA*02XX) and Lr-0.12 (DRB1*06XX-DQB1*07XX-DQA*01XX) occurred at frequencies of 14.37 and 12.46%, respectively.

Conclusions

Meanwhile, our lab contributed to several vaccine correlation studies (e.g., PRRSV, CSFV, ASFV, FMDV, swine influenza A virus, human HPV) elucidating the immunodominance in the T-cell response with antigen-specificity dependent on certain SLA-I and SLA-II haplotypes. Moreover, these SLA-immune response correlations could facilitate tailored vaccine development, as SLA-I Lr-04.0 and Lr-32.0 as well as SLA-II Lr-0.15b and Lr-0.12 are highly abundant haplotypes in European farmed pigs.

Notes:

V-089 - *Clostridium perfringens* sporulation vaccines prevent chicken necrotic enteritis

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Session: Vaccinology

Objective

Although the of pathogenesis of chicken necrotic enteritis (NE) remains largely unresolved, the severity was often associated with the toxin-releasing of *Clostridium perfringens*. Previously we found that chickens immunized with *C. perfringens* sporulation vaccines reduced chicken NE. The objective of this study was to investigate the most effective doses in relation with the vaccine mechanism.

Methods

The sporulation supernatant proteins from two *C. perfringens* isolates were used to prepare vaccines CP1 and CP2. Induction of inflammatory response in epithelial CMT-93 and macrophage Raw 264.7 cells were performed using CP1. Birds were vaccinated at d 0 and 10 with different doses and then infected with sporulated *Eimeria maxima* M6 oocysts at d 16 and *C. perfringens* at d 20. Chicken body weight was measured at d 0, 16, 20 and 21. Birds were sacrificed at d 21 to collect blood, ileal tissue and content for analysis of antibody titer, histopathology, inflammation and colonization levels.

Results

CP-1 and CP-2 were detected enterotoxin (CPE) positive by Western Blot. CP-1 induced cell death in CMT-93 and Raw cells. *E. maxima* and *C. perfringens* induced clinical NE of severe intestinal inflammation with ileal villus shortening and necrosis, immune cell infiltration, and crypt hyperplasia. Vaccines CP1 and CP2 attenuated intestinal inflammation and prevented NE-induced body weight gain (BWG) loss during d 20-21 (NE phase). Among these vaccine groups, birds vaccinated with CP1-3 (0.7μg/bird) had the highest daily BWG during NE phase compared to NE birds (25 vs -3g/bird/day). ELISA assay showed that antibody titer in CP1-3 was increased by 49 and 69%, respectively, compared to those in NC (negative control) and NE (positive control) birds. Real time PCR results showed that *C. perfringens* in ileum luminal and tissue of CP1-3 birds was reduced by 5 and 3 logs, respectively, compared to those in NE birds.

Conclusions

In conclusion, vaccinating chickens with CP-1 and CP-2 effectively reduces chicken NE, possibly through inducing protective antibody and reducing *C. perfringens* colonization and invasion.

Financial Support
Arkansas Biosciences Institute; USDA National Institute of Food and Agriculture (NIFA) Hatch project 1012366, NIFA Hatch/Multi State project 1018699, NIFA SAS 2019-69012-29905, NIFA project 2020-67016-31346 to Xiaolun Sun.; Poultry Federation Scholarship to Ying Fu

Notes:

V-090 - T cell epitope content comparison (EpiCC) analysis between PCV2 vaccines and field strains

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Session: Vaccinology

Objective

As porcine circovirus type 2 (PCV2) continues to evolve, the genetic gap between available vaccines and field strains grows. Vaccines that contain T cell epitopes well-matched to the circulating strains are more likely to induce strong T cell-mediated memory responses upon challenge or field exposure. To quantify the relatedness between PCV2 vaccines (three monovalent and one bivalent) and field strains, we predicted and compared the T cell epitope content contained in their capsid proteins.

Methods

614 PCV2 field strains, including genotype PCV2a (n=108), PCV2b (111), and PCV2d (396) viruses from Asia (76), Europe (246), and America (293), were compared to three PCV2a monovalent vaccines and one bivalent vaccine (combination of PCV2a and PCV2b; VacAB). Utilizing PigMatrix, vaccines and field strains were screened to identify putative class I and II T-cell epitopes. Field strains were then compared to vaccines to generate a relatedness score. These T cell epitope content comparison (EpiCC) scores assess epitope content shared between field strains and vaccines. For each field strain, we calculated the percentage of T cell epitope covered by the vaccines. For the bivalent vaccine, we quantified the individual and combined contributions of its components to the shared epitope content.

Results

Overall, the bivalent vaccine had the highest EpiCC scores globally and by geographical region. Considering all the analyzed strains, putative VacAB epitopes shared with the PCV2 field strains covered, on average, 82.4% of their total epitope content. VacAB T cell epitope coverage was 13%-19% higher than that of monovalent vaccines. For the bivalent vaccine, 75.3% of the epitope content shared with field strains was found in both vaccine components, which illustrates the depth of the bivalent vaccine coverage.

Conclusions

This study demonstrated that the bivalent PCV2 vaccine has greater T cell epitope overlap with field strains than monovalent vaccines. This result suggests that the bivalent vaccine might offer broader T cell-mediated immune response and protection against circulating PCV2 strains.

Financial Support
Zoetis

Notes:

V-091 - A structure-based vaccine for bovine respiratory syncytial virus using NDV vector

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Session: Vaccinology

Objective

Bovine Respiratory Syncytial Virus (BRSV) is the major cause of calf pneumonia, which causes significant economic losses to the cattle industry. At present, there is no satisfactory live-attenuated or inactivated vaccine available for the prevention of BRSV infection. BRSV fusion (F) protein has been proposed as a major vaccine candidate. In this study, we used a vaccine LaSota strain of NDV (rNDV) as a vector for expressing the BRSV fusion (F) protein.

Methods

Using reverse genetics, we have made four rNDVs carrying BRSV F, the pre-fusion form of F (pre-F), and the ectodomain of BRSV F or pre-F gene that were fused with NDV F protein transmembrane and cytoplasmic tail, respectively.

Results

The correct sequences of BRSV genes were confirmed by sequence analysis using a set of primers for fully sequencing the F and Pre-F genes of BRSV. The recovered viruses have been characterized in vitro for their ability to replicate and their expression levels of BRSV F protein and BRSV pre- F protein. The insertion of BRSV F protein did not affect the replication capacity of recombinant NDV. The expression of BRSV F and pre-F proteins were characterized in chicken DF1 cells by using F-specific antibodies. We found the rNDVs expressing ectodomain of BRSV F or pre-F gene that were fused with NDV F protein transmembrane and cytoplasmic tail expressed higher levels of F and pre-F proteins, respectively.

Conclusions

Hence, these two rNDV carrying BRSVF F genes will be selected for small animal and calve studies.

Financial Support
U.S. Department of Agriculture, Animal and Plant Health Inspection Services; U.S. National Institute of Allergy and Infectious Diseases

Notes:

V-092 - Injection of Zn, Se, Cu and Mn enhances antibody response to *Bovine Coronavirus* vaccination in beef cattle.

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Session: Vaccinology

Objective

Bovine coronavirus (BCoV) is a causal agent of neonatal calf diarrhea (NCD). Vaccination of pregnant cows, adequate passive transfer, and hygiene are crucial to prevent NCD. Trace mineral (TM) deficiencies impair the response to vaccination in cattle. The objective was to evaluate the effects of injectable trace minerals (ITM: Zn, Se, Cu & Mn) concurrent with NCD vaccination on serum and colostrum neutralizing antibody (SNA & CNA) responses to BCoV and subsequent passive transfer in beef cattle.

Methods

Twenty-two pregnant Angus cows (6.5 mo of gestation) were administered 2mL of an NCD vaccine containing BCoV, BRV, & *E. coli* (ScourGuard® 4K) IM, and randomly assigned to receive an ITM solution (ITM, n=11; Se, Cu, Zn, & Mn; Multimin®90, 1 mL/ 200Lb) or saline (Control, n=11), SC. A second dose of the vaccine and ITM or saline were given 21 d later. Blood samples were collected weekly for determination of BCoV-SNA and TM concentrations. Colostrum and calves' blood samples were collected to determine BCoV neutralizing antibodies as a measure of passive transfer. PROC GLIMMIX and T-test of SAS® were used for comparisons overtime and between groups.

Results

At initiation of the study cows had marginal serum Cu, Se, and Zn concentrations below normal reference values. Offspring's perinatal mortality was reported in four cows (18.2%, two in each group). Treatment with ITM significantly increased serum Se and Cu concentrations at calving compared to values before treatment ($P< 0.01$) and those in the control group ($P<0.001$ for Cu and $P<0.05$ for Se), which remained marginally deficient. There was an effect of time on BCoV-SNA titers ($P<0.05$). Cows treated with ITM tended to have greater BCoV-SNA and -CNA titers ($P=0.1$) than control cows. Calves born to ITM cows had significantly greater BCoV SNA titers during the first ($P=0.05$) and second ($P=0.07$) week of age.

Conclusions

In conclusion, administration of ITM improved Se and Cu status and enhanced BCoV antibody response to vaccination and subsequent passive transfer to the offspring in beef cattle with marginal trace mineral deficiencies.

Financial Support
University of Georgia; Multimin USA, INC

Notes:

V-093 - Evaluation of baseline hematological and biochemical parameters in water buffaloes (*Bubalus bubalis*)

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Session: Preventive Medicine

Objective

Water buffalo farming (*Bubalus bubalis*) plays an important role in the economy of Italian animal productions. For animal health and welfare reasons, the monitoring of the health status of animals is crucial, and hematological and biochemical tests may provide an important support to the needed periodical clinical examinations. The aim of this study was to determine standard hematological and biochemical parameters in a controlled population of healthy water buffaloes during a one year period in Italy.

Methods

Twenty-six buffaloes (15 females and 11 males) aged between four and 16 months were considered and selected from a herd where animals were subjected to serological tests for brucellosis, infectious bovine rinotracheitis (IBR) chlamydiosis (*Chlamidia psittaci*), parainfluenza 3, bovine viral diarrhea (BVD), Q fever (*Coxiella burnetii*) and *Yersinia enterocolitica* O:9. The animals were housed in standard farming conditions, and underwent a daily clinical check-up. Serum and EDTA blood were taken weekly from T0 until the 16th week, after every two weeks, for a one year period.

Results

Among the parameters evaluated (RBC, RDW, HGB, MCHC, HDW, MCH, HCT, WBC, MCV, PLT, MPV, albumin, creatinine, total proteins, ALP, AST, uric acid, total bilirubin, calcium, amylase, BUN, ALT, GGT, triglycerides, glycaemia, cholesterol) no important seasonal changes were observed during the observation period, with the exception of the BUN, for which evident changes are observed over time.

Conclusions

Hematological and biochemical parameters of animals are essential to confirm clinical diagnosis and to estimate the severity of diseases. Our results show that the hematological and biochemical parameters of the investigated buffaloes do not present significant variations influenced by seasonal changes. Standard values for hematological and biochemical parameters for healthy water buffaloes are proposed and discussed.

Notes:

V-094 - *In-silico* evidence for enhancement of AIV H9N2 virulence during co-infection with avian infectious bronchitis virus

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Session: Virology

Objective

In the last few decades, frequent incidences of avian influenza (AI) H9N2 outbreaks have caused high mortality in poultry farms resulting in colossal economic losses in several countries. In Egypt, the co-infection of H9N2 with the infectious bronchitis virus (IBV) has been observed extensively during these outbreaks. However, the pathogenicity of H9N2 in these outbreaks remained controversial. The current study reports isolation and characterization of the H9N2 virus recovered from a concurrent IBV infected broiler chicken flock in Egypt during 2011.

Methods

The genomic RNA was subjected to RT-PCR amplification followed by sequencing and analysis. The deduced amino acid sequences of the eight segments of the current study H9N2 isolate were compared with those of Egyptian H9N2 viruses isolated from healthy and diseased chicken flocks from 2011 to 2013.

Results

In the phylogenetic analysis, the current study isolate was found to be closely related to the other Egyptian H9N2 viruses. Notably, no particular molecular characteristic difference was noticed among all the Egyptian H9N2 isolates from apparently healthy, diseased or co-infected with IBV chicken flocks. Nevertheless, in-silico analysis, we noted modulation of stability and motifs structure of Hemagglutinin (HA) antigen among the co-infecting H9N2 AI and the IBV and isolates from the diseased flocks.

Conclusions

The findings suggest that the putative factor for enhancement of the H9N2 pathogenicity could be co-infection with other respiratory pathogens such as IBV that might change the HA stability and function.

Notes:

V-095 - A virulent and pathogenic infectious clone of Senecavirus A

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Session: Virology

Objective

Senecavirus A (SVA) is a picornavirus that circulates in swine populations worldwide causing outbreaks of vesicular disease (VD). Vesicular diseases are among the most economically important diseases of livestock and could have catastrophic economic consequences due to international trade restrictions. Thus, a better understanding of SVA infection biology and the viral mechanisms is critical to establish control strategies for SVA and for other picornaviruses. To begin to study the molecular interactions of SVA, we generated a reverse genetics system for SVA based on the wild type SVA strain SD15-26.

Methods

The full-length cDNA genome of SVA was cloned into a plasmid under a T7 RNA polymerase promoter. Following *in vitro* transcription, the genomic viral RNA was transfected into BHK-21 cells and rescue of infectious virus (rSVA SD15-26) was shown by inoculation of highly susceptible H1299 cells.

Results

In vitro characterization of the rSVA SD15-26 showed similar replication properties and protein expression levels as the wt SVA SD15-26. A pathogenesis study was conducted in finishing pigs to evaluate the pathogenicity and infection dynamics of the rSVA SD15-26 virus in comparison to the wt SVA SD15-26. Animals from both rSVA- and wt SVA SD15-26-inoculated groups presented characteristic SVA clinical signs followed by the development of vesicular lesions on the snout and/or feet. The clinical outcome of infection, including disease onset, severity and duration was similar in rSVA- and the wt SVA SD15-26-inoculated animals. All animals inoculated with rSVA or with wt SVA SD15-26 presented a short-term viremia, and animals from both groups shed similar levels of virus in oral and nasal secretion, and feces.

Conclusions

Our data demonstrates that the rSVA SD5-26 clone is fully virulent and pathogenic in pigs, presenting comparable pathogenesis and infection dynamics to the wt SVA SD15-26 strain. The SVA infection clone generated here is a useful platform to study virulence determinants of SVA, and to dissect other aspects of SVA infection biology, pathogenesis and persistence.

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

Notes:

V-095 - Age-related susceptibility of ferrets to SARS-CoV-2 infection

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Session: Virology

Objective

Susceptibility to SARS-CoV-2 and the outcome of COVID-19 have been linked to underlying health conditions and the age of affected individuals. Here we assessed the effect of age on SARS-CoV-2 infection in ferrets.

Methods

We compared the susceptibility of young (6-month-old) and aged (18- to 39-month-old) ferrets to four different doses of SARS-CoV-2. Viral replication, viral load and shedding in respiratory secretions and feces were monitored by rRT-PCR, virus isolation and titrations for 14 days post-inoculation (pi), while seroconversion was assessed by virus neutralization assays. The virological and serological findings were used to estimate the median infectious dose (ID50) of SARS-CoV-2 in young and aged ferrets. Additionally, expression of ACE2 and TMPRSS2 were assessed in upper and lower respiratory tract of ferrets and correlated with outcomes of SARS-CoV-2 infection and replication.

Results

By using infectious virus shedding in respiratory secretions and seroconversion, we estimated that the infectious dose of SARS-CoV-2 in aged animals is ~32 plaque forming units (PFU) per animal while in young animals it was estimated to be ~100 PFU. We showed that viral replication in the upper respiratory tract and shedding in respiratory secretions is enhanced in aged ferrets when compared to young animals. A higher level of ACE2 and TMPRSS2 expression levels were detected in the nasal turbinate of older ferrets when compared to young animals ($p<0.05$).

Conclusions

Together these results suggest that the higher infectivity and enhanced ability of SARS-CoV-2 to replicate in aged individuals is associated – at least in part – with expression levels of ACE2 and TMPRSS2 at the sites of virus entry. The young and aged ferret model developed here may represent a great platform to assess age-related differences in SARS-CoV-2 infection dynamics and replication.

Financial Support
Cornell University

Notes:

V-096 - Genetic and antigenic characterization of an expanding H3 Cluster IV-A influenza A virus clade in US swine

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Session: Virology

Objective

The genetic and antigenic diversity of influenza A virus (IAV) in swine is shaped by transmission and persistence of human IAV within pig populations. The relative frequency of the phylogenetic clade H3 CIV-A that circulated for 20 years in US swine declined to 7% in 2017, but rose to 32% in 2019. To determine putative mechanisms associated with increased detection, we conducted phylogenetic and phenotypic analyses of representative strains.

Methods

To visualize the expansion, spatial spread, and genetic evolution of C-IVA, a Nextstrain web application was developed. We performed phylodynamic analysis of the HA to quantify relative genetic diversity. Internal gene constellations were analyzed for reassortment. Hemagglutination inhibition (HI) and neuraminidase inhibition (NI) assays determined antigenic drift.

Results

Two C-IVA clades emerged in 2017 and cocirculated in multiple US states. Low relative genetic diversity from 2017 to 2019 suggested clonal expansion of genetically similar viruses. The clade with the majority of detections was associated with an N156H amino acid substitution, but HI assays demonstrated no significant antigenic drift associated with this mutation. The minor clade was paired with N2-02B.2 clade in ancestral strains, but acquired an N2-02A.2 in 2016. An 8-fold change in NI titers between the N2 from 02B.2 and 02A.2 clades was observed, indicating antigenic drift between the N2 clades associated with the two H3 clades. The major clade HA gene was tightly linked with the nucleoprotein (NP) of the H1N1pdm09 lineage, indicating reassortment to replace the North American swine lineage NP.

Conclusions

These data demonstrate that increased detection of the H3 Clade IV-A was not associated with increased genetic or antigenic diversity of the HA, but was associated with antigenic diversity of the NA and acquisition of the H1N1pdm09 NP. Defining factors driving spatial and temporal patterns in IAV-S diversity in swine is essential to informing vaccine strain selection and strategies to reduce the expansion and spread of potentially zoonotic swine-origin IAV.

Financial Support
Iowa State University; U.S. Department of Agriculture, Agriculture and Research Services

Notes:

V-098 - Assessment of the feasibility of using neutralizing monoclonal antibody to protect pigs against swine influenza virus

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Session: Virology

Objective

Passive immunity is an effective tool to offer rapid protection against infectious diseases. The traditional approach involved transferring of purified antibody to animals is costly and not practical in agriculture animals. One alternative approach is to use a viral vector to deliver the genes encoding a neutralizing monoclonal antibody (mAb). In this study, swine influenza virus (SIV) was used as a model infectious agent to evaluate the feasibility of rapid induction of protective immunity in pigs through the use of a viral vector expressing a neutralizing mAb.

Methods

Thirty-nine hybridoma clones were generated, of which 3 clones exhibited neutralizing activity. One neutralizing clone was cultured in large quantity and the antibody was purified for a passive transfer experiment in pigs. Four SIV-negative piglets were injected with 28 mg of purified mAb (principal group), while the four control piglets were injected with PBS. The pigs were challenged one day after immunization by an intratracheal inoculation with a virulent H3N2 SIV strain at a dose of 105.0 TCID50 per piglet.

Results

Principal group sera had hemagglutination inhibition titers of 1:40 before infection. After challenge with a H3N2 strain, the principal group shed significantly less virus than the control group ($p < 0.001$). The principal group exhibited lower microscopic and gross lung lesions than the control group ($p < 0.001$). To improve the practical potential of this application, a chimeric mouse x pig mAb genes was constructed and packaged into an adeno-associated virus serotype 2 (AAV2) vector. In vitro studies show that AAV2-construct was capable of transducing porcine kidney epithelial cells and produces detectable levels of anti-SIV antibody.

Conclusions

Passive-transfer of neutralizing mAb protects pigs against infection with SIV. These results lay the groundwork for our future study on the use of passive immunization for control of animal infectious diseases.

Financial Support
U.S. Department of Agriculture, Animal Health Formula Funds

Notes:

V-101 - The effects of host factors in the replication of PRRSV and swine influenza viruses

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Session: Virology

Objective

Understanding the host factors critical for viral infection is a key step for devising a novel approach to prevent and control viral diseases. Swine influenza A virus (SIAV) and porcine respiratory and reproductive syndrome Virus (PRRSV) are important viral diseases in pigs. PRRSV is a member of the family *Arteriviridae* and causes reproductive failure in pregnant sows and respiratory disease in young pigs. SIAV belongs to the family *Orthomyxoviridae* and causes influenza outbreaks in pigs. Protein disulfide isomerases (PDIs) are oxidoreductases that play key roles in proper protein folding in the ER and reported to be involved in the replication of some viruses. Therefore, we aimed to elucidate the effects of PDIs in the replication of PRRSV and SIAV.

Methods

A porcine macrophage cell line that expresses the receptor for PRRSV (CD163) was previously generated. Using porcine cell lines that support PRRSV or SIAV, we determined the effects of various PDIA enzymes in the replication of these viruses and investigated the underlying mechanism. To establish transgenic knockout mice lacking a PDI gene and their parental wild-type mice, heterozygous mice were purchased and bred. The wild-type mice were tested for their susceptibility to various influenza viruses to establish the baseline for subsequent infection of knockout mice.

Results

Porcine macrophage cell lines expressing CD163 showed similar inhibition in PRRSV replication as non-porcine originated cell lines following knockdown of PDI genes using specific siRNAs. Similarly, various strains of SIAV were also significantly inhibited in the porcine originated cells. The preliminary results using the wild-type mice with mixed genetic background showed a range of susceptibility to various influenza strains, which are indicated by lung viral titers and body weight loss.

Conclusions

These findings suggest that the PDI genes are important for PRRSV and SIAV replication in cells and may serve as a potential target for new intervention strategies for PRRSV infections.

Financial Support
U.S. Department of Agriculture

Notes:

V-102 - Rapid genotyping of porcine reproductive and respiratory syndrome virus using Minion nanopore sequencing

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Session: Virology

Objective

The global spread and constant evolution are challenges to the control of the Porcine reproductive and respiratory syndrome virus (PRRSV), one of the most important viruses affecting the swine industry. Effective control of PRRSV benefits from genotyping, which currently relies on Sanger sequencing.

Methods

Here we describe protocols for portable real-time genotyping of PRRSV directly from clinical samples based on partial (AmpliSeq) and targeted whole genome sequencing (WGS) using the MinION nanopore platform. Protocols were validated on 134 clinical samples with Cts ranging from 15 to 35. The WGS protocol targets type 2 PRRSV, the most prevalent in the U.S.A and China

Results

Seventy-four whole genome sequences were obtained using the WGS protocol, representing a success rate of 55%. Fifty out of sixty sera (83.3%) had at least 80% of genome covered at a minimum of 20X sequencing depth. Full genomes were obtained within the first hour of sequencing for a subset of samples with Cts below 24.9. The AmpliSeq protocol was developed with the aim to sequence the complete ORF5 and partial ORF4 and ORF6 of PRRSV types 1 and 2. After 5 min of sequencing, identities to the reference sequence were above 99%. AmpliSeq enabled the classification of clinical samples into lineages 1,5 and 8.

Conclusions

The rapid turnaround time, portability and ease of use of this technology offer significant advantages over traditional diagnostic approaches or other sequencing platforms. The protocols presented here may become valuable tools with potential for field applications during PRRSV elimination programs.

Financial Support
National Pork Board

Notes:

V-103 - Examination of bovine herpesvirus 1 gene expression during stress-induced reactivation from latency

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Session: Virology

Objective

The latency-reactivation cycle of bovine herpesvirus 1 (BoHV-1) is crucial for virus transmission. Since BoHV-1 belongs to the alpha-herpesvirinae subfamily, it is assumed that neurons are the primary site for life-long latency. However, BoHV-1 DNA is readily detected in pharyngeal tonsils (PT) during latency suggesting this is an alternative site for latency. Additional studies revealed that the synthetic corticosteroid dexamethasone triggered reactivation from latency in sensory neurons and PT. The goal of this study was to examine viral gene expression in PT during latency and during early stages of reactivation from latency.

Methods

For these studies, calves were infected with BoHV-1 via the ocular and nasal cavity. At 60 days after infection (latency), calves were treated with a single IV injection of dexamethasone to trigger reactivation from latency. PT were collected at 30, 90, 180, and 240 minutes after dexamethasone treatment. Total RNA was prepared from PT and cDNA libraries were prepared and then RNA-sequencing was performed. Viral genes that were expressed were subsequently identified by bioinformatic analysis. PT was also formalin fixed and paraffin embedded for immunohistochemistry analysis and histological studies.

Results

Strikingly, the BoHV-1 bICP4 transcript, which encodes an essential transcriptional regulatory protein, was readily detected 30 minutes in PT after latently infected calves were treated with dexamethasone. Ninety minutes after DEX treatment bICP4 and to a lesser extent bICP0 were detected. Within three hours after dexamethasone treatment, all lytic cycle viral genes were expressed. Immunohistochemistry studies confirmed bICP4 was detected in PT during early stages of reactivation. Prior to dexamethasone treatment, the latency related (LR) gene, which is abundantly expressed in latently infected sensory neuron within trigeminal ganglia (TG), was not readily detected. In contrast, the tegument viral protein VP16 and bICP0 are detected prior to bICP4 in TG neurons during dexamethasone induced reactivation.

Conclusions

These studies indicate dexamethasone triggers a novel program of viral regulatory genes in PT relative to TG during early stages of reactivation from latency.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture; U.S. National Institutes of Health; Oklahoma State University; USDA-NIFA

Notes:

V-104 - Histo-blood group antigen-like producing bacteria inhibit rotavirus A infection of intestinal epithelial cells

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Session: Virology

Objective

Direct binding of group A rotavirus (RVA) by histo-blood group antigen (HBGA)-like substances on bacteria surface represents and additional mechanisms utilized by probiotics that can mitigate rotavirus disease severity. The aim of this study was to evaluate the ability of HBGA-positive/negative commensal bacteria from *Firmicutes (Lactobacillus rhamnosus GG* (LGG)*, Streptococcus bovis (SB), Lactobacillus brevis* (LB), *Clostridium clostridioforme* (CC), *Enterococcus faecalis* (EF)), *Bacteroidetes (Bacteroides thetaiotaomicron (BT)) Proteobacteria (Escherichia coli G-58* (G-58), *Escherichia coli Nissle 1917* (EcN)) *and Actinobacteria (Bifidobacterium adolescentis* (BA), *Bifidobacterium longum (*BL) phyla to block group A rotavirus (RVA) infection *in vitro.*

Methods

The presence of HBGA-like substances on bacterial surface was assessed by flow cytometry using the anti-HBGA monoclonal antibodies (Biolegend, CA). To evaluate the ability of the bacteria to directly bind RVA particles preventing their infection of intestinal porcine epithelial cells (IPEC-J2), the bacterial strains were pre-incubated (with bacteria-to-virus ratio 10,000:1) with trypsin-activated RVAs: G4P[6], G1P[8], G9P[13], G5P[7], obtained supernatants were used to inoculate IPEC-J2. Cell culture immune fluorescent assay was used to quantify RVAs amounts.

Results

The results demonstrated that bacteria expressing significantly higher amounts of HBGA were able to reduce the RVA replication in IPEC J2 cells in a strain-specific manner: (SB, BT, BA - G1P[8], BL - G4P[6] and CC - G5P[7]). In contrast, bacteria with low-level (EcN, LB, LGG, EcN) or no HBGA expression (G58) had moderate or no effect on RVA titers. We also observed high-to-moderate effect LGG, LB and BL-pre-treatment in inhibiting different strain RVA infection. This indicates that a cocktail of bacteria may be a more feasible approach to treat genetically diverse RVA infections.

Conclusions

Our findings confirmed that the presence of HBGA-like on bacterial surface may reduce RV infection via direct binding of viral particles *in vitro*.

Financial Support
International Development Research Centre

Notes:

V-105 - Targeting porcine viral pathogens using a bait-capture method

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Session: Virology

Objective

Viral respiratory disease is an important issue in swine production worldwide. Sequencing of viral genomes from porcine respiratory samples can be used to increase the understanding of these diseases. However, low viral titers and high levels of host genomic material are some of the challenges faced when using field samples. Furthermore, many assays target single viruses, which limits the ability to understand co-infection dynamics. Finally, viral culture is often needed to enrich genomic material prior to sequencing, which can introduce bias into the resulting genomic sequences. To overcome these limitations, "bait capture and enrichment" method using cDNA biotinylated "baits" can be applied. The aim of this study was to design a custom bait panel for important swine pathogens and employ it on field samples.

Methods

A list of 31 swine viruses was compiled. Full-length genomes were identified and downloaded using the NCBI Complete Genomes for Viruses. For influenza A, B, and C, nucleotide sequences were downloaded from the NCBI Influenza Virus Resource, selecting only those identifying the host as swine. These sequences were used as input into bait design by CATCH algorithm, with the following parameters: length of probe = 120bp; the number of potential mismatches between probe and target = 30bp; coverage of targets genomes = 100%.

Results

The number of accessions with complete genomic sequences varied widely, with a low of just 1 for classical swine fever virus and a high of 5,995 for influenza virus. In total, 10,672 genomes across all 31 viruses were included in the panel design. The final bait set was comprised of 19,957 unique baits, which together covered 100% of each of the input genomes. These baits are currently being tested on both field and lab-based mock samples of known and unknown viral status.

Conclusions

In conclusion, this work represents successful completion of a necessary step within the workflow of the overall objective, which is to validate an assay that can provide unbiased full-genome sequences of numerous potentially co-infecting viruses from porcine field samples.

Financial Support
National Pork Board; USDA Hatch Capacity Grant Funding; Minnesota Agricultural Research, Education and Extension Technology Transfer Program; Pipestone MorriSTONE Research Award; Agriculture and Food Research Initiative

Notes:

V-106 - Development of a model for susceptibility to SARS-CoV-2 based on expression of ACE2 and TMPRSS2 in avian cells

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Session: Virology

Objective

The SARS-CoV-2 (SC2) virus has caused a world-wide pandemic because of the virus's ability to transmit efficiently human-to human. A key determinant of infection is the attachment of the viral spike protein to the host receptor angiotensin-converting enzyme 2 (ACE2) and there have been multiple predictions of host susceptibility based on this attachment. Multiple experimental animal studies have been performed to determine if they can act as biological vectors. In an effort to have a better predictive model of animal host susceptibility to SC2 we developed the avian fibroblast cell line, DF1, to express the ACE2 and/or transmembrane serine protease 2 (TMPRSS2) genes from human and multiple animal species.

Methods

Using lentivirus transduction, avian DF1 cells expression human ACE2 and TMPRSS2 genes, were purified by FACS analysis. Expression of both genes was determined by RTPCR and immunohistochemistry. Dual positive cells were grown in 96-well plates and inoculated at an MOI of 1 with the USA-WA1/2020 isolate of SARS-CoV-2. To further test the model we developed seven additional transgenic cell lines of ACE2 and TMPRSS2 combination insertions in the DF1 backbone from cat, horse, pig, goat, hamster and two different bat species.

Results

Titers of SC2 in the DF1 cell lines expressing human ACE2 and TMPRSS2 peaked at 24 hours post infection (10^6 TCID50/ml) and were comparable to those observed in Vero cell lines. Results demonstrate permissive replication of SC2 in cat, hamster and goat species, but not pig or horse which correlates well with those species that have been experimentally challenged. Interestingly, both bat species initially had increased titers (appx 2 log10) for up to 12 hours, but no increase was observed after this and might be related to the truncated TMPRSS2 protein relative to the human protein.

Conclusions

The development of this model system allows for more efficient testing of potential susceptibility of animal species to be infected and act as biological vectors to SC2 and SC2 variants to humans or other animals.

Notes:

V-107 - An established catfish cell line to aid in fish virus studies

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Session: Virology

Objective

Though cell lines are a very relevant research tool in virology, cell lines originated from ictalurid catfish are limited. The ictalurid cell line (channel catfish ovary (CCO - ATCC® CRL-2772)) previously available from ATCC cell repository has recently been reported as cross-contaminated by brown bullhead (BB) cells. Lack of host-specific cell lines and contamination issues necessitated initiation of cell cultures from the fin tissues of hybrid catfish (♀ channel catfish, *Ictalurus punctatus* x ♂ blue catfish, *I. furcatus*).

Methods

A combination approach involving tissue explantation and enzymatic digestion methods were employed to develop the hybrid catfish fin (HCF) cell line. The HCF cell line has been characterized, maintenance conditions optimized, species of origin molecularly authenticated, and its susceptibility to fish viruses evaluated.

Results

The fin cell cultures were passaged over 100 times and transitioned into an established cell line. The HCF cell line has been characterized, maintenance conditions optimized, species of origin molecularly authenticated, and its susceptibility to fish viruses evaluated. Susceptibility of HCF cell line to catfish viruses demonstrated the potential of these cells to propagate ictalurid viruses.

Conclusions

Susceptibility of HCF cell line to catfish viruses demonstrated the potential of these cells to propagate ictalurid viruses. This established ictalurid catfish cell line could serve as an efficient tool for virus studies, antiviral agent screening, and vaccine development benefitting catfish aquaculture.

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

Notes:

V-108 - Genome rearrangement as live attenuated influenza vaccines platforms.

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Session: Vaccinology

Objective

Influenza A virus is an important pathogen for poultry, swine and humans. Live attenuated influenza vaccines (LAIVs) provide a more broadly protective immune response by mimicking a natural infection and stimulating both humoral and cellular immune responses. To generate efficacious LAIVs, we generated viruses carrying different genome rearrangement strategies.

Methods

Different attenuation strategies were generated by genome rearrangement cloning the open reading frame of NS2 (RANS), M2 (RAM) or M42 (RAM-42) downstream of PB1 in segment 2. Additionally, multiple stop codons were introduced in segment 8 (RANS) or segment 7 (RAM and RAM-42) to prevent the expression of either NS2 or M2 or M42 respectively. Within the background of an avian IAV strain rearranged viruses of the H9N2 and H5N8 subtype were obtained. Likewise, rearranged viruses of the H1N1 and H3N2 subtype in the background of a swine IAV strain were attempted. Stability and growth properties of the different viruses were analyzed in vitro; whereas the efficacy of the RAM-H1N1 as LAIV was evaluated in mice. Clinical signs, viral loads and histopathological analysis were monitored after lethal challenge to estimate the vaccine efficacy.

Results

Viruses of the H5N8 and H9N2 subtype were obtained in the RANS, RAM, and RAM-42 platforms. These viruses were stable and grew to similar levels in comparison to the wild type (wt) viruses. However, and despite similar polymerase complex activity, viral titers of rearranged viruses were reduced at 41ºC compared to the wt isogenic strains. Efficient rescue of RAM-H1N1 and RAM-H3N2 viruses was also observed. Mice vaccinated with RAM-H1N1 strain were completely protected from challenge with a 10,000-mouse lethal dose 50 of a prototypic pandemic 2009 H1N1 strain.

Conclusions

The findings support the goal of generating LAIVs based on genome rearrangement strategies. Further studies are underway to better understand the immunogenicity and protection conferred in poultry and mammalian species.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture; U.S. National Institute of Allergy and Infectious Diseases

Notes:

V-109 - Antiviral and immunomodulatory therapy in BRSV: Effects on immune cells, lung pathology, and gene expression

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Session: Immunology - 1

Objective

Bovine respiratory syncytial virus (BRSV) causes respiratory disease in calves and is analogous to RSV in human infants. We hypothesized that treatment of calves with an antiviral drug and an immunomodulatory NSAID would decrease clinical signs, lung pathology, and alter gene expression. To evaluate these therapeutic modalities we performed a placebo-controlled study on calves. We studied the effect of treatment with a fusion protein inhibitor (FPI) for BRSV compared with placebo, NSAID alone or dual therapy.

Methods

Pre-ruminant calves were divided into 6 treatment groups: ibuprofen days (D) 3-10, ibuprofen D 5-10, placebo, FPI D 5-10, FPI and ibuprofen D 5-10, and FPI and ibuprofen D 3-10. Daily clinical scores and viral shedding were measured in nasal swabs by qRT-PCR. T cell subsets were evaluated by flow cytometry. On D10 lung tissue with lesions (LL) and without lesions (LN) was collected, total RNA extracted, and RNA sequencing performed. Differential gene expression analysis was conducted with Gene ontology (GO) and KEGG pathway enrichment analysis. Quantitative histopathology on H & E lung sections was evaluated using canonical discrimination analysis to determine the structural level where differences between treatments occurred.

Results

Dual therapy decreased clinical scores, most significantly for D3-10. Differential gene expression in lung tissues was compared for LL and LN: oxidative stress and cell damage was greatest and innate and adaptive immune functions were reduced in LL. Dual therapy starting D3 showed greatest difference in gene expression patterns compared to placebo, especially in pathways related to innate and adaptive immune responses. Ibuprofen, as monotherapy increased viral shedding. Analysis of histopathology showed maximal separation from placebo for dual therapy at the levels of the alveolus, septum, and bronchus.

Conclusions

Clinical benefits of dual FPI and ibuprofen therapy extend at least in part from histopathological changes in the lung when treatment was started D3. Ibuprofen as monotherapy started on D3 decreased lung injury, but not when started on D5. In dual therapy Ibuprofen enhanced the specific antiviral effect of FPI, due to its ability to reduce the damaging effect of prostanoids and oxidative stress.

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

Notes:

V-110 - Alternatives to antibiotics: neonatal immunomodulation to improve disease resistance in animals

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Session: Antimicrobial Alternatives

Objective

An evolving field of immunomodulation is innate training, which relies on cellular epigenetic modifications that lead to a heightened (ie, trained) response upon repeated microbial stimulation of innate immune cells. Our overall goal is to utilize innate training as an approach to enhance disease resistance. Prior experiences show bacillus Calmette-Guerin (BCG) exposure increases cytokine production by myeloid-lineage cells upon secondary microbial stimulation, and epidemiological data suggest improved disease resistance in humans administered neonatal BCG vaccine.

Methods

Inoculated pigs and cattle with BCG (or mock) and evaluated subsequent impact on clinical disease or shedding with relevant animal health or foodborne pathogens. In addition, mononuclear cells isolated from mock and BCG inoculated animals were subjected to epigenetic assays and restimulation in culture to evaluate trained phenotype. Training is defined as increased cytokine transcription/production after *in vitro* exposure to lipopolysaccharide (LPS) in cells from BCG versus mock treated animals.

Results

Upon *in vitro* LPS exposure, monocytes isolated from pigs after intravenous (IV) BCG administration produced more IL-1b and TNFa cytokine than monocytes from mock pigs. The innate trained phenotype was not observed in pigs given BCG by the subcutaneous (SC) or intramuscular route, despite detectable adaptive T cell responses. Thus, neonatal piglets were administered IV BCG early summer, with influenza virus challenge to follow. Calves were inoculated SC with BCG in early summer, with bovine respiratory syncytial virus challenge to follow. Previous SC BCG in cattle induced a peripheral trained phenotype. Blood leukocyte populations will be preserved for transcriptomic and epigenetic evaluation (ChIP-seq and ATAC-seq) in both trials.

Conclusions

BCG administration induced a trained phenotype in porcine and bovine peripheral cells, primarily monocytes. Trials are ongoing to understand if BCG induced training translates into protection again respiratory viral infection in pigs and cattle.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture; USDA-ARS; Iowa State University

Notes:

V-111 - Rural raccoons are not drivers of antimicrobial resistant human Salmonella cases in southern Ontario, Canada

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Session: Antimicrobial resistance/use

Objective

Non-typhoidal *Salmonella* infections represent a substantial burden of illness to human health, and the increasing prevalence of antimicrobial resistance among these infections is a growing concern. Using a combination of short-read whole genome sequence data from bacterial isolates from human *Salmonella* cases, raccoons, livestock and environmental sources, and epidemiological modeling, our objective was to determine if there was evidence for potential transmission of *Salmonella* and associated antimicrobial resistance determinants (determined in silico) between these different sources in the Grand River watershed in Ontario, Canada.

Methods

Outputs from bioinformatics analyses were used in logistic regression models to assess for potential associations between source type and the presence of select resistance genes and plasmid incompatibility (Inc) types. A total of 608 isolates were obtained from the following sources: humans (n=58), raccoons (n=92), livestock (n=329), and environmental samples (n=129).

Results

Resistance genes of public health importance, including AmpC producers (i.e., *bla*$_{\text{CMY-2}}$) were identified in humans, livestock, and environmental sources, but not in raccoons. Population structure based on the 3002-loci cgMLST scheme revealed that human *Salmonella* isolates were often more similar to isolates from livestock and environmental sources, than with those from raccoons. Rare instances of serovars *S.* Heidelberg and *S.* Enteritidis in raccoons likely represent incidental infections and highlight possible acquisition and dissemination of predominantly poultry-associated *Salmonella* by raccoons within this ecosystem. Raccoon-predominant serovars were either not identified among human isolates (*S.* Agona, *S.* Thompson) or differed by more than 350 loci (*S.* Newport).

Conclusions

Collectively, our findings suggest that the rural population of raccoons on swine farms in the Grand River watershed are likely not major contributors to antimicrobial resistant human *Salmonella* cases in this region.

Notes:

V-P001 - Antimicrobial resistance of *Escherichia coli* and *Salmonella typhimurium* isolated from poultry in Armenia

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Session: Antimicrobial use/resistance/stewardship

Objective

There are growing concerns about the antibiotic resistance of poultry-related bacteria, as antibiotics are widely used in this field for both preventive and growth promoting purposes. Within the framework of the presented research, we have studied the antibiotic resistance of some of bacteria isolated from commercial poultries in Armenia.

Methods

Samples were collected from meat and internal organs of slaughtered commercial poultry. Isolation and identification of *Escherichia coli* and *Salmonella typhimurium* have been performed based on traditional bacteriological and biochemical methods. We determined the resistance of microorganisms to antibiotics by the disc-diffusion method using 39 antibiotic discs representing antibiotics which are available in both human and animal health sectors in Armenia. We defined 5 rating groups according to the degree of bacterial stability or susceptibility based on the zone of inhibition surrounding the antibiotic discs.

Results

E. coli isolated from poultry were highly resistant to lincomycin, cefixime, ceftazidime, erythromycin, with slightly less resistance to an additional 14 antibiotics. *E. coli* showed high sensitivity for trimethoprim and less sensitivity for 15 other antibiotics. Overall, *E. coli* isolated from poultry showed resistance to 35.8% and sufficient susceptibility to 43.5% of antibiotics tested. *S. typhimurium* has shown high resistance against cephalothin, lincomycin, chloramphenicol, cephalexin, ceftriaxone, carbenicillin and less resistance against 19 additional antibiotics. At the same time, they showed high sensitivity for gentamicin, moxifloxacin, trimethoprim and a less sensitivity for 9 additional antibiotics. *S. typhimurium* isolated from poultry showed resistance to 53.8% and sufficient susceptibility to 28.2% of antibiotics tested.

Conclusions

The results show that AMR is present in the commercial poultry sector of Armenia and that specific surveillance programs need to be developed to assess and monitor antimicrobial use and AMR issues.

Notes:

V-P002 - Determination of antimicrobial resistance of *Staphylococcus aureus and Staphylococcus albus* isolated from lymph nodes of pigs

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Session: Antimicrobial use/resistance/stewardship

Objective

Antimicrobial resistance (AMR) remains as a serious problem for both public health and animal health. As there is not enough research in Armenia in the field of veterinary in this direction, we have undertaken this study to determine the degree of resistance of *staphylococcus* spp. isolated from slaughtered pigs.

Methods

We collected samples of lymph nodes from slaughtered pigs at slaughterhouses. Isolation and identification of *staphylococcus* spp. have been performed based on conventional bacteriological and biochemical methods. We have determined the resistance of microorganisms to antibiotics by the disc-diffusion method, using 39 antibiotic discs from various types and groups which are available in Armenia. According to the degree of bacterial stability or susceptibility, we defined 5 rating groups ranging from highly resistant to highly sensitive based on the distance of bacterial growth surrounding the antibiotic disc.

Results

Bacteria of *S. aureus* and *S. albus* were isolated from the pig lymph nodes. *S. aureus* isolates were highly resistant to the following: cefixime, cefuroxime, ampicillin, levofloxacin, cephalexin, and ceftazidime, with less resistance against 2 other antibiotics. The same bacteria showed high sensitivity for cephalothin, amoxicillin, and carbenicillin. Overall, *S. aureus* isolated from pigs showed resistance to 25.6% and sensitivity to 17.9% of antibiotics tested. Results for *S. albus* showed high resistance against cephalothin, and bacitracin with less resistance against 7 other antibiotics. They showed high sensitivity for amoxicillin and cefazolin and less sensitivity for 15 additional antibiotics. *S. albus* isolated from pigs showed resistance to 23.0% and sensitivity to 41 % of the antibiotics tested.

Conclusions

We have shown that AMR isolates of *S. aureus* and *S. albus* are present in the pig breeding sector in Armenia with both showing high resistance to fluconazole and nystatin. It is necessary to develop surveillance programs to monitor both antimicrobial use and development of AMR

Notes:

V-P003 - Efficacy of tulathromycin for treatment of respiratory disease in goats

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Session: Antimicrobial use/resistance/stewardship

Objective

Respiratory infections represent a significant disease burden on goat production in the U.S., however, the single antibiotic labeled for treatment of respiratory disease in goats is not effective for all respiratory pathogens affecting this species. Veterinarians and producers are thus frequently faced with the need to treat animals for respiratory disease with antibiotics labeled in other species that lack efficacy data in goats. Previous work performed by our research team strongly suggests that the long-acting macrolide antibiotic, tulathromycin, may be an effective treatment for respiratory disease in goats, but additional research is needed to gain label approval. Our goal is to complete the research necessary for successful application to the FDA for label approval of the use of tulathromycin for treatment of respiratory disease in non-lactating goats. To achieve this goal, our research team will complete a randomized controlled trial of naturally occurring respiratory disease in goats to pursue the following objectives:
1. Determine the clinical efficacy of tulathromycin for treatment of naturally occurring respiratory disease in non-lactating goats
2. Document the current species distribution of bacterial causes of pneumonia in non-lactating goats in the United States
3. Collect antimicrobial resistance data necessary for establishment of species-specific breakpoints for respiratory pathogens in goats

Methods

At this time, we are in the process of obtaining protocol concurrence through the FDA CVM Office of New Animal Drug Evaluation for completion of the clinical efficacy trial for respiratory disease in non-lactating goats, and anticipate beginning animal studies in spring 2022.

Results

None available at this time.

Conclusions

None available at this time.

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

Notes:

V-P004 - Ceftiofur resistance trends in Gram-negative bovine mastitis isolates from Wisconsin: A retrospective report

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Session: Antimicrobial use/resistance/stewardship

Objective

Ceftiofur is one of the most common antibiotics used to treat Gram-negative bovine mastitis. Therefore, monitoring ceftiofur resistance in common Gram-negative mastitis pathogens is important for animal health and improved antibiotic stewardship. This study aims to describe the proportion of Gram-negative bacteria resistant to ceftiofur that was isolated from mastitic milk submitted for culture and susceptibility testing to WVDL between 2007-2019.

Methods

Antimicrobial susceptibility testing (AST) data from 2007-2019 was collected from Escherichia coli (n=1518), Pasteurella species (n=110), Serratia species (n=167), and Enterobacter species (n=89). The broth microdilution method of in vitro AST was conducted using the bovine mastitis panel. In order to determine resistance, the CLSI ceftiofur breakpoint for Escherichia coli (mastitis) and Pasteurella multocida (bovine respiratory disease) were applied to other Gram-negative pathogens. With our preliminary data, we have reported the proportion of susceptible isolates.

Results

Based on our preliminary results, we observed that the proportion of ceftiofur resistant E. coli ranged from 0-21.4% with little fluctuation except for years 2018-2019 in which resistance peaked. The proportion of susceptible Serratia, Pasteurella, and Enterobacter species to ceftiofur was relatively high (85.7-100%), with few changes in the proportion of susceptible isolates.

Conclusions

Although we observed some changes in ceftiofur resistance, the majority of the Gram-negative bacteria tested remained susceptible to ceftiofur in vitro. Resolution of Gram-negative mastitis using ceftiofur may not align with our results since only E. coli had specific breakpoints for determining resistance. Breakpoints for other Gram-negatives would need to be established to better understand resistance of those pathogens to ceftiofur. Given ceftiofur's clinical relevance, it is important for this antimicrobial to remain effective against these common Gram-negative bacteria and to continue monitoring ceftiofur resistance.

Notes:

V-P006 - Production and economic outcomes of restricting antimicrobial use in U.S. hog production: A systematic review

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Session: Antimicrobial use/resistance/stewardship

Objective

In the United States, sub-therapeutic levels of in-feed antimicrobials are traditionally used in livestock production to promote growth and improve feed efficiency and health outcomes. Long-term antibiotic use, even at sub-therapeutic levels, raises concern for increased antimicrobial resistance (AMR) that may decrease the effectiveness of antibiotic treatments in humans and animals. In response to global concern for AMR, the United States Food and Drug Administration implemented the Veterinary Feed Directive (VFD) on January 1st of 2017, requiring medicated feed to be used under the supervision of a veterinarian. The objective of our study was to understand the potential production and economic impacts of restricting in-feed sub-therapeutic antimicrobial use in U.S. hog production under the VFD.

Methods

A systematic review was conducted using the databases AgEcon Search, PubMed, CAB/Medline, and google scholar. Literature was screened based on set inclusion criteria and data from qualifying studies was synthesized in Excel. Overall, 41 studies qualified for inclusion.

Results

The most common outcomes included average daily gain, average daily feed intake, feed to gain ratio, annual profit, net profit per head, and producer and consumer surpluses. Results indicated that the average daily gain associated with antibiotic use ranged from 0.18kg to 0.94kg and was predicted to decrease up to 0.45% under restriction. Average daily feed intake ranged from 0.35kg to 2.58kg and was also predicted to decrease under restriction, with overall feed to gain ratios increasing up to 1.13%. Predicted values for net profit per head and annual profit ranged from a loss of $4.82 to a gain of $4.42, and a loss of $488 million to a gain of $117.2 million, respectively.

Conclusions

While the lasting effects of the VFD may not be evident for many years, this review provides a summary of expected outcomes as predicted by existing literature. Moving forward, comparisons of expected versus actual outcomes can be used to inform policy and future research.

Notes:

V-P007 - Genetic molecular characteristics of Enterobacteriaceae isolates harboring metallo-β-Lactamase NDM-5 from Korean companion dogs

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Session: Antimicrobial use/resistance/stewardship

Objective

The increasing prevalence of carbapenemase-producing Enterobacteriaceae (CPE) is considered as a global threat, listed by the World Health Organization (WHO) as priority 1 critical pathogens. Based on the current situation, we investigated for CPE in companion animals and their genetic characteristics.

Methods

We screened a total of 520 clinical bacterial isolates from companion dogs and cats which were hospitalized to Veterinary Medical Teaching Hospital, Seoul National University from 2018 to 2020. After screening, suspected strains were identified by PCR, and subtyped by pulsed field gel electrophoresis (PFGE) and Multilocus sequence typing (MLST). Plasmid DNA was isolated and sequenced with the Illumina MiSeq Sequencing System.

Results

In our study, 5 strains of metallo-β-Lactamase NDM-5-producing *Escherichia coli* (n=3) and *Klebsiella pneumoniae* (n=2) were isolated from 4 different dogs. All isolates showed resistance to various antibiotics, including carbapenems. Multilocus sequence typing (MLST) result showed that all *E. coli* strains were ST410, and all *K. pneumoniae* strains were ST378. We describe in this study the first description of NDM-5 producing carbapenem-resistant *K. pneumoniae* ST378. Plasmid analysis showed that $bla_{\text{NDM-5}}$ is carried on IncX3 plasmid, showing high concordance rate with those being detected worldwide human and animal isolates. The bla_{NDM} gene was associated with ble_{MBL} gene and the IS*Aba125* element truncated with IS5 element.

Conclusions

Our results suggested that CPE might be spreading between the animals, especially companion animals. Additional emergence of CPE is indicating either the “Off-label” use of carbapenems towards companion animals or horizontal transmission from the host’s owner. Therefore, further investigation is necessary to unveil the role of NDM-5 carrying IncX3 plasmids from companion and food animals in our country. This study was supported by National Research Foundation (NRF-2020R1A2C2008794), BK21 FOUR Future Veterinary Medicine Leading Education and Research Center and Research Institute for Veterinary Science, Republic of Korea.

Financial Support
National Research Foundation of Korea; BK21 FOUR Future Veterinary Medicine Leading Education and Research Center and Research Institute for Veterinary Science, Republic of Korea

Notes:

V-P008 - Applying survival analysis to antimicrobial resistance trends in *Staphylococcus pseudintermedius* isolated from canine pyoderma

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Session: Antimicrobial use/resistance/stewardship

Objective

Antimicrobial resistance (AMR) emergence in *Staphylococcus pseudintermedius*, a companion animal pathogen, and transference to humans through contact with dogs presents a potential threat to public health. This study aimed to examine patterns of AMR in canine *S. pseudintermedius* and investigate survival analysis as a supplement to typical AMR surveillance methods.

Methods

We analyzed *S. pseudintermedius* (n = 1,170) isolated from canine skin cultures in the Northeastern United States between 2007 and 2017. First, minimum inhibitory concentrations (MIC) were categorized as resistant and susceptible with CLSI breakpoints. Antibiograms were created and trends in the prevalence of resistance assessed with Cochran-Armitage (CA) tests. We modeled MIC distributions of antimicrobials without a detectable trend using survival analysis (SA); growth inhibition was the "event" and antimicrobial concentration was "time." A Cox proportional hazards regression analyzed changes in probability of growth inhibition across antimicrobial concentrations to compare MIC distributions between years.

Results

Antibiograms showed a high prevalence of resistance to penicillins, with 19 to 89% of isolates resistant in a given year, tetracyclines (64 to 100%), and macrolides (33 to 55%), while aminoglycosides (0 to 21%) and ansamycins had the lowest prevalence (0 to 2.5%). 19 to 47% were methicillin resistant. CA trend tests indicated increasing resistance to chloramphenicol, clindamycin, erythromycin, and trimethoprim/sulfamethoxazole. Although an increase in fluoroquinolone resistance was not detected with CA trend tests, SA showed an increase in marbofloxacin and enrofloxacin MICs from 2014 to 2017.

Conclusions

This population of *S. pseudintermedius* showed increasing resistance to several antimicrobials from 2007 to 2017. SA exposed decreasing fluoroquinolone susceptibility, which may indicate the SA method is well suited for AMR that occurs incrementally. AMR monitoring can be improved by detecting resistance trends unapparent in antibiograms and CA trend tests through the use of SA on interval censored MIC data.

Notes:

V-P009 - Evaluation of a bacteriophage enzyme, PlyC, for treatment of *S. uberis*-associated bovine mastitis

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Session: Antimicrobial use/resistance/stewardship

Objective

Bovine mastitis is the most economically significant disease affecting dairy cattle and contributes substantially to the overall use of antibiotics on dairy farms. Although mastitis is caused by many different bacterial species, *Streptococcus uberis* is one of the most prevalent Gram-positive pathogens associated with the disease. Concerns among consumers regarding antimicrobial use and resistance have led to development of alternative strategies for controlling mastitis. One alternative to traditional antibiotics is the use of cell wall hydrolase enzymes, derived from bacteriophage, that rapidly lyse *S. uberis* on contact. One such enzyme is PlyC. The goal of this study is to evaluate PlyC against *S. uberis in vitro* and to develop an *S. uberis* bovine mastitis model for future efficacy studies with PlyC.

Methods

The activity of PlyC was determined tubidometric lytic assays 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. Bovine mastitis pilot studies were performed with four primiparous Holstein-Friesian dairy cows and *S. uberis* strain 0140J.

Results

Our results demonstrate that PlyC possesses antimicrobial activity against all *S. uberis* strains tested. Moreover, PlyC attained three logs of killing in raw, mastitic milk derived from clinically affected cows. High titer antibodies directed against PlyC were unable to neutralize its lytic activity. Additionally, PlyC was non-toxic to MAC-T bovine mammary cells in culture and found to be non-irritating in rabbit epidermis and mucous membrane models. Pilot cow studies were conducted to determine the optimal dose of *S. uberis* needed to develop clinical symptoms of bovine mastitis in anticipation of clinical trials. Trial 1 evaluated a dose of 125 cfu *S. uberis* 0140J per quarter and Trial 2 evaluated 1,000 cfu/quarter.

Conclusions

The *in vitro* and *in vivo* findings to date support advancement of PlyC to *S. uberis*-associated bovine mastitis clinical trials.

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

Notes:

V-P010 - Comparative genome analysis of *Mycobacterium intracellulare* reveals the molecular basis of multidrug resistance

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Session: Antimicrobial use/resistance/stewardship

Objective

Non-tuberculous mycobacteria (NTM) are ubiquitous bacteria that are widely distributed in natural environments. *Mycobacterium intracellulare* is an opportunistic pathogen that causes pulmonary NTM infections. In a previous study, our group analyzed the distribution and the antibiotic resistance profile of NTM isolated from soil. Among the *M. intracellulare* isolates, 7 multidrug resistant isolates were analyzed to delineate the uniqueness of the environmental strains with respect to antibiotic resistance and virulence factors by single-molecule sequencing.

Methods

The seven strains of *M. intracellulare*, S1-36A, S1-32, S2-11, S2-8, B1-4, N6-8, and ATCC13950 were part of a previously published collection that were identified based on the sequences of the *rpoB*, *hsp65*, and *16S rRNA* genes. Whole-genome sequencing for *de novo* assembly was performed using PacBio Sequel. The pangemone analysis was performed by Roray. Functional analysis was performed based on Clusters of Orthologous Groups. The virulence factors were searched with three different databases (VFDB, Victors, PATRIC) and the antimicrobial resistance (AMR) were analyzed using the four databases (ARG-ANNOT, NCBI, Lahey, CARD).

Results

The phylogenetic and pangenome analysis revealed that B1-4 and N6-8 did not belong to *M. intracellulare*, although the previous result of *rpoB*, *hsp65*, and *16S rRNA* gene amplification were classified as *M. intracellulare*. Among the five *M. intracellulare* isolates, the virulence factors *mosR* and *mce8F* appeared only in the sequence of S2-11 and also *adhD* only in S1-32. Seven antimicrobial resistance genes, *efpA*, *murA*, *tuf*, *aviRb*, *mtrA*, *mfpA*, and *rbpA*, were commonly identified in all five *M. intracellulare* isolates. However, the AMR genes are not consistent with the phenotypic drug-susceptibility testing.

Conclusions

Our results showed that antimicrobial mutations could not be correctly characterized and some resistant phenotypes could not be ascribed a causative mutation. In addition, these results may provide a path to discover markers that can differentiate the *M. intracellulare* complex.

Financial Support
This work was carried out with the support of "Cooperative Research Program of Center for Companion Animal Research (Project NO. PJ013985)" Rural Development Administration, Republic of Korea.

Notes:

V-P011 - Phenotypic differences in *Streptococcus suis* and *Glaesserella parasuis* when grown in co-culture

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Session: Antimicrobial use/resistance/stewardship

Objective

Streptococcus suis and *Glaesserella parasuis* are common commensals residing in the tonsil of the soft palate of healthy pigs, but are also known to cause serious disease. Despite several studies in mono-culture, little is known about how these organisms interact in tonsils and how they shift from a benign to virulent state. *S. suis* serovars 2 and 9, and *G. parasuis* serovars 5 and 3 are prevalent in Canada and various parts of the world. The purpose of this study is to identify phenotypic changes in co-cultures of *S. suis* and *G. parasuis* and characterize changes in virulence-associated gene expression. We hypothesize that co-culture of *S. suis* with *G. parasuis* will cause a shift of both organisms from sessile to planktonic phase in a serovar-specific manner. We also predict that co-culture will increase growth rates and planktonic cell release.

Methods

Strains include a clinical isolate of *S. suis* serovar 2 (SS2c) and 9 (SS9c), a non-clinical serovar 9 (SS9h), and low- and high-virulence isolates of *G. parasuis* serovar 3 (GP3) and 5 (GP5), respectively. Strains were grown in mono- and co-culture for comparison.

Results

To date, we have shown that *S. suis* growth rate remains unchanged in co-culture relative to mono-culture, while GP3 is decreased with SS2c at hours 11 and 12 ($p<0.01$), and with SS9c at hours 10, 11, and 12 ($p<0.001$). Biofilm assays showed significant biomass increases in GP3 with SS9c at hours 48 and 72 ($p<0.001$), and increases in GP5 with SS9c at hours 24, 48, and 72 ($p<0.01$). An increase in planktonic cells was also seen in GP5 co-cultures with SS9c at hours 24, 48, and 72 ($p<0.01$). These results suggest that co-culture has no effect on *S. suis* growth rate but adversely affects *G. parasuis* growth, while co-culture with SS9c increases biofilm formation.

Conclusions

Future work will explore the effect of co-culture on resistance to antibiotics, serum, and saliva. Finally, mechanisms involved in observed phenotypic changes will be identified using RNA sequencing. This work provides insight into how *S. suis* and *G. parasuis* interact and how their phenotype shifts in co-culture.

Financial Support
Natural Sciences and Engineering Research Council of Canada

Natural Sciences and Engineering Research Council of Canada | Conseil de recherches en sciences naturelles et en génie du Canada

Notes:

V-P012 - Factors associated with antimicrobial-resistant enterococci across the beef production system: A scoping review

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Session: Antimicrobial use/resistance/stewardship

Objective

This study aims to examine factors associated with antimicrobial-resistant enterococci within Canadian beef production along the farm to fork continuum. Factors are identified through a scoping review conducted in partnership with the iAM.AMR pan-Canada research initiative.

Methods

A scoping review was conducted to identify interventions associated with an increase or decrease of AMR enterococci in cow-calf operations, feedlot, abattoir, meat processing, and retail environments. Five databases were searched using variants of "beef", "enterococci" and "antimicrobial resistance". Articles were excluded if they did not contain primary research, were not specific to beef cattle, the context was not applicable to North America, or the study was conducted *in vitro.* Articles not written in English and conference abstracts were also excluded. The search protocol was registered in advance of screening (http://hdl.handle.net/1880/113592). Article screening and data extraction were conducted using Covidence systematic review software (Veritas Health Innovation, Melbourne, Australia).

Results

Title and abstracts were screened for 725 studies. 255 studies were assessed via full text. 30 studies met the selection criteria and are undergoing data extraction at the time of submission. Preliminary findings indicate that 33% of included articles were published in the past three years, implying a recent increase of articles addressing antimicrobial-resistant enterococci in beef production. 93% of studies occurred at cow-calf and feedlot operations. Factors were most commonly discussing specific antimicrobial removal or variation (n = 11), raising cattle without antimicrobials (n = 6), and environmental differences between different cattle management systems (n = 4).

Conclusions

There is limited research on enterococci resistance in the abattoir and retail space and a lack of standardization in antimicrobial resistance measurement and reporting. The diversity of study designs and reporting styles may introduce difficulties when comparing studies or performing a meta-analysis.

Financial Support
Government of Alberta

Notes:

V-P013 - Whole-genome sequencing of *Escherichia coli* to characterize antimicrobial resistance within a One Health continuum

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Session: Antimicrobial use/resistance/stewardship

Objective

This study will determine the genetic relatedness of temporospatially restricted generic *Escherichia coli* isolates obtained within a One Health continuum. Further, it aims to identify the chromosomal and mobile genetic elements that confer antimicrobial resistance (AMR) to the isolates, and define the frequency of occurrence and epidemiological patterns of these resistance determinants.

Methods

E. coli isolates are available from routine surveillance projects of feedlot beef and broiler chicken production operations (fecal samples), locally sourced beef and chicken retail meats, post-treatment wastewater and private well water collected in Alberta, Canada from 2018 to 2019. A total of 288 isolates will be selected by stratified random sampling according to phenotypic class-level resistance, and short- and long-read whole genome sequencing will be performed, with the resultant contigs then combined bioinformatically to produce hybrid sequences. Antimicrobial resistance genes (ARGs) will be identified by BLAST search of AMR databases.

Results

Hybrid assemblies will allow resolution of mobile genetic elements (MGEs) such as plasmids, transposons, integrons, and integrative conjugative elements. To assess isolate relatedness, phylogenetic trees will be constructed. Regression analysis with adjustment for clustering will identify associations in the pattern and frequency of resistance elements.

Conclusions

Evaluating AMR transmission within a One Health context requires high-resolution determination of not only resistance genes, but also ARG organization onto MGEs. In this study, temporal and geographical restriction will allow a robust epidemiological comparison of the relatedness and AMR carriage of generic *E. coli* isolates from multiple loci within a One Health continuum. Determination of the structure of identified MGEs through hybrid sequences will address a significant deficit in the literature. Factors identified in this evaluation will inform important AMR mitigation strategies and risk assessment models.

Notes:

V-P014 - Engineered probiotics to deliver antimicrobials targeting the pig pathogen *Streptococcus suis*

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Session: Antimicrobial use/resistance/stewardship

Objective

Streptococcus suis is an important pathogen infecting pigs. The first line of treatment is antibiotics; however, antibiotic resistance is a major global health issue and alternative strategies are lacking. This project aims to engineer a probiotic bacterium to produce and release antimicrobial molecules to target the pig pathogen *Streptococcus suis*

Methods

Using in-house developed tools a library of chimeric lysins (n=24) is constructed. Each lysin will have an unique combination of a peptidoglycan cell wall binding domain (CWB) and lytic domain (LD). In addition, each lysin will encode a short peptide tag to facilitate luminescence in the presence of the luminescent substrate. The library of chimeric lysins will be cloned *Limosilactobacillus reuteri*. To release the recombinant proteins, native prophages of *L. reuteri* will be activated to lyse the engineered probiotic and subsequenlty release the therapeutic molecules. By normalizing the luminescence levels we wil normalize the levels of recombinant lysin. Subsequenlty, filter-sterilized lysates will be assessed for their antimicrobial activity against select *S. suis* strains.

Results

Since the luminescent tagging system had not been used in *L. reuteri*, we first validated its application. As a proof-of-concept, we fused the the luminescent tag to leptin. Leptin concentration, as determined by ELISA, positively correlated with luminescence levels. Next, we cloned the gene encoding the functionally characterized endolysin PlySs under the control of an inducible promoter, which we established in *L. reuteri.*

Conclusions

As of August 2021, we already validated and optimized the application of a luminescent tag in *L. reuteri*. Since the *S. suis* endolysin PlySs is not toxic to *L. reuteri*, we are in the exciting position to generate derivaties of PlySs with different combinations of cell-wall binding and lysin domains whereby we can normalize protein levels by luminescence. These can be tested accordingly for their antimicrobial activity, and offers a foundation to target an important pathogen in hog farming.

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

Notes:

V-P015 - Bovine lactoferrin, a promising therapeutic for avian necrotic enteritis

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Session: Antimicrobial use/resistance/stewardship

Objective

While avian necrotic enteritis (NE) can be effectively controlled by antibiotics, growing concerns about antimicrobial resistance (AMR), a public health risk, makes this treatment option undesirable. Research efforts for effective antibiotic alternatives like bovine lactoferrin (bLF), are increasing. 'Generally Recognized as Safe', bLF is widely available, but little is known about its safety and fate in chickens. Likewise, its ability to counter the $6 billion in annual losses resulting from necrotic enteritis B-like (NetB)-positive *Clostridium perfringens* type A (CP) infections is unknown. Widely known for its multifunctionality, we evaluated the biodistribution of this natural protein in chickens, and tested its efficacy against CP, *in vitro*. We hypothesized that bLF would inhibit CP growth, be safely tolerated by chicks and localized in tissues prone to NE lesions.

Methods

Use of bLF in birds is supported by its shared structural and functional homology with ovotransferrin, an essential defense protein. Its diverse effects have been attributed to lactoferricin B (LFcin B), a 25 aa -amino terminal peptide. Therefore, we evaluated the efficacy of LFcin B (100 µg/mL) against the growth of wildtype CP in Tryptose Sulfite Cycloserine (TSC) broth. Meanwhile, following 5 days of bLF supplementation (250 mg/kg) in 22 healthy 5-week-old specific-pathogen-free White Leghorn chickens, we observed and periodically sacrificed chicks over a 14-d period to evaluate bLF biodistribution using antibody-based assays. Slides were imaged on a Leica scope and staining intensity quantified using ImageJ software.

Results

In addition to evidence of a 1.5-fold decrease in the number of colonies grown, relative expression of the NetB gene was significantly downregulated. Moreover, while bLF was detected in the liver and intestinal villi of supplemented chicks, no signs of adverse effects were observed.

Conclusions

Our preliminary findings suggest bLF will effectively mitigate losses due to subclinical and clinical NE either on its own or as an adjunct therapeutic.

Financial Support
Boehringer Ingelheim Animal Health; Office of Research, College of Veterinary Medicine, Western University of Health Sciences

Notes:

V-P016 - Impact of bovine leukemia virus infection on disease incidence and severity in dairy cattle

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Session: Dairy cattle physiology, immunology or disease

Objective

Enzootic bovine leukosis is caused by bovine leukemia virus (BLV), a type of delta-retrovirus. BLV infects cattle, which leads to decreased milk production, longevity, and immune system dysfunction. An estimated 46% of all dairy cattle in the U.S. are estimated to be infected with BLV. This study aims to determine the impact BLV has on predisposing dairy cattle to common diseases by; 1) Determining the effect of BLV infection status has on host responses to experimentally infected *E. coli* 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, 24 Holstein dairy cattle will be enrolled based on BLV status. Animals will be experimentally infected with *E. coli* mastitis and immune system markers will be measured. For Objective 2, dairy cattle from commercial dairy farms will be enrolled in cohorts with calving dates 60-67 days from the first sample collection date. A total of 1063 animals have been enrolled from each of 8 farms (~130/farm). Animals will be tested for BLV status and monitored for disease incidence over a lactation cycle.

Results

For Objective 2 in the first 2 cohorts, 88 animals were enrolled at a single farm. A total of 5 animals sero-converted between enrollment and 60 days post 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 around calving, mean lymphocyte counts for BLV+ animals became non-significant from BLV- animals ($p>0.05$). Further BLV ELISA data and disease incidence will be added when animals have completed the observed lactation cycle.

Conclusions

Lymphocytes counts were significantly higher in BLV+ animals at dry-off. Changes in PVL and lymphocyte counts may be influenced by stress associated with dry-off and/or calving. The observed decrease in lymphocyte counts between dry-off and post-calving in BLV+ cows may also be explained by lymphocyte trafficking for colostrum production. ELISA status remained consistent over the lactation cycle and remains an essential tool in BLV infection status determination.

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

Notes:

V-P017 - Development and characterization of poultry-specific immune reagents and immunoassays

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Session: Diagnostic testing/disease modeling/precision agriculture

Objective

Limited availability of immune tools for poultry immunology research hinders progress in developing vaccines and novel immunotherapeutics for poultry species. In this report, progress of the NIFA-funded grant on poultry immune reagent development to identify chicken cytokines and chemokines and their function will be presented.

Methods

Thirteen chicken cytokines and chemokines were identified, their proteins expressed in bacterial and mammalian expression systems and mouse monoclonal antibodies (mAbs) against them were generated anc characterized using Western blots, ELISA, immunocytochemistry, qPCR and cell proliferation. During the third-year grant period, total 65 new mAbs have been developed and their specificities were validated using several assays including ELISA, immunohistochemistry, Western blot, flow cytometry, qPCR, and cell proliferation.

Results

All the target molecules that we selected have shown to have critical functions in host defense against pathogens and these include 13 cytokines (interleukin-4, 7, 10, 12, 13, 17F, 21, 22, 23, 26, 34, IFN-alpha, IFN-kappa, TNF-alpha, CSF-1, and TGF-beta) and 3 chemokines (CXCLi2, CCL4 and 5). Functional similarity and dissimilarity were discovered between the immune molecules of poultry and mammals although most functional aspects of these immune molecules have been preserved through evolution. Antigen-capture ELISAs were developed using a pair of capture and detecting mAbs for each target proteins. All the target molecules that we characterized showed functional similarity to mammals and exerted critical functions in host innate and adaptive immune responses.

Conclusions

These new poultry immune reagents will be useful for analyzing host immune response to vaccines and antibiotic alternatives, and new antigen capture ELISAs for chicken cytokines and chemokines will facilitate the discovery phase of their roles in host immune response against various infectious diseases of poultry

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

Notes:

V-P018 - Animal disease spread models can be improved by including human behavior

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Session: Diagnostic testing/disease modeling/precision agriculture

Objective

Human behavior and decision-making affect the spread of animal diseases and their economic impacts. Our research sought to parameterize and simulate the effects of human behavior on disease transmission in the swine industry. We were especially interested in how information and message construction affect decision-making.

Methods

The team deployed experimental games designed to collect data on decision-making in simulated disease outbreak scenarios. We explored compliance at the operational level in one set of scenarios. In another set of scenarios, we explored decision-making to invest in biosecurity at the tactical level. Post-game surveys collected additional information on risk perceptions. We recruited participants from the university community, the swine industry, and an online workforce, Amazon Mechanical Turks. We then integrated epidemiologic information, human risk perception, and socio-economic influences into an agent-based model to simulate how human behavior can affect disease spread. This work was supported by the USDA National Institute of Food and Agriculture, under award number 2015-69004-23273.

Results

Graphical information led to better compliance compared to numeric or worded messages. Knowing disease incidence of nearby farms led to greater investment in biosecurity, whereas knowing the level of biosecurity adoption nearby did not. We did not find a difference in decision-making between industry “insiders” and other participants. The distribution of behaviors and their change over time was then incorporated into agent-based models, with results mirroring the trends seen in PEDv incidence over time.

Conclusions

By knowing what information and types of messages lead to better compliance with biosecurity protocols and greater investment in biosecurity, we can put a damper on disease spread. Encoding this information within agent-based models allows us to explore the degree of change we can expect across a livestock system. This approach can be applied to other livestock and crop production systems to study how best to improve biosecurity and protect health.

Financial Support
US Department of Agriculture-National Institute of Food and Agriculture

Notes:

V-P019 - MicroRNA profiling in *Mycobacterium avium* subsp. *paratuberculosis*-infected cattle at different stages of infection

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Session: Diagnostic testing/disease modeling/precision agriculture

Objective

Mycobacterium avium subsp. *paratuberculosis* (MAP) is the causal agent of Johne's disease (JD), and it causes diarrhea and weakness in cattle, finally leading to death. During a long subclinical stage, infected animals without clinical signs shed pathogens through feces, causing infections in the cohabitation. Therefore, the diagnosis of JD during a subclinical stage is very important, but it is difficult to diagnose with current diagnostic methods. Circulating miRNAs draw attention as useful biomarkers in various veterinary diseases because of their stability and features that their expression changes according to the state of the disease. Based on current knowledge, circulating miRNAs extracted from bovine serum were used to develop a diagnostic tool of JD.

Methods

Experimental animals were divided into 4 groups based on fecal shedding, the presence of antibody, and clinical signs. Gene expression was analyzed by miRNA-sequencing for each group and selected biomarker candidates were validated by qRT-PCR. We also used Ingenuity Pathway Analysis for network analysis and showed the relationship between differentially expressed miRNAs and predicted genes.

Results

Eight miRNAs commonly found in each group were selected as biomarker candidates based on significant differences in the expressing miRNAs. We then validated it via qRT-PCR, and the results were two upregulated miRNAs (bta-miR-374b, bta-miR-2887) and two downregulated miRNA (bta-miR-147, bta-miR-346) showed the same trend. Network analysis showed the immune response that appears on subclinical stage with molecules related to the Tregs in the center of the network.

Conclusions

Taken together, our data suggest that two miRNAs (bta-miR-374b, bta-miR-2887) play major roles in the immune response to MAP infection during the subclinical stage.

Financial Support
BK21 FOUR Future Veterinary Medicine Leading Education and Research Center; The Strategic Initiative for Microbiomes in Agriculture and Food

Notes:

V-P020 - The novel diagnostic platform for *Peste des Petits Ruminants* (PPR) in Georgia

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Session: Diagnostic testing/disease modeling/precision agriculture

Objective

Peste des petits ruminants (PPR) is a highly contagious and often fatal disease of small ruminants with significant economic, food security, and livelihood impacts. The disease is caused by a morbillivirus (PPRv) closely related to the rinderpest virus. PPR is considered one of the topmost damaging animal diseases of small domestic ruminants. Sheep meat comprises a significant proportion of the global food supply. Thus, PPR poses a severe threat to the small ruminant industry, particularly to developing countries, including the Republic of Georgia. There are four distinct lineages (I–IV) of PPRv that have been recognized based on partial sequences of the F, N, and H genes. PPRv detected in Georgia in February 2016 belonged to lineage IV.

Methods

Considering this scenario, SLA developed a novel diagnostic platform for the early detection of PPRv. The rapid detection assay is based on RT-qPCR previously developed (Batten et al. 2011) The TaqMan-based assay targets PPRv N gene using forward primer 5'-AGAGTTCAATATGTTRTTAGCCTCCAT-3', reverse primer 5'-TTCCCCARTCACTCTYCTTTGT-3' and FAM-5-CACCGGAYACKGCAGCTGACTCAGAA-TAMRA probe and uses the qScript™ XLT One-Step RT-qPCR Tough Mix kit (QuantaBio, MA, USA).

Results

In this study, we selected twenty PPRv-positive field samples together with proficiency test (PT) samples (PT organized by EU Reference Laboratory for Peste des Petits Ruminants, CIRAD, France*)*. The assays were run on an ABI7500-Fast platform under the following cycling conditions: RT step at 50ºC for 10 min, followed by a hold step at 95ºC for 1 min, and 45 cycles at 95ºC for 15 min and 60ºC for 60 sec. The results of 20 selected samples were positive, as expected.

Conclusions

The novel diagnostic platform established at SLA allows us to expand our detection capacity by identifying lineage III PPRV.

Financial Support
U.S. Defense Threat Reduction Agency

Notes:

V-P021 - Determining the correlation between blood and saliva glucose levels and saliva pH levels in veterinary patients

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Session: Diagnostic testing/disease modeling/precision agriculture

Objective

Determining glucose levels in small and large animal patients currently requires venipuncture to obtain blood to use in a glucometer or to run a full panel of bloodwork. Because of this, most canine and feline diabetic patients must be routinely brought to the vet to have glucose levels checked. When it comes to equine patients, nutritional status and oral health can be assessed using glucose levels, but obtaining blood is not always practical, especially in field situations. In both small and large animal patients, salivary pH levels can be a good indicator of oral health and could correlate to the glucose levels in the saliva. The aim of this study was to determine if a correlation exists between blood and salivary glucose levels and saliva pH levels in hopes of developing an alternative, less invasive method of obtaining glucose levels in veterinary patients.

Methods

Blood was drawn from patients that presented to Tuskegee University Veterinary Teaching Hospital to check glucose levels with a glucometer, a pH indicator strip was used to determine the saliva pH, and saliva was collected to be used in the glucose assay.

Results

There was no correlation determined between blood and saliva glucose levels or saliva pH levels, but factors such as hypersalivation and stress were associated with decreased and increased saliva glucose levels, respectfully.

Conclusions

Although no correlation was determined in this study, further research in a more controlled environment could yield a correlation.

Financial Support
Boehringer Ingelheim Animal Health; The Health and Human Services Center of Excellence (HHS COE) Veterinary Scholar Program (COE-VSP), Tuskegee Veterinary Scholars Program

Notes:

V-P022 - Enhancing field detection tools for bovine tuberculosis using mycobacterial-specific biomarkers

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Session: Diagnostic testing/disease modeling/precision agriculture

Objective

Discovery and validation of pathogen-specific-biomarkers (Pks5, MB2515c and MB1895c) for bovine tuberculosis in multiple animal species has opened various avenues for disease control. One method is to improve the stringency of disease detection in the field. Our work focuses on utilization of these biomarkers as a target for disease detection with the help of highly specific DNA aptamers.

Methods

Short segments of the biomarkers were recombinantly expressed and were used as targets for DNA aptamer selection via sequential salt elution following one-step aptamer selection. The selected aptamer pool was amplified, TA cloned and then Sanger sequenced to identify redundant candidates predicted to be stable by mFold. Once selected, these aptamers were checked for specificity against the biomarkers Pks5, MB2515c and MB1895c using a southwestern blot.

Results

We expressed biomarker-peptides MB2515c and PKs5. These two were then used for aptamer selection. A total of five aptamer sequences qualified for specificity testing, and one anti-MB2515c aptamer sequence was confirmed to bind our target.

Conclusions

Selected DNA aptamers will be validated with infected serum samples before development of a prototype lateral flow assay. The assay will significantly support the disease control program through enhanced detection of infected animals.

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

Notes:

V-P023 - Inflammation and bile acid composition determine *C. perfringens*-induced enteritis in mice

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Session: Diagnostic testing/disease modeling/precision agriculture

Objective

Clostridium perfringens is a prevalent foodborne bacterial pathogen responsible for human enteritis and chicken necrotic enteritis with limited mechanism understanding. *C. perfringens* and coccidia infection easily recapitulates chicken necrotic enteritis, while no reliable animal model is available to mimic *C. perfringens*-induced human enteritis. In this study, we aimed to investigate the pathogenesis of *C. perfringens*-induced human enteritis.

Methods

At day -14 before infection, BL6 wild type (WT) and *Il10*$^{-/-}$ mice were fed with Chenodeoxycholic acid (CDCA) diet (0 or 1.5 g/kg). At day -2 before infection, the mice were subcutaneously injected with a single dose of 7 mg/kg indomethacin or PBS control. The mice were then infected with *C. perfringens* at 10^9 CFU/mouse or PBS at days 0 and 1. The mice were euthanized at day 2. Small intestinal tissue and content were collected for histopathology, molecular biology analysis, and bile acid quantification by HPLC/MS-MS.

Results

The results showed that CDCA diet increased this bile level in the small intestine content and reduced cholic acid level. *C. perfringens* infection alone failed to induce intestinal inflammation in mice fed either basal or CDCA diet. Interestingly, indomethacin induced more intestinal inflammation in *Il10*$^{-/-}$ mice fed CDCA diet compared to WT mice fed CDCA diet, while indomethacin induced mild intestinal inflammation in WT and *Il10*$^{-/-}$ mice fed basal diet. Notably, *C. perfringens* induced very severe enteritis in small intestine of *Il10*$^{-/-}$ mice exposed to indomethacin and CDCA diet compared to WT mice with indomethacin and CDCA diet, while the pathogen induced mild intestinal inflammation in WT and *Il10*$^{-/-}$ mice with indomethacin and basal diet. The severe intestinal inflammation showed as massive immune cell infiltration, blunted villi, and crypt hyperplasia and fusion.

Conclusions

Together, these results showed that we have developed a *C. perfringens*-induced human enteritis model using BL6 WT and *Il10*$^{-/-}$ mice. Preexisting intestinal inflammation and CDCA dominance are the key elements for driving *C. perfringens*-induced enteritis.

Financial Support
Arkansas Biosciences Institute; USDA National Institute of Food and Agriculture (NIFA) Hatch project 1012366, NIFA Hatch/Multi State project 1018699, NIFA SAS 2019-69012-29905, NIFA project 2020-67016-31346 to Xiaolun Sun; Poultry Federation Scholarship to Ying Fu

Notes:

V-P024 - Artificial intelligence algorithms to predict estrus in Holstein cattle across genetics and environments

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Session: Diagnostic testing/disease modeling/precision agriculture

Objective

The profitability of dairy cattle herds is impacted by the capability of the cows to return to estrus promptly after calving. Cyclicity is influenced by many genes of small effect in addition to non-genetic factors. Despite the challenges to detecting estrus and the commonly used practice of timed artificial insemination, estrus detection remains elusive. The objective of this study was to investigate the capability of artificial intelligence approaches to improve estrus detection.

Methods

Support vector machine and random forest algorithms were used to develop an estrus prediction tool. A support vector machine identifies a hyperplane that accurately separates estrus outcomes, and the combination of input weights in the plane is used to predict estrus in additional cows. Random forest uses a combination of multiple decision trees, aiming to predict estrus and strengthening the final prediction. Records on the detection of estrous cyclicity at 50 days post-calving from approximately 2500 Holstein cows across multiple U.S. regions, states, herds, and seasons were studied. The genotype of more than 770,000 single nucleotide polymorphisms (SNPs) was available.

Results

Prediction factors included region, state, farm, season, and SNPs associated with estrus at $0.01 < \text{P-value} < 0.000001$. The accuracy of the support vector machine and random forest prediction approached 100% in the training dataset and 77% and 67% in the test dataset. With the SNPs decreasing in number and increasing in association, the accuracy of the random forest did not change substantially, yet the accuracy of the support vector machine stabilized after 400 SNPs. Investigation of variable weights identified SNPs in chromosome 17 contributing to the prediction performance.

Conclusions

Our results suggest that artificial intelligence algorithms are useful tools for estrus detection. Consideration of additional physiological indicators such as parity may further improve the predictor. The support of USDA-NIFA-AFRI-003542 and the University of Illinois at Urbana-Champaign Center for Digital Agriculture is appreciated.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture; Center for Digital Agriculture, University of Illinois at Urbana-Champaign

Notes:

V-P025 - Comparison of DNA extraction methods for *Mycoplasma* detection testing in veterinary biologics

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Session: Diagnostic testing/disease modeling/precision agriculture

Objective

Current detection methods for *Mycoplasma* contamination in veterinary biological products are culture- or nucleic acid-based assays. The PCR detection has advantages in decreased testing time but requires broth enrichment for adequate sensitivity. Our objective was to compare PCR sensitivity and robustness in *Mycoplasma* detection using an automated extraction (AE) versus a manual extraction (ME) with a spin-column kit.

Methods

Three species were evaluated: *Mycoplasma (M.) hyorhinis, Acholeplasma (A.) laidlawii*, and *M. orale*. Each species underwent 10-fold serial dilutions in Mycoplasma broth diluent. For ME, a Qiagen QIAamp Fast DNA Stool Mini Kit was utilized. The Promega RSC Whole Blood DNA Kit was used for AE. Technicians extracted 0.5mL of the serially diluted samples for each evaluated dilution and subjected it to Touchdown PCR and electrophoresis. Each experimental set was conducted by three technicians in multiple replicates. Robustness was calculated as the percent of contaminated experimental sets by method. For sensitivity, a liberal and conservative limit of detection (LOD) were assessed for each technician by species for all experimental sets without contamination. A liberal LOD required one or more replicates to amplify, whereas the conservative LOD required all replicates to amplify.

Results

The AE process had 100% non-contaminated experimental sets (n=12) across all technicians while the ME resulted in 54.5% contamination of the experimental sets (n=11). In all cases, AE resulted in at least 3-log lower LOD than ME for all species evaluated for the liberal analysis. The conservative analysis was similar with a 2-log lower LOD for AE compared to the ME for all species evaluated.

Conclusions

Based on the robustness and sensitivity differences between AE and ME, CVB's Protocol 1006, Polymerase Chain Reaction for the Detection of *Mycoplasma* Contamination, will be revised to incorporate AE usage for detecting *Mycoplasma* in the future. These preliminary studies will be supplemented with additional data.

Notes:

V-P026 - X-ray crystallography at GM/CA@APS assisting in the fight against pandemics and infectious diseases

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Session: Diagnostic testing/disease modeling/precision agriculture

Objective

The National Institute of General Medical Sciences and National Cancer Institutes' structural biology facility at the Advanced Photon Source (GM/CA@APS) operates a national user facility for crystallographic structure determination of biological macromolecules, including two undulator beamlines (23ID-B and 23ID-D), with an emphasis on challenging, high-impact projects.

Methods

GM/CA beamlines provide stable, intense X-ray beams of user-selectable size down to 5-micron diameter, an intuitive user interface for experiment control, and an automated pipeline for data processing. Developments at GM/CA@APS have helped to transform modern structural biology. Major GM/CA@APS contributions include development of micro-crystallography capabilities that have enabled experiments with projects that might otherwise seem impossible.

Results

For example, structures of numerous membrane proteins have been determined, such as for G protein-coupled receptors (GPCRs), where crystals are typically grown in lipidic cubic phase and are small, fragile, and optically invisible. Many targeted structures relate to immunology and infectious diseases, such as COVID-19, influenza, HIV, Ebola, and Zika. This poster provides a current overview of GM/CA@APS beamline capabilities, along with a display of selected scientific highlights from beamline users.

Conclusions

Scientists working with One Health diseases are encouraged to pursue structural-biology efforts and to apply for beam time with GM/CA@APS (https://www.gmca.aps.anl.gov).

Financial Support
U.S. National Institute of General Medical Sciences; U.S. National Institutes of Health; Acknowledgments: GM/CA@APS has been funded in whole or in part with Federal funds from the National Cancer Institute (ACB-12002) and the National Institute of General Medical Sciences (AGM-12006). This research used resources of the Advanced Photon Source, a U.S. Department of Energy (DOE) Office of Science User Facility operated for the DOE Office of Science by Argonne National Laboratory under Contract No. DE-AC02-06CH11357. The Eiger 16M detector was funded by an NIH–Office of Research Infra

Notes:

V-P027 - Presence of brucellosis among dogs in the Republic of Armenia

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Session: Epidemiology and/or public health

Objective

Brucellosis is endemic in Armenia. The test-slaughtering method is used in the fight against Brucellosis of large and small ruminants. Cross contamination among the various types of animals with various types of the causative agent may be contributing to the permanent existence of the epizootic chain. The objective of the study is to determine the presence of brucellosis among the stray dogs in Yerevan (Capital of RA).

Methods

We collected 384 blood samples from stray dogs in Yerevan and performed the Rose Bengal test (RBT). Positive samples by RBT were then tested by serum agglutination test (SAT) and two types of ELISA, indirect and competitive. The competitive ELISA detects only the specific antibodies against *B. abortus, B. melitensis, B. suis* and differentiates the strain of *Brucella* 19. Confirmation of brucellosis requires samples must be positive by RBT followed by positive results by SAT or ELISA.

Results

Out of 384 samples tested by RBT, 17 (4.4%) were positive. Of these 17 samples, 9 samples (52.9%) tested positive by SAT, which comprises 2.3% of the total samples, 7 samples (41.1%) tested positive by indirect ELISA, which comprises 1.8% of the total samples, and 14 samples (82.3%) tested positive by competitive ELISA, which comprises 3.6% of the total samples.

Conclusions

We have shown the presence of *Brucella* spp. among stray dogs in Yerevan. We feel it is necessary to implement additional studies among dog (particularly shepherds dogs) populations in marzes with higher prevalence of brucellosis. These additional results will provide an opportunity to evaluate the role of the dogs in assuring a stable epizootic chain of brucellosis among the agricultural animals and will contribute to a design strategy for preventing the spread of disease.

Notes:

V-P028 - Seroprevalence, spatial distribution and risk factors of *Francisella tularensis* in Georgia and Jordan

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Session: Epidemiology and/or public health

Objective

Francisella tularensis is a gram-negative intracellular coccobacillus that multiplies in macrophages. Two of the four subspecies of *F. tularensis;* namely *tularensis* and *holarctica,* are disease agents for humans. The pathogen survives for months in soil, water and dead animals but is easily killed by heat and water chlorination. *F. tularensis* has a broad spectrum range of hosts including birds, domestic and wild mammals and can be transmitted by several species of ticks, flies and mosquitoes.
The disease is considered to be endemic for Georgia within a few focal areas. Small and large outbreaks are periodically reported (*Velidjanashvili I, 1992;* Sakvarelidze LA, *et al.*, 1983; Chitadze N, et al, 2009). There are no data on the seroprevalence of *Francisella tularensis* in Jordan.
The goal of this study was to determine the seroprevalence, spatial distribution, and risk factors of *F. tularensis* in Georgia and Jordan population

Methods

A total of 300 (150 – target population; 150- control group) Georgian and 900 (203 – target population; 697 - control group) Jordanians were serologically tested for *F. tularensis* by enzyme linked immunosorbent assay. Additionally, 506 (cattle – 314, Sheep – 98, Goat – 54, Dog - 40) and 730 (Cattle – 305, Sheep – 260, Goat – 95, Camel – 45, Horse - 25) animal samples from Georgia and Jordan, respectively were collected and tested. Veterinarians and farmers from both countries filled out a self-administered questionnaire to collect demographic and risk factor information. Bivariate and multivariate logistic regressions were performed to determine which variables associate with seropositivity.

Results

The overall seroprevalence of *F. tularensis* was 12.7% (15/110) from farmer group and 10% (4/40) from veterinary group and none of the control group individuals were positive from Georgia; Out of 506 animal samples, 4.3% (22/506) were positive on *F. tularensis* Ab. In Jordan, 9.8% (20/203) from the target population and 6.5% (46/697) from the general population were seropositive on tularemia; 3.7% (27/730) of animal samples turned to be positive on tularemia Ab.

Conclusions

This is the first study to address the seroprevalence of *F. tularensis* in Georgia, Jordan among veterinarians and farmers. It provides a first look at the potential exposure of animal workers and the general population to zoonotic diseases. Veterinarians and farmers are at increased risk of tularemia infections.

Financial Support
U.S. Defense Threat Reduction Agency

Notes:

V-P029 - Managing bovine leukemia virus by integrating surveillance of young stock and whole herd scans

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Session: Epidemiology and/or public health

Objective

Our research strives to improve the sustainability of the US dairy industry by developing and testing methods to reduce the prevalence of Bovine Leukemia Virus (BLV). It was previously believed that BLV primarily affected older cows, but field trial data indicated that first lactation cows were infected with BLV. Our study will aid in identifying when BLV infections occur among young stock while continuing to identify and manage infected cows in the milking herd.

Methods

Dairy farms will be surveyed to learn calf and cow management protocols before enrollment into the study. Blood samples will be collected from female neonates (24 hours to 8 days in age), then, one year in age, first pregnancy check, and at 60 days in milk for each lactation. Following collection, blood samples will be tested for BLV antibodies (via ELISA) and provirus (via qPCR). This longitudinal profiling will provide evidence of BLV infection dynamics within infected herds. In tandem with profiling, the milking herd will be screened for BLV every other year. Milk samples will be collected and tested for BLV antibodies. Blood samples will then be collected from cows with BLV antibodies to quantify provirus. Summarized results will be conveyed to producers to use for mitigation strategies.

Results

Currently, five Michigan farms are enrolled in the study. Three farms milk >500 cows with a 38% (± 6.98) average BLV prevalence and two farms milk <500 cows with a 55.5% (± 0.77) average BLV prevalence. Seven months since the commencement of the project, 171 female neonates have been sampled and therefore enrolled in the project to be profiled throughout their life span. Of those, 33.33% have BLV antibodies and 1 calf has detectable provirus. The first round of screening the milking herds on all 5 farms is planned for completion by January 2022.

Conclusions

Our integrated approach of tracking BLV incidence and managing cows already infected is novel and will give producers an integrated approach to managing BLV. This will work to improve animal welfare and work towards a more sustainable industry.

Financial Support
U.S. Department of Agriculture

Notes:

V-P030 - Risk behaviors and compliance with COVID-19 guidelines among university students and personnel

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Session: Epidemiology and/or public health

Objective

The purpose of this study is to characterize the association of risk behaviors with COVID-19 test-positivity and vaccination status among students, faculty, and staff at the University of Georgia (UGA). By improving our understanding of compliance with university COVID-19 guidelines, this study also aims to identify targets for educational campaigns and preventive measures to decrease SARS-CoV-2 transmission among the university community.

Methods

Students and personnel at UGA who had been tested for COVID-19 in the previous 14 days were invited to complete an anonymous online survey. Participants were asked about demographic information, COVID-19 test results, vaccination status, behaviors on- and off-campus, and compliance with recommended preventive measures.

Results

Between April 14 and October 13, 2021, 84 individuals who were eligible for participation completed the survey. Their roles at the university included faculty (20%); staff (24%); graduate (19%), professional (10%), and undergraduate (20%) students. No positive COVID-19 test results were reported, and 92% of participants had received at least one dose of a SARS-CoV-2 vaccine. The most commonly reported risk behaviors included dining outdoors or utilizing pickup services at an off-campus restaurant, attending social gatherings of ≤ 10 people, and shopping at a grocery store or large retail location. The frequency with which respondents reported attending social gatherings of > 50 people increased after August 1, 2021 (23%) compared to previous months (6%). Self-reported compliance with COVID-19 prevention practices was higher on the UGA campus (mean: 95%; range: 0-100%) than off-campus (mean: 87%, range: 41-100%).

Conclusions

These preliminary results suggest that education efforts concerning the prevention of SARS-CoV-2 transmission at UGA have been successful, particularly in on-campus settings. Moving forward, this survey will provide a means to continue to monitor compliance with COVID-19 guidelines, risk factors for test-positivity, and incidence of vaccine breakthrough cases among the university community.

Notes:

V-P031 - Transport and trophic effects of animal movement on gastrointestinal nematode infection

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Session: Epidemiology and/or public health

Objective

The ecological effects of mobile hosts on resident animals in the habitats they traverse typically manifest in two ways: via transport of parasites, and trophic modification of resources in ways that may constrain parasite dissemination. The research outlined in this presentation will use an integrated framework to link transport and trophic effects with movement intensity and duration to identify and test fundamental predictions about the impact of animal movement on gastronintestinal nematode (GIN) transmission dynamics. We hypothesize that movement of migrants through resident habitats increases GIN intensity in resident species and the strength of this effect is modified by the intensity and duration of the movement.

Methods

We are conducting a field study in Serengeti National park, Tanzania, focusing on wildebeest as migratory nematode vectors, and both wild and domestic ungulates as resident hosts. We will quantify GIN density in the environment and GIN intensity and prevalence in wildebeest and resident species before/after wildebeest migration. We will capture six migration events over three years to generate data on a range of movement intensity-duration combinations. Following each migration event, we will use wildebeest intensity and duration as covariates in our analyses of GIN density. We will map wildebeest occupancy using an established camera-trap grid with 200 active cameras. For fecal sampling, we will visit camera trap sites following a rotation that maximizes our ability to sample sites varying in terms of expected WB intensity and duration during the migration. For pasture L3 sampling, we will use a randomly-selected subset of camera sites to assess changes in GIN density in the environment. We will also collect continuous data on a number of key environmental covariates at a subset of 100 randomly selected camera trap sites.

Results

We describe work done to date on site selection and the establishment of our camera grod and environmental sensor network.

Conclusions

None at present.

Notes:

V-P032 - Prevalence of *Histophilus somni* in Spain

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Session: Epidemiology and/or public health

Objective

The aim of this study was to evaluate the prevalence of *H. somni* as a pathogen involved within the BRD complex in the Spanish national herd, along with comparing results obtained by culture versus qPCR.

Methods

The presence of *H. somni* was assessed in 1,396 samples from animals with suspected BRD from January 2017 to January 2020, representing 346 Spanish farms. Samples were passively obtained by a private diagnostic laboratory (EXOPOL) with the aim of determining the causal agents at the time of a BRD problem. All the samples were individually tested by culture. Nevertheless, qPCR was run with a commercial kit on pools of up to 5 samples from the same farm.

Results

When both techniques were compared, bacterial culture failed to detect 73.0% of the positive samples identified by qPCR. Consequently, the qPCR results were selected for evaluation of the prevalence of *H. somni* which in this study was 23.0%, whilst differences were observed over the 3 years (18.4%, 17.8% and 28.9% prevalence was detected in 2017, 2018 and 2019, respectively). When analysed by the production system, 26.4%, 20.2% and 9.3% of the samples were positive in feedlots, veal units and adult cattle, respectively. With regard to the type of sample, the highest detection rate was found in tracheal scrapes (33.3%), followed by bronchoalveolar lavages (30.2%), organs (19.3%) and finally 17.8% in the case of respiratory tract swabs.

Conclusions

The difference in detection of *Histophilus somni* between culture and qPCR is likely associated with the limitation of growing these fastidious bacteria and common practices implemented in the field such as the use of therapeutic antibiotics. The results of this study confirm the use of qPCR assays for improving the diagnosis of bacteria and can be considered to be representative of Spanish geography, detecting a 23.0% prevalence of *H. somni*. These results highlight the importance of including measures for *Histophilus somni* prevention on Spanish cattle farms such as vaccination programmes, together with measures against other *Pasteurellaceae* and viruses, to minimise the impact of BRD.

Notes:

V-P033 - The evolutionary ecology of pathogen emergence via cross-species transmission in the avian-equine influenza system

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Session: Epidemiology and/or public health

Objective

Viral emergence poses a constant threat to humans and animals and we are neither able to predict which viruses will emerge, nor where, when, or which populations will be affected. The overall aim of this project is to determine how environmental, host, and virus factors influence host-pathogen interactions and transmission dynamics of potentially emerging viruses. Avian influenza viruses (AIVs) provide unique opportunities to address this because they have jumped into humans, dogs, pigs and horses, with significant consequences on public health, food security, and the global economy. We will focus on the interspecies transmission and emergence of AIVs to horses because AIV strains have emerged in horse populations on independent occasions.

Methods

We propose to perform field work in a well-defined ecosystem that favors avian-to-horse AIV transmission and also to perform laboratory experiments using avian and equine influenza viruses with different levels of "equine fitness" - ability to infect and transmit in horses. Our laboratory experiments will use genetic engineering to capture changes in fitness due to virus evolution. Results obtained will be combined in a mathematical framework that will enable the estimation of risk of viral emergence, including the effects of herd immunity.

Results

Results from the first field campaign show promising variation in influenza infection in horses which is related to animal age and proximity to sources of AIVs.

Conclusions

This multidisciplinary research will provide new insights on the mechanisms that underpin viral emergence and will aid the design of more effective intervention measures to control future events of viral emergence.

Financial Support
USDA-NIFA; BBSRC

Notes:

V-P034 - A cross sectional survey of helminth parasites of North American bison in the central United States: 2021 update

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Session: Epidemiology and/or public health

Objective

Bison bison bison host a wide array of internal parasites. It is estimated that up to 5% of bison deaths in the US are a result of parasitic infections. There are few published studies discussing the parasites of bison residing in the United States over the last 20 years.
The purpose of this study is to investigate the diversity of internal parasites in North American bison using a variety of parasitological techniques.

Methods

In this ongoing study, bison herds belonging to National Bison Association producers and some national park herds were recruited by phone and/or email from August 2021. Fresh fecal samples were collected and shipped to us by participating producers. Anthelminthic usage was also obtained to study the efficacy of anthelmintics in the recruited bison herds.
Each sample was quantitatively analysed to obtain the trichostrongyle eggs and Eimeria oocysts per gram of feces using the Mini-Flotac technique with a sensitivity of 5 eggs per gram (epg) and the McMaster test in triplicate with a sensitivity of 33.3 epg. The presence of lungworm larvae and fluke eggs were assessed using a baerman test and sedimentation respectively.

Results

We present parasitological data from herds distributed among the plains states. We compare quantitative data from McMaster and Mini-Flotac techniques. We present the fecal egg count reduction in herds that used anthelmintics.

Conclusions

This will provide information needed to fill the knowledge gap in the epidemiological understanding of the effects of bison parasites in the United States.

Financial Support
South Dakota State University; Center of Excellence for Bison Studies at South Dakota State University

Notes:

V-P036 - African swine fever surveillance: Challenges and opportunities of non-invasive sampling of warthogs

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Session: Epidemiology and/or public health

Objective

Non-invasive sampling of wildlife has become an viable means of confirming disease status in an area. It has been used with success in wild boars in the northern hemisphere, however, te utility of these methods for assessming African swine fever (ASF) status of vertebrate sylvatic cycle hosts in sub-Saharan Africa has not been explored. As it represents a potentially powerful means of determining the ASF status of warthogs, we undertook a preliminary assessment of rope bait sampling in South Africa.

Methods

A range of rope baits were assessed, namely (i) pig feed wax baits, (ii) pig feed wax baits infused with truffle oil, (iii) cotton ball baits with a molasses attractant, (iv) cotton ball baits with a fermented yeast attractant and (v) traditional maize baits. These were deployed in both natural and transformed settings, targeting wild and habituated warthog, respectively. All interactions with the baits were monitored using camera traps deployed at the baiting sites.

Results

Our results indicate that warthogs in natural areas interact the least with the baits, whereas interaction with baits occurs readily at transformed sites, particularly if non-target wildlife species such as baboon display an interest in the baits. The warthogs most likely to interact with and consume the baits are those that are habituated to areas where access to feed occurs on a regular basis. Non-target species interacting with the baits differed between the different baiting environments.

Conclusions

Despite the limited success with oral baits in natural areas, rope baits hold potential for sampling warthogs at transformed sites where access to feed is consistent. This suggests that with additional refinement tope baits may provide a cost-effective alternative to warthog surveillance efforts that traditionally relied on immobilization for sampling, particularly in those areas where an active wildlife-domestic interface exists and the risk of ASF transmission is highest.

Financial Support
U.S. Department of Agriculture

Notes:

V-P037 - Suitability modelling of Ornithodoros spp. in California to identify ASFV high-risk areas with climate variation

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Session: Epidemiology and/or public health

Objective

Emerging from international trade routes, African swine fever virus (ASFV) is a risk for global animal health, leading to devastating economic consequences. In the United States (U.S.) ASFV could be transmitted either directly between Sus scrofa (domestic, feral, and wild swine) or by soft ticks of the genus Ornithodoros. Several species of Ornithodoros are present in California, thus, knowledge of vector-host spatial co-occurrence is necessary in order to mitigate the spatial risk of ASFV establishment in the U.S.

Methods

Recorded presence of Ornithodoros spp. was retrieved from literature searches, the GBIF network database and records from the Essig and the Bohart Entomology Museum collections. Maximum entropy species distribution modeling (Maxent) was used to predict the current distribution of Ornithodoros spp. based on current climatic trends and future projected climate changes. Swine suitability data and GIS analyses of their distributions were used to ascertain the spatial co-occurrence of hosts and tick vectors.

Results

The results predicted previously unrecognized areas of California with suitable habitat for Ornithodoros spp. and identifies high-risk areas for ASFV, where the suitable habitat of pigs and the tick vector overlap. Moreover, global climate models predict an increase in the distribution of suitable habitat in the Costal and Valley regions of California for Ornithodoros spp., with an increased associated risk.

Conclusions

Our predictions are useful to support risk-based decision-making for targeting surveillance efforts for the genus Ornithodoros in areas of high risk in California, increasing the efficiency and effectiveness of public health investigations.

Notes:

V-P038 - Genetics of retroactive measures of stress response and relationships with disease resilience in nursery and grow-finish pigs

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Session: Host or pathogen genomics or proteomics

Objective
Infectious disease and other external stressors are large cost components in pork production and are detrimental to animal welfare. Resilience is defined as the ability of an animal to perform and thrive despite exposure to stressors (infectious and/or non-infectious) and has been shown to have an important genetic component. However, selection for improved resilience is hampered by lack of phenotypes that can be collected on selection candidates in breeding populations, which must be kept in facilities with high biosecurity. In ongoing research we have developed methods to identify pigs that are more resilient to disease in biosecure environments. Extensive phenotypes have been collected on 3500 pigs exposed to a polymicrobial natural disease challenge. These include body weights, daily feed and water intake, and mortality and morbidity, as well as immune assays and -omics data prior to entry into the challenge. The objective here is to capitalize on the natural challenge data and samples by evaluating response to stress in a retrospective manner based on cortisol and DHEA in hair, with the overall goal to develop and refine indicators of resilience that can be collected on pigs in commercial farms and/or in nucleus herds. Specific objectives are to evaluate the genetic basis, as well as the phenotypic and genetic relationships of differences in retrospective measures of stress response in healthy nursery pigs and in disease-challenged grow-finish pigs with performance and disease resilience, and to evaluate phenotypic and genetic associations of the blood transcriptome of healthy nursery pigs with their retrospective measures of stress response both prior to and during the disease-challenge.

Methods
Hair samples will be collected on 960 pigs at three time points to capture hair growth, and circulating cortisol and DHEA levels over the following periods: 1) birth to early nursery, 2) nursery disease challenge, and 3) recovery in the finisher. Resulting data will be used to estimate phenotypic and genetic parameters of retroactive measures of stress response and to determine genomic regions using genome-wide association studies. Phenotypic and genetic correlations and associations between the stress response traits with performance and disease resilience traits collected on the same pigs will be estimated and investigated. Phenotypic and genetic associations of the blood transcriptome and of the complete blood count of healthy nursery pigs with retrospective measures of stress response of healthy nursery pigs and of disease-challenged grow-finish pigs will be evaluated.

Results
No results are available yet from this recently initiated project.

Conclusions
Project deliverables will impact the industry through development of the use of hair as a source of indicators of resilience, as well as the scientific community by providing knowledge on the relationship between disease resilience and resilience to other external stressors.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture; Genome Canada; Genome Alberta; Genome Prairie; PigGen Canada

Notes:

V-P039 - Multiple-locus variable number tandem repeats analysis of five *Brucella suis* strains isolated in Ukraine

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Session: Host or pathogen genomics or proteomics

Objective

Brucella suis is a pathogen of worldwide zoonotic significance. Among *Brucella* molecular typing techniques, Multiple-Locus Variable Number Tandem Repeat Analysis (MLVA) is widely used and highly informative. The objective of this study was to provide for the first time MLVA data of local *B. suis* strains from Ukraine and to put them in a global context.

Methods

The genomes of five Ukrainian *B. suis* strains, isolated from pig herds (Poltava, Chernihiv, Luhansk and Kherson Oblasts) between the years 1960-1999 were sequenced using the Illumina technique. Genomes were subjected to *in silico* MLVA-16 typing (A. Dahouk et al. 2007). Obtained data was compared with the MLVA results of 851 *B. suis* isolates from various countries (P.M. Muñoz et al., 2019). Clustering analysis was conducted with BioNumerics software using data as character dataset with categorical distance coefficient and UPGMA.

Results

All five Ukrainian *B. suis* isolates were confirmed as belonging to *B. suis* biovar 2. Four of the strains clustered within the Eastern European group, whereby three strains formed a tight cluster and one strain clustered separately. The one remaining strain was significantly different from the others and clustered with strains from Sardinia. The highest number of differences was found among VNTR markers Bruce09, Bruce12 and Bruce30. The strain isolated in Kherson Oblast in 1960, showed high similarity with the Italian strains, whereas *B. suis* from Luhansk obtained in 1964 was clustered together with Croatian and French strains. Three other strains from 1990s formed their own cluster group and are more closely connected to the *B. suis* strains from different EU countries.

Conclusions

MLVA-16 revealed genetic diversity among the five strains indicating genetic heterogeneity among Ukrainian *B. suis* isolates and presumably also indicates different geographical origins. However, more isolates must be analyzed in order to get a clear picture of the Ukrainian *B. suis* population and their origin. MLVA-16 will be a useful tool for future outbreak analysis and source tracking.

Notes:

V-P040 - Dual RNA-seq for revealing host-pathogen interaction between human macrophage and *M. paratuberculosis* at initial stage of infection

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Session: Host or pathogen genomics or proteomics

Objective

Mycoabcterium avium subsp. *paratuberculosis* (MAP) is a causative agent of Johne's disease. MAP is also considered a potential cause of human Crohn's disease with regard to the isolation of this bacterium from the patients. Pathogenic mechanism of MAP infection is associated with its persistency in the intracellular environment. It is known that MAP can survive in the macrophages inhibiting phagosome-lysosome fusion. However, additional strategies are needed in terms of energy acquisition and stress response to survive and proliferate inside the macrophages. From this perspective, transcriptomic changes both in THP-1 and MAP at initial stage of infection was analyzed using dual RNA-seq.

Methods

Differentiated THP-1 macrophages were inoculated with MAP strain K-10 at a multiplicity of infection of 10:1 and incubated for 3 h. For the dual RNA-seq, bacterial RNA was enriched by eliminating most of host RNA using centrifugation of total cell lysate. Total RNA was subjected to RNA-seq.

Results

Compared to the broth cultured MAP, THP-1 infected MAP showed many differentially expressed genes (DEGs). Up- and down-regulated genes were annotated to the VFDB and KEGG database to determine the function of DEGs. Within the macrophage, MAP up-regulated genes responsible for various stress environment, including several two component systems (mprA/B, tcrX/Y, prrA/B, and trcS/R). In addition, iron metabolism related genes were up-regulated including mbt and esx-3 genes.

Conclusions

The host-pathogen interaction in terms of stress response and nutritional immunity was analyzed by the transcriptomic data using dual RNA-seq. Transcriptomic profiles revealed in this study may explain intracellular persistent mechanism and provide insights for further virulence determination of MAP. This work was supported by the Strategic Initiative for Microbiomes in Agriculture and Food, MAFRA (No. IPET918020-4), Basic Science Research Program through the NRF funded by the ME (No. NRF-2019R1I1A1A01063387), BK FOUR and the Research Institute for Veterinary Science, Republic of Korea.

Notes:

V-P041 - Genomic screens to identify causative polymorphisms accounting for Marek's disease genetic resistance in chicken

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Session: Host or pathogen genomics or proteomics

Objective

Marek's disease (MD), a lymphoproliferative disease of chickens caused by the pathogenic Marek's disease virus (MDV), is a serious disease problem of chickens. Despite widespread use of vaccines, more virulent MDV strains have repeatedly arisen. Consequently, alternative control methods, such as improving MD genetic resistance, are needed. In prior work, we demonstrated that genes showing differential gene expression in response to MDV infection account for 83% of the genetic variance. This submission is designed to identify the causative variants.

Methods

Integrating Hi-C, ChIP seq for MDV Meq and chromatin marks that identify promoters and/or enhancers, and RNA seq to identify transcripts, we identify candidate regulatory elements that contain the causative polymorphisms. In Experiment 1, we use splenic-derived lymphocytes from uninfected and MDV-infected chickens to reveal regulatory elements with specific transcription factors (TF) motifs that regulate gene expression in response to MDV infection. In Experiment 2, the same design will be used except MDV will lack Meq, the viral oncogene and a bZIP transcription factor. In Objective 3, we validate our predictions by progeny-tested commercial layer sires.

Results

Despite clear differential gene expression and clustering of samples, Hi-C results do not indicate clear differences in topologically associating domains (TADs) between sample groups. Contingencies querying samples from cultured cells (e.g., control and MDV-infected fibroblasts) will commence once pandemic restrictions are removed.

Conclusions

There are two likely possibilities. First, our approach is not sensitive enough to pick up the subtle chromatin differences when only a small fraction of the cells are infected. Or second, differential transcriptional start sites (TSSs) and not enhancers are the regulator elements that we should focus on. [Results from this work was used in a 2021 Science (372:984) publication - 3D genomics across the tree of life reveals condensin II as a determinant of architecture type.

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

Notes:

V-P042 - Big-data genomic investigation to improve dairy cattle health

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Session: Host or pathogen genomics or proteomics

Objective

Animal health is important for the dairy industry regarding profit, sustainability, animal welfare, and consumer expectations. Mastitis and other diseases cost 230 million dollars to the U.S dairy industry each year. Although host genetics only contribute to a small amount of variation in disease risk, genetic selection of health traits provides an economic and sustainable approach to deal with this issue. Leveraging the US dairy genomics database and other functional data resources, our goal is to uncover the genetic mechanism of host resistance to mastitis and to apply these genomic discoveries to improve cattle disease resistance and profitability of the dairy industry.

Methods

The Council of Dairy Cattle Breeding and USDA Animal Genomics and Improvement Laboratory have included six common diseases including mastitis into the national genomic evaluation in 2018. Leveraging the US dairy genomics database and other functional data resources, our genomic investigation and application will reveal the genetic basis of disease resistance and deliver a set of health SNPs to the dairy industry to improve the selection of robust cows. Specific aims of this project include: 1) Identify genomic regions and candidate genes associated with mastitis by using big data genome-wide association analysis of mastitis and other immune-related diseases; and 2) Integrate sequence-level GWAS, transcriptome and functional validation of immune cells to identify health SNPs and apply them to optimize genomic selection of disease traits.

Results

We expect to identify a set of disease SNP that are associated with mastitis and other disease resistance, which will be added to the newest SNP chips for the national genomic evaluation of dairy cattle. The new set of disease SNP will help accelerate the genetic improvement of disease resistance in cattle.

Conclusions

This project will be the largest such genomic study of disease resistance in dairy cattle and is expected to have a major impact to the dairy industry on both profitability and animal health.

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

Notes:

V-P043 - Transcriptomic profiling of *Babesia bigemina* reveal differences in kinete and blood developmental stages

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Session: Host or pathogen genomics or proteomics

Objective

Babesiosis is an important disease that occurs worldwide by apicomplexan intracellular parasites of the genus *Babesia*. Bovine babesiosis causes high morbidity and mortality, generating substantial economic losses for livestock production. Most experimental anti-*Babesia* vaccine candidates are based on molecules involved in the merozoite stage, but the current knowledge for processes involving *Babesia* intra-tick developmental stages is limited. In this study, we investigated the gene expression of two stages of the biological cycle of *B. bigemina*, one in the cattle (blood-stage) and other in the tick (kinete).

Methods

Three naïve splenectomized Holstein calves were infected with *B. bigemina*. *Rhipicephalus microplus* ticks were allowed to feed on the infected animals during an ascending parasitemia. To obtain *B. bigemina* blood-stage parasites, blood of each infected calf was collected and placed in individual short-term *in vitro* culture. *B. bigemina* kinetes were collected from the engorged female ticks' hemolymph. Blood-stage parasites and kinetes were suspended in TRIzol and total RNA were sequenced using a HiSeq 2500 (Illumina).

Results

Approximately 40 million reads were obtained from the analysis of RNA samples extracted from *B. bigemina* blood-stage or kinete. Differential expression on a total of 3,721 genes was analyzed, and of these, 2,282 genes (61.3%) were differentially expressed at a statistically significant level (cut-off 0.05) between the two hosts. In the kinete, 1,072 genes were up-regulated, with the most differentially expressed gene having a 56,767-fold increase. In the blood-stage, 1,210 genes were up-regulated, with the highest at a 9,686-fold increase. In blood-stage parasites, we identified genes involved in erythrocyte invasion like SBP and AMA-1. In kinetes, KSP (>20,000-fold increase), ROM (>14,000-fold) and TRAP (>2,900-fold) genes were highly transcribed.

Conclusions

In this study involving *B. bigemina*, an important bovine parasitic agent, several candidate genes were identified as possible new vaccine candidates to block transmission of babesiosis.

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

Notes:

V-P044 - Production of complete equine parvovirus capsids *in vitro*

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Session: Host or pathogen genomics or proteomics

Objective

The recently discovered equine parvovirus-hepatitis (EqPVH) has been proposed as the causative agent of Theiler's disease (TD) in horses, a syndrome associated with veterinary administration of horse serum products. Hepatitis causation specifically by virus-contaminated serum is supported by molecular data; conversely EqPVH DNA has a prevalence of 7- 17% in healthy horse populations worldwide. Serum positive for EqPVH at the limit of detection by qPCR is infective. To date the virus has not been cultured, inhibiting investigations into its biology, including hepatitis causality. To establish causality between the virus and the disease, an EqPVH synthetic genome was constructed and tested for its ability to generate virus.

Methods

Serum from horses suffering from hepatitis and positive by PCR for EqPVH was kindly donated by Dr. Divers at Cornell University. Standard cloning methods were unable to generate a plasmid carrying the viral genome. The genome was instead amplified in two pieces, double digested with *Bam*HI and *Nhe*I and ligated. The ligation reactions were then amplified using a modified megaprimer protocol. The synthetic genome was verified by next generation sequencing (NGS), then transfected into RK13B cells, incubated for 2 days then harvested for PCR and electron microscopy (TEM).

Results

Circular synthetic genome contains the parvovirus NS and VP genes and a single copy of the palindrome flanking linear parvovirus genomes. NGS found the genome was >99% identical to viral sequence in the positive serum from Cornell. Synthetic genome transfection into RK13B generated parvovirus capsids detected by PCR and TEM.

Conclusions

A synthetic genome of the recently discovered equine parvovirus-hepatitis was used to produce EqPVH capsids *in vitro*. The packaged genome sequence, if any, is not yet confirmed. Synthetically generated virus has no potential contamination with other horse pathogens, and upon inoculation into horses will fulfil Koch's postulates to determine the role of equine parvovirus-hepatitis in development of Theiler's Disease.

Notes:

V-P045 - Forecasting metritis cases in dairy cattle using deep learning of genomic and management information

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Session: Host or pathogen genomics or proteomics

Objective

Uterine health influences the capability of dairy cattle to conceive and produce calves. Shortly after calving, dairy cattle can develop metritis, a uterine disease that hinders fertility and milk yield. Early prediction of at-risk cattle is critical to prevent or reduce the rapid progression of this disease that is influenced by management and genetic factors.

Methods

Deep learning approaches are well suited to predict events impacted by many interacting factors of small effects such as metritis. This study aimed to explore the use of one-dimensional convolutional neural networks to predict a metritis event. These neural networks enable accounting for linear and nonlinear relationships between genomic and environmental features to predict metritis. Metritis events recorded on 2182 Holstein cows from 2012 to 2016 across 16 U.S. herds and two seasons were analyzed. In the population studied, metritis incidence ranged from 12% to 52% across herds. Genomic information from 1,304,717 SNPs was used to develop convolutional neural networks within individual chromosomes.

Results

The predictions were trained in 85% of the cows and tested on the remaining 15%. The networks' sensitivity and specificity to predict metritis averaged 98% for the training data across chromosomes. The average sensitivity was 71% in the test data set, whereas the average sensitivity was 33%. Notable was the high sensitivity to predict metritis obtained using genomic information from some chromosomes that may reflect genetic associations with metritis.

Conclusions

Our results demonstrate that deep learning approaches are well-suited to identify cows at high risk for metritis and support management decisions to prevent or diminish the incidence of uterine disease. The support of USDA-NIFA-AFRI-003542 and the University of Illinois at Urbana-Champaign Center for Digital Agriculture is appreciated.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture; Center for Digital Agriculture, University of Illinois at Urbana-Champaign

Notes:

V-P046 - Calf expressed microRNAs associated with dams infected with bovine leukemia virus

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Session: Host or pathogen genomics or proteomics

Objective

The 2007 USDA survey estimated 89% of dairy operations in the United States were seropositive for Bovine Leukemia Virus (BLV). Bovine leukosis is the disease caused by BLV and leads to increased susceptibility to opportunistic infections. Cattle infected with BLV often suffer impaired immune function. Further, about 5% of infected cattle will develop lymphomas. MicroRNAs are a small, noncoding RNA species that are involved in post-transcriptional gene regulation. Research that explores the associations between BLV and host microRNA profiles is lacking. While research provides evidence that microRNAs influence immune development in calves, no research has addressed the potential influence of maternal BLV infection status on progeny microRNA expression. Our project aims to identify calf microRNAs influenced by a dam's BLV status.

Methods

Blood samples from dam and calf pairs were collected from animals at the Dairy Cattle Teaching and Research Center at Michigan State University. Dams were sampled 14 to 30 days prior to parturition, and again within 24 hours of parturition (n= 51). Antibodies for BLV were assessed via ELISA, as well as the amount of provirus or proviral load (PVL) via qPCR for all dams. Calves were sampled on days 0, 7, 14, and 21 of life (n=51). White blood cells (WBCs) were extracted from all calf samples and dams within 24 hours of parturition. In further analysis, RNA will be extracted from WBC samples to sequence microRNAs.

Results

Counts of calf microRNA sequences will be assessed to determine associations between dam BLV infection status and the calf.

Conclusions

We hope to identify expression of microRNAs in calves born to both ELISA positive and ELISA negative dams and account for the varying levels of PVL in ELISA positive dams. We will continue to investigate the potential impact that dam BLV infection has calf development. It is imperative to look further into these potential associations as microRNAs play a role in gene function, and this may impact the development of calves on a dairy operation.

Notes:

V-P047 - Comprehensive landscape of nidovirus–host protein interactions by comparative proteomics

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Session: Host or pathogen genomics or proteomics

Objective

Porcine reproductive and respiratory syndrome virus (PRRSV) and porcine epidemic diarrhea virus (PEDV) are responsible for severe economic losses worldwide. Our project aims to gain a better understanding of the molecular mechanisms of interactions between animal nidoviruses and their hosts in order to develop new antiviral strategies. We hypothesized that the tight interactions between host and viral proteins define the fate of nidoviral infection and pathogenesis.

Methods

Virus-host interactions are highly dynamic, leading to important changes in the intracellular levels of host proteins. Virus-induced modulation of the intracellular environment creates a more favorable condition for viral infection and spread. Consequently, quantitative comparative proteomic profiling of the nidovirus-infected cells in a time-resolved manner will provide dynamic and global mapping of virus-host interactions. Specifically, we analyzed proteomic patterns in host cells during nidoviral infection and characterized protein composition of the virions and extracellular microvesicles (EMV) and exosomes produced by PEDV or PRRSV infected cells.

Results

We found that PRRSV and PEDV infections affected the abundance of numerous host proteins associated with EMV. Our data showed that nidovirus infection resulted in significant alterations in the host cell proteome. We also found that both viruses induced specific changes, unique to their molecular pathogenesis, e.g., the abundance of proteins involved in immune responses was changed in PEDV infected cells. Interestingly, in PEDV infected cells, host proteins involved in cell cycle regulation and the cytoskeletal system were affected in abundance. Moreover, PEDV significantly modulated biological pathways such as entry into the host cells, type I IFN signaling, defense response to viral infection, etc.

Conclusions

Dynamic proteomics greatly facilitates our understanding of the molecular details of virus-host interactions. PEDV and PRRSV infections modulate intracellular environment, suggesting that host proteins may influence viral replication capacity and pathogenesis.

Financial Support
Natural Sciences and Engineering Research Council of Canada; Fonds de recherche du Québec ; GFR was summer student at LA laboratory and she was supported by Mitacs Globalink Research Internship fellowship

Natural Sciences and Engineering Research Council of Canada | Conseil de recherches en sciences naturelles et en génie du Canada

Notes:

V-P048 - Characterization of the respiratory microbiome and virome associated with bovine respiratory disease

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Session: Microbiome and bacteriology

Objective

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.

Methods

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)).

Results

To date, nasal swabs have been collected from herds in the US and Ireland for year one and year two, and evaluation of the bacterial and viral populations has begun through next-generation sequencing and QPCR. In the US herd, year one samples from the feedlot after weaning were evaluated. Calves diagnosed with BRD had an increased abundance of *Mycoplasma sp., Sneathia sp., Bergeyella sp.,* and *Corynebacterium sp.* compared to control cohorts. Bovine coronavirus and bovine respiratory syncytial virus were also detected in feedlot calves diagnosed with BRD.

Conclusions

Further evaluation of year two samples will occur in 2021 and 2022.

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

Notes:

V-P050 - Longitudinal investigation of early fecal microbiota in pigs from nine cohorts in Ontario and Quebec

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Session: Microbiome and bacteriology

Objective

The microbiota is integral to swine health, growth and disease susceptibility. It is vital to understand healthy gut microbiota composition throughout early life stages when environments are changing and immunity is developing. There are limited large-scale longitudinal studies classifying healthy succession of swine microbiota. The objectives of this project are 1) study swine fecal microbiota succession from a few days after birth to post-weaning on conventional and raised without antibiotic farms, 2) investigate the association of pig gut microbiota composition with health and growth performance.

Methods

Fecal samples were collected from nine cohorts of 40 pigs(n=360) from distinct farrowing sources in Ontario and Quebec, Canada at four timepoints from birth to one-week post-weaning. Pig body weight was recorded at each fecal sampling. Bacterial community DNA was extracted from fecal samples and subjected to MiSeq Illumina sequencing of V3-V4 regions of 16S rRNA genes. Sequence data were processed using the mothur bioinformatics pipeline, and microbiota community data were analyzed with a compositional approach.

Results

Samples had a sequencing depth of 28 053 ± 39 623(median ± interquartile range). The fecal microbiota was dominated by 4 major phyla; including Firmicutes, Bacteroidetes, Proteobacteria and Fusobacteria. Hierarchical clustering showed three distinct community clusters of fecal microbiota that appeared successively across early life stages. Further analysis will be conducted to elucidate differentially abundant taxa between clusters and identify influential external factors, such as sow factors, rearing conditions and pig health that may be associated with microbiota composition.

Conclusions

The data show that swine undergo several shifts in gut microbiota throughout their early stage of production and data will allow identification of external variable that can be used to manipulate microbiota variation and improve health. The project findings will decrease antimicrobial use, increase animal welfare and have positive economic impacts.

Notes:

V-P051 - Relative and absolute quantification of the ocular microbiome in infectious bovine keratoconjunctivitis

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Session: Microbiome/Bacteriology

Objective

Infectious bovine keratoconjunctivitis (IBK) represents a major welfare and economic concern in commercial beef operations worldwide. IBK is thought to be caused by *Moraxella bovis*, yet this pathogen is inconsistently isolated from cases and other bacteria have been implicated in disease development. This study, still in progress, will identify therapeutic targets and improve diagnostics for IBK through 1) relative assessment of the ocular microbiome using 16s rRNA gene amplicon sequencing (16S) and 2) absolute assessment using quantitative PCR (qPCR). It is expected that animals with IBK will have markedly different ocular microbiomes when compared to normal animals.

Methods

Ocular swabs are being obtained from a large cohort of normal cattle and cattle with IBK. DNA will be extracted from ocular swabs with approximately half of this DNA used for 16S and the remainder used for qPCR. For the former, the V4 hypervariable region will be amplified. Following 16S DNA quantification, samples will be sent for 16S sequencing. Following pre-processing of the data using the DADA2 pipeline, Phyloseq and QIIME2 will allow for the calculation of relative proportions of bacterial species. These proportions will be compared using UniFrac analysis between animals with/without IBK to determine significance. A qPCR panel containing multiple bacterial targets has been designed based on prior microbiome surveys of IBK in cattle. qPCR Ct values will be compared between IBK and normal groups using multivariate modelling.

Results

It is expected that components of the bovine ocular microbiome which contribute to homeostasis of the healthy eye and those which are present in IBK will be identified using relative 16S sequencing. This will further inform the design of our novel qPCR panel. This combined approach will allow for identification of novel therapeutic targets for IBK, improved diagnostic test design, and ability to compare our results with future studies.

Conclusions

We anticipate that this approach will lead to novel insights into the bovine ocular microbiome in both healthy animals and those with IBK.

Financial Support
U.S. Department of Agriculture

Notes:

V-P052 - Assessing the gut microbiome as a tool for the mitigation of respiratory disease in nursery pigs

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Session: Microbiome/Bacteriology

Objective

Porcine reproductive and respiratory syndrome virus (PRRSV) causes the most costly disease to swine production in the United States. Disease caused by this virus often involves secondary bacterial pathogens, which exacerbates respiratory disease and increases antimicrobial administration in young growing pigs. Although commercial vaccines are used to reduce the effects of PRRSV on swine health, the currently available vaccines are considered inadequate for disease control. Alternative strategies for control of PRRSV is needed to maintain swine health and welfare while lessening the economic effects of this disease on pork producers. The goal of this work is to investigate the gut microbiome as an alternative tool for PRRSV control due to its impact on the immune system and clinical outcome after infection. Objectives of the work include investigating the effects of microbiome modulation on outcome of swine with respiratory disease and identifying what beneficial microbes are associated with improved health. We anticipate the data generated in this project will allow us to characterize and determine the gut microbes which improve pig health in the presence of PRRSV. Our goal is to determine how beneficial gut microbes may be used as a preventative medicine tool to reduce the effects of respiratory disease and decrease the need for antimicrobials in swine. The impacts of this work will improve animal welfare and animal health, lessen the economic losses to producers with PRRSV-positive herds, and reduce the risk of antimicrobial resistance in swine.

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

Notes:

V-P053 - A novel neuroendocrine-microbiome mechanism mediating heat stress and infection in chickens

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Session: Microbiome/Bacteriology

Objective

The long-term goal of this project is to utilize a novel mechanistic approach to identify underlying mechanisms by which heat stress in chicken broilers can lead to the emergence of avian pathogenic Escherichia coli (APEC). The performance of this 3year project, which began in 2021, will lead to novel approaches to prevent colibacillosis due to APEC by providing neurochemical biogeographical mapping of the broiler intestinal and respiratory systems in conjunction during heat stress conditions in conjunction with comprehensive microbiome analysis. The aim of the initial findings reported here was to determine the concentrations of stress-related neurochemicals in the tissue and luminal content of the colon of the broiler intestinal tract, and to investigate if this biogeography changes with age of the bird.

Methods

Broiler chickens raised under ideal conditions were euthanized (n=12 chickens per age group) at 2, 4, or 6 weeks of age. Quantification of neurochemicals in full-thickness tissue and luminal content of the broiler chicken colon was determined using ultra-high performance liquid chromatography with electrochemical detection. Data were analyzed by two-way ANOVA with Tukey's post-hoc test.

Results

The catecholamines norepinephrine, epinephrine, and dopamine were detected in colon tissue. Norepinephrine was identified in the colon content. Whereas norepinephrine and epinephrine concentrations were affected ($P<0.05$) by age of the bird, dopamine was not significantly altered ($P>0.05$). Bird age-related changes were also observed ($P<0.05$) for colonic concentrations of serotonin, its metabolite 5-hydroxyindole acetic acid as well as for histamine.

Conclusions

As *E. coli* typically colonize the colon, it is this gut region that is a focal point of our research effort. Although we have just initiated this 3-year project, initial results have been attained. Our results indicate stress-related neurochemicals with demonstrated mechanistic roles in mediating *E. coli* pathogenicity are present in tissue and content of the colon throughout the broiler's production lifespan.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture; USDA Agricultural Research Service

Notes:

V-P054 - An investigation of *Streptococcus suis* in nursery pigs

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Session: Microbiome/Bacteriology

Objective

Streptococcus suis commonly colonizes the upper respiratory tract of pigs and under certain circumstances, systemic disease occurs. Pigs may shed *S. suis* in feces, but the relationship between virulent strains of *S. suis* in the gastrointestinal tract and outbreaks of systemic disease is not well known. The objectives of this study were to monitor the *S. suis* fecal shedding and *S. suis* disease in nursery pigs on a farm with a history of *S. suis* disease outbreaks.

Methods

A total of 480 pigs from 4 cohorts of weaned pigs were monitored and rectal swabs were obtained at weaning (Day 1), Day 7, and 37 (cohort 1 and 2) or from diarrheic pigs (all cohorts). Pigs were euthanized if neurological signs or severe lameness were observed. Tissue samples from systemic and non-systemic sites were cultured for the isolates serotyped by multiple PCR.

Results

To date, 417 rectal swabs from 296 pigs were collected and *S. suis* was recovered from 16 (5.4%) pigs. At weaning, *S. suis* was isolated from 15 (3.6%) pigs but no pig tested positive after Day 5 post-weaning. Only 7/108 (6.5 %) diarrheic pigs shed *S. suis* within the first week post-weaning. Isolates belonged to serotypes 15 (n=7), 31 (n=3), or untypable (n=6). Three (0.6%) pigs developed neurological signs and 64 (13.3%) showed signs of lameness, but *S. suis* was not recovered from their rectal swabs. All 3 pigs with neurological signs and 18 (85.7%) severely lame pigs were euthanized. Serotype 9 (meninges), 31 (tonsil), and untypable (meninges, tonsil) were recovered from pigs with neurological signs. Serotypes 9 (meninges, tonsil) 15 (spleen, tonsil), 16 (tonsil), 29 and 33 (nasal swabs), and untypable (meninges, tonsil, lung) were identified in lame pigs.

Conclusions

Weaned pigs carry *S. suis* in their gastrointestinal tract, but serotypes of these *S. suis* may be different than isolates found from systemic sites in pigs showing clinical signs. Isolation of *S. suis* in fecal samples in nursery pigs may have no diagnostic value to predict the outbreak of disease or help identify the serotype involved in the disease.

Financial Support
Ontario Pork; Swine Innovation Pork; Ontario Ministry of Agriculture, Food, and Rural Affairs Research (OMAFRA); Grand Valley Fortifiers

Notes:

V-P055 - Optimizing an infection model for *E. coli* F4+ in newly weaned pigs

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Session: Microbiome/Bacteriology

Objective

Post-weaning *E. coli* diarrhea is an important cause of mortality, poor growth performance, and drug use on swine farms. It is necessary to have a predictable infection model for enterotoxigenic *E. coli* so novel treatment options can be investigated. The objective of this study was to investigate the effects of infectious dosage, age, and susceptibility on post-weaning diarrhea in newly weaned pigs challenged with *E. coli F4+*.

Methods

Three trials were conducted. In each trial a group of piglets were tested for presence of mucin gene by RFLP-PCR and 12 susceptible (S) and 12 resistant (R) pigs assigned to 4 groups (3 S and 3 R in each group), including control (no challenge), low dose (LD; challenged with 10^8 CFU/mL), moderate dose (MD; 10^9 CFU/mL), and high dose (HD; challenged with 10^{10} CFU/mL). Pigs were challenged once at age of 15 days (Trial 1) and 19 days (Trial 2), and twice at age of 22-23 days (Trial 3). Pigs were monitored for diarrhea and rectal swabs were collected and tested for presence of *E. coli* F4+ quantitatively. All pigs were euthanized 5 days post-challenge and tissue samples were collected for histopathology examination.

Results

All control pigs except one tested negative for presence of *E. coli* F4 across all 3 trials. Diarrhea was observed in 44.4, 55.6, and 55.6% of pigs in LD, MD and HD group (p=0.003), but the presence and number of *E. coli* F4+ (mean log10 of CFU/ml) was not significantly different among LD (4.7), MD (5.5), and HD (6.3) group (p=0.14). There was no significant difference in diarrhea in susceptible (59.3%) and resistant (44.4%) pigs (p=0.28). However, the mean log10 of CFU/ml in susceptible and resistant pigs was 6.03 and 5.03, respectively (p=0.007). Regardless of infectious dose and susceptibility, 33.3, 44.4, and 77.8% of pigs had diarrhea associated with the challenge strain in trial 1, 2, and 3, respectively (p=0.021).

Conclusions

Diarrhea could be induced in pigs challenged with moderate or high dosage at age of 3 weeks. However, some resistant pigs demonstrated diarrhea which needs to be further investigated by histopathology examination.

Financial Support
Ontario Pork; Ontario Ministry of Agriculture, Food, and Rural Affairs Research (OMAFRA); Swine Innovation Porc

Notes:

V-P056 - A challenge study investigating the relationship between *Streptococcus suis* disease and nursery diet in pigs

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Session: Microbiome/Bacteriology

Objective

Streptococcus suis related diseases in nursery pigs result in significant economic losses due to decreased growth performance, mortality, and increased drug use. Multiple factors related to bacteria (virulence factors), environment (other diseases, stress, diet), and host (genetics) may contribute to *S. suis* disease. The objective of this study was to determine the impact of nursery diet on *S. suis* disease in pigs experimentally infected with *S. suis* serotype 2 or serotype 9.

Methods

Two trials were conducted. In each trial, 30 pigs were weaned at age of ~3wks (D1) and assigned to receive either a standard nursery diet (high complexity; HC; n=15) or a less expensive low complexity (LC, n=15) diet containing a higher amount of soybean meal during the entire trial. The pigs were sub-divided to 6 groups including HC/control (HCC; no challenge), HC/S2 (challenged with serotype 2), HC/S9 (challenged with serotype 9), LC/control (LCC; no challenge), LC/S2 (challenged with serotype 2), and LC/S9 (challenged with serotype 9). Pigs were challenged with *S. suis* inoculum at D18, D24, and D30 (trial 1) and at D7 and D14 (trial 2) and assessed for clinical signs of *S. suis* disease and bacterial colonization for 6wks (trial 1) and 4wks (trial 2).

Results

In total, 5 (50%) and 1 (10%) of the pigs in HC/S2 and LC/S9 developed disease, respectively ($p<0.05$). Tonsil and/or nasal cavities of 5 (50%) pigs in HC/S2 and 2 (20%) pigs in LC/S2 were colonized with serotype 2 ($p<0.05$). No pigs in HC/S9 and LC/S9 group were colonized with serotype 9. In trial 1, there was a significantly elevated post-challenge rectal temperature (PCRT) in HC/S2, LC/S2, and LC/S9 groups ($p<0.05$). However, in trial 2 only the pigs in LC/S2 were more likely to have elevated PCRT ($p<0.05$) on D15.

Conclusions

Pigs that received LC diet and challenged with serotype 2 were less likely to demonstrate *S. suis* disease, however, this needs to be interpreted cautiously and evaluated on farm where multiple risk factors are present. Also, further work is needed to evaluate the response in pigs fed HC or LC diet when infected with serotype 9.

Financial Support
Ontario Pork; Swine Innovation Porc; Ontario Ministry of Agriculture, Food, and Rural Affairs Research (OMAFRA)

Notes:

V-P057 - Recovery and time-series analysis of metagenome assembled genomes within the fecal microbiome of commercial swine

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Session: Microbiome/Bacteriology

Objective

Metagenome-assembled genomes (MAGs) may be used to investigate microbiome dynamics within commercial swine production systems by improving characterization of bacterial strains within metagenomic data. We hypothesized that common environment and management exposures would increase the proportion of MAGs shared between pens of swine sampled over time. To investigate this, the project aimed to characterize MAGs obtained from fecal metagenomes at different timepoints in the life of commercial swine.

Methods

Composite pen-level fecal samples obtained from a group of swine (N = 36 pigs across 12 pens) as part of a recent National Pork Board funded study were selected for analysis. For each pen, samples from three unique life stages were analyzed: at weaning, post vaccination, and just prior to marketing. MAGs were assembled following the methods described in the PIGC pipeline with minor workflow alterations. Processed samples underwent metagenomic binning using metaWRAP and dereplication using dRep. Both original and dereplicated MAGs underwent taxonomic classification using GTDB-TK, and these results were used for further pen-level analysis.

Results

Preliminary results at the weaning time point yielded 1,057 MAGs across all samples and dereplication yielded 521 non-redundant MAGs, suggesting that about half of all bacterial strains were common to at least 2 pens. All dereplicated MAGs were classified to the genus level and 335 were classified to the species or subspecies level, representing 191 unique species. MAGs are being assembled for the two remaining time points and the resulting MAG profiles will be used for further comparison of pen-level variation in draft genome quality, phylogenetic analysis, and relative abundance.

Conclusions

Current MAG workflows still have many limitations for metagenomic datasets, though additional refinement of these approaches may further our understanding of the impact that group structure and environmental factors have on complex microbial communities, particularly host-host “sharing” of commensal microbes under varying commingling scenarios.

Financial Support
The Agricultural Research, Extension and Education Transfer Technology (AGREET) Program through the State of Minnesota; This work is supported by Agricultural and Food Research Initiative grant no. 2021-68015-33499 and grant no. 2019-67017-29110 from the USDA National Institute of Food and Agriculture; Boehringer Ingelheim VSS Program and the College of Veterinary Medicine, University of Minnesota; Minnesota Agriculture Research, Education and Extension Technology Transfer Program; Funding, wholly or in part, was provided by the National Pork Checkoff.

Notes:

V-P058 - Characterization of the beef nasopharyngeal microbiome associated with bovine respiratory disease

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Session: Microbiome/Bacteriology

Objective

Bovine respiratory disease (BRD), a significant cause of morbidity and mortality affecting feedlot cattle, is diagnosed using clinical respiratory scoring (CRS) and thoracic ultrasonography (TUS). Identifying BRD associated microbes is paramount in the development of targeted therapeutics. Typical means of bacterial identification require culture dependent techniques which delay species identification and thus delay veterinary medical intervention. Next generation sequencing eliminates the use of pathogen culture and furthermore allows discovery of non-culturable pathogens. The nasopharyngeal microbiome is reported to closely resemble the bacterial communities that populate the lung.

Methods

In this study, 60 spring-born suckler bred weaned calves were transported to the Research Centre and housed indoors. Nasal swabs were collected 24 h post arrival (day (d) 0), on the day of BRD detection (d BRD) and at day 28 (d 28) post-arrival. All calves were vaccinated on d 0 against bovine respiratory syncytial virus (BRSV), parainfluenza-3-virus (PI-3), *Mannheimia haemolytica* A1, and bovine herpesvirus type (BoHV)-1. DNA was extracted from the nasal swabs using DNeasy, PowerSoil DNA kit and the 16S rRNA gene V3 to V4 hypervariable region was amplified and sequenced using an Illumina Miseq instrument. Positive and negative controls were included in the analysis. Amplicon sequence variants (ASVs) were generated in DADA2 and microbiome analysis was performed using Phyloseq.

Results

A PCA revealed similar clustering on d 0 and d BRD between healthy and BRD animals, while on d 28 clustering was distinct between the cohorts. On d 28 of the study, calves diagnosed with BRD had a significant abundance of *Filobacterium sp* compared to the healthy cohort.

Conclusions

Filobacterium sp has been associated with tracheitis, an infection of the trachea in cattle and is not routinely characterised in Irish BRD cases. Further investigation of *Filobacterium sp* presence and association with BRD is warranted.

Notes:

V-P059 - Reverse vaccinology approaches using the genome of invading North American *Haemaphysalis longicornis*, the Asian longhorned tick

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Session: Parasitology and/or ticks

Objective
The goal of this research is to mitigate the threat to United States agriculture posed by the introduction and expansion of the invasive Asian longhorned tick, *Haemaphysalis longicornis*, using reverse vaccinology approaches. First detected in 2017 in Hunterdon County, NJ, this tick has since been discovered in over 100 counties in fifteen states. In addition to being a vector of known human pathogens, it is an economically damaging livestock ectoparsite throughout its native east Asian range, is able to reproduce parthenogenetically, and vectors the protozoan *Theileria orientalis* group of pathogens that are causative agents of bovine anemia. These factors together make *H. longicornis* a paramount threat to the U.S livestock industry. Treatment of animals and pastures with chemical acaricides remains an accepted method of tick control yet carries a wide range of potentially negative consequences, including the development of acaricide resistance and the possibility for residual contamination of milk and/or meat. As such, here we will pursue the development of anti-tick vaccines - a strategy that relies on host immunization with synthetic recombinant proteins derived from candidate homologs encoded by the tick itself - as an alternative tick control strategy.

Methods
A highly contiguous and well-curated *Haemaphysalis longicornis* genome will yield multiple candidates for anti-tick vaccine development. In collaboration with the USDA Ag100Pest initiative, an *H. longicornis* genome will be produced and annotated using PacBio Sequel II single-molecule long read sequencing with template from invading New Jersey ticks. The resulting gene catalog will be mined using both model-driven approaches and manual homology searches to produce a ranked list of gene products that represent vaccine candidate antigens. Recombinant antigens will be synthesized using expression vectors, screened for antigenicity in vaccinated cattle, and assessed for anti-tick activity in stall trials. In addition, we will pursue an artificial feeding system to deliver such antibodies to *H. longicornis* in the lab such that future trials can be conducted in a simplified and rapid timeframe.

Results
This work is funded by a USDA-NIFA Agriculture and Food Research Initiative grant beginning 01 July 2021.

Conclusions
It is clear that *H. longicornis* poses a multi-pronged threat to the U.S livestock industry. Our proposal would address the concerns of producers by giving them new tools to control and prevent infestations on their herds. Typically tick infestations are controlled by acaricide treatments and dips. Anti-tick vaccines are considered to have several advantages over acaricide treatment, which must be done continuously and raises concerns about residual contamination of animal products. Development of effective vaccines holds promise to lessen the contribution of acaricides in an integrated tick management program.

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

Notes:

V-P060 - A tick salivary immunogen-based vaccine against tick feeding and TBD infections in cattle

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Session: Parasitology and/or ticks

Objective

This project seeks a previously unexplored strategy: to make a novel vaccine based on multiple tick salivary proteins (TSP) for use in cattle against tick infestation and to prevent tick-borne disease (TBD) infections of cattle and other livestock. The proposed vaccine will be developed using the *Amblyomma americanum* tick and cattle model, and will target multiple TSP that are conserved in major economically important tick species of livestock: cattle fever ticks (CFT, *Rhiphicephalus microplus* and *R. annulatus*), the new invasive tick species, Asian longhorned tick (*Haemaphysalis longicornis*), and the blacklegged tick (*Ixodes scapularis*).
Objective 1: Define baseline immune status of repeatedly infested cattle that acquire protective immunity against tick feeding.
Objective 2: Validate efficacy of a tick vaccine based on multiple TSPs

Methods

Objective 1 Experimental summary: There are two major experiments under objective 1 (i) repeated tick infestation of cattle and, (ii) collection of sera and PBMCs to determine antibody titers and quality of peripheral B and T cells using the EliSpot assay. Tick infestation and ELISA assays will be done at TAMU (PD lab), B and T cell EliSpot assays will be done in the Co-PD lab at Kansas State
Working hypothesis: We will test the hypothesis that anti-tick immunity observed in repeatedly infested cattle is definable by yet undetermined immune response (antibody titers, B and T cell composition) bench-mark.
Objective 2 Experimental summary: There are three major experiments under this objective: (i) expression of recombinant antigens, (ii) use immune response bench marks from the first objective to determine the effective antigen dose, and (iii) validate the protective efficacy of the selected effective dose. The design of dendritic cell targeted antigens will be done in the Co-PD's lab; expression of recombinant antigens will be done the PD's lab; immunogenicity and effective antigen dose analyses will be shared by the two labs; and validating protective efficacy will be done at TAMU (PD lab).
Working hypothesis: A vaccine formulation based on multiple tick salivary proteins will provoke protective anti-tick immunity in cattle that will mimic acquired immunity that is observed in repeatedly infested cattle.

Results

Proposed experiments in both objectives 1 and 2 are ongoing, and results are pending

Conclusions

Proposed work is ongoing, conclusions are pending

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

Notes:

V-P061 - Artificial reproduction of *Cyprinus carpio* and *Clarias gariepinus*

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Session: Physiology or Immunology

Objective

The objective of this work was to set up an experimental protocol concerning artificial reproduction in two species of fish: cat fish '*Clarias gariepinus*' and royal carp '*Cyprinus carpio*' with the induction of spawning using the GnRH.

Methods

We conducted this study at an aquaculture farm in Khemis Meliana (Ain Defla, the Center of Algeria). In our work, we used six royal carp broodstock including 4 females and 2 males, and five African catfish broodstock, 3 females and 2 males. The hormonal injection was made into the back muscle below the fin. The doses of GnRH were determined according to the weight of each parent. The fertilization was done artificially using the dry method. After incubating the eggs, a binocular magnifying glass was used to check the condition of the eggs and the development of the embryos over time.

Results

The results obtained show that the artificial reproduction of these species is possible, as well as the survival and growth of the larvae. Indeed, after injection of GnRH, the female carp succeeded in laying eggs and the protocol was broken in the middle because of the poor control of latency time. For *C. gariepinus*, successful ovulation, fertilization and larval hatching, as well as larval monitoring, were noted. For this species, a latency period of 22 hours has been recorded, obtaining approximately 35.700 larvae of *Clarias*. The outbreak rate was 48%.

Conclusions

At the end of this experiment, we can conclude that it is possible to improve reproduction by properly using hormonal stimulation techniques and by improving the diet and abiotic factors that are dominant in fish farming.

Notes:

V-P062 - Determining the effects maternal programming on calf health and immunity during postnatal life

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Session: Physiology or Immunology

Objective

Neonatal dairy calves are prone to digestive and respiratory diseases resulting in high calf mortality. However, little is known about the role fetal programming may have in increasing the susceptibility of calves to disease. We hypothesize that maternal mastitis infection and/or high milk production during pregnancy will result in reduced growth and an increased inflammatory response in the offspring.

Methods

Holstein bull calves were transported to the university farm within 24 hours of birth. Calves were born to dams that were classified as high producers (HI; Top 25% for M305; n =7), high producers with mastitis (HIMAST; SCC ≥ 200,000 cells/mL; n = 15), moderate producers (MOD; lower 60% for herd M305; n = 15) and moderate producers with mastitis (MODMAST; n = 6). Body weight, crown rump length (CRL), skull length (SL), skull width (SW), girth and height were collected weekly. Blood was collected at 24, 48 and 72 hours post travel and weekly for analyses of circulating factors. Data were analyzed in SAS using PROC MIXED with time as a repeated measure when appropriate.

Results

No effect of maternal milk production and/or mastitis infection was observed on calf BW, CRL, SL, SW, girth or height ($P > 0.05$). Similarly, no difference in circulating haptoglobin concentrations, Malondialdehyde or C-reactive protein were observed ($P > 0.05$). Post travel, HIMAST and MODMAST calves exhibited reduced circulating glucose concentrations compared to MOD calves ($P \leq 0.04$). Ca 2^{+} was greater in HI calves at week 1 compared with MOD ($P = 0.05$). At week 3 these concentrations were greater in HIMAST calves when compared to HI and MOD calves ($P \leq 0.02$). Circulating AST and TP concentrations were greater in HIMAST compared to MOD and MODMAST calves ($P \leq 0.03$) at 8 weeks.

Conclusions

These data demonstrate that maternal mastitis infection during pregnancy affects blood biomarkers postnatally. We are completing histological analyses, RNA-seq on tissue samples collected as well as analyzing samples from an in vitro LPS challenge to better understand these results and the effects of fetal programming on these animals.

Financial Support
USDA-NIFA

Notes:

V-P063 - The effects of weaning pace on blood metabolites of Holstein dairy calves

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Session: Physiology or Immunology

Objective

Weaning is one of the most challenging events in dairy calves' life, and may stimulate body response and affect metabolites related to the inflammation. Therefore, the objective of this study was to determine role of weaning method (abrupt vs. gradual) on blood metabolites related to inflammation.

Methods

Forty Holstein dairy calves, 20 male and 20 female, were blocked by gender and body weight at birth and randomly assigned in two weaning paces (abrupt and gradual). The abrupt weaning consisted of 3 days, whereas the gradual weaning consisted of 14 days. Blood samples were collected from the jugular vein at d 3 of age, one-day before weaning, and one-day post-weaning. Glucose, haptoglobin, b-hydroxybutyrate (BHBA), non-esterified fatty acids (NEFA), albumin, and antioxidant capacity were determined in spectrophotometric methods. Data were analyzed using mixed model of SAS with significance declared at $P \leq 0.05$ and the tendency at $P < 0.10$.

Results

Serum BHBA, antioxidant capacity, and NEFA concentration showed a weaning pace × time ($P < 0.01$; 0.06, and < 0.001, respectively) interaction while haptoglobin was increased in abrupt method comapred with that for the gradual method (0.65 vs 0.74; $P = 0.07$). These interactions are attributed to the changes in serum BHBA, NEFA concentration, and antioxidant capacity between the treatments during the post-weaning period; however, no difference was observed at d 3 of age and pre-weaning. Calves weaned abruptly showed a lower BHBA and antioxidant capacity, and a greater NEFA concentration. There were no interaction or treatment effect for albumin ($P > 0.23$) and glucose concentration ($P > 0.59$).

Conclusions

In summary, under our experimental conditions the blood metabolites of dairy calves were affected by weaning pace.

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

Notes:

V-P064 - Colonocytes demonstrate an age-dependent defect in epithelial restitution in a pig model of colonic ischemia and repair

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Session: Physiology or Immunology

Objective

To improve intestinal health in young pigs, we aim to understand the mechanisms that regulate mucosal barrier injury and repair. This study examines colonic epithelial restitution and barrier recovery in suckling and weanling pigs following ischemic injury. We hypothesize that suckling pigs will demonstrate a relative defect in colonic epithelial restitution.

Methods

Ischemia was induced in the colon of anesthetized suckling (2-week-old) and weanling (6-week-old) pigs. Injured mucosa was mounted on Ussing chambers for *ex vivo* recovery. Barrier integrity was assessed by transepithelial electrical resistance (TEER) and histology.

Results

All pigs demonstrated a significant, time-dependent effect of ischemia on colonic epithelialization ($P<0.0001$). Suckling pigs also demonstrated a significant, time-dependent effect of recovery on colonic epithelialization ($P=0.03$). In suckling pigs, 45-minutes ischemia reduced epithelial coverage to 72.2±11.8%, which declined to 45.6± 24.0% during *ex vivo* recovery. In weanlings, 45-minutes ischemia reduced epithelial coverage to 63.3±17.2, which was maintained at 64.2± 20.2 during *ex vivo* recovery. All pigs demonstrated decreased mean TEER during *ex vivo* recovery, although barrier integrity was significantly upheld in injured suckling pig mucosa compared to uninjured controls ($P<0.0001$) .

Conclusions

Preliminary data suggests age-dependent restitution of colonic epithelial cells following ischemic injury. Similar to prior investigations on small intestine, these studies reveal a defect in colinic epithelial restitution of suckling pigs. Notably, some amount of injury appears to prime the intestines in suckling pigs for enhanced mucosal repair, as demonstrated by changes in mean barrier resistance. This age-dependent defect in colonic barrier repair will be further characterized by additional barrier function (flux) assays, scanning electron microscopy, and single-cell RNAseq. Defining age-dependent differences in colonic repair will inform future studies examining mechanisms of and clinical interventions for upregulating intestinal barrier repair in pigs.

Financial Support
COHA Translational Fellowship funded by U01 TR002953

Notes:

V-P065 - Effects of inflammatory signals and anti-inflammatory supplementation on inflammatory markers in pigs

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Session: Physiology or Immunology

Objective

Viral infection during gestation elicits the release of maternal inflammatory signals that can affect the immunological homeostasis of the offspring after birth. Anti-inflammatory supplementation during the gestation and lactation phases could minimize the disruption of immunological processes by maternal infection that, in turn, could impact organ function, health, and performance of the offspring later in life.

Methods

In the present study, we assessed the effects of porcine reproductive and respiratory syndrome virus (PRRSV) infection during gestation on the levels of pro- and anti-inflammatory cytokines in the blood of three-week-old female and male pigs. All sows received a corn and soybean meal-based diet that matched the nutritional requirements and half of the pigs were nursed by sows supplemented with docosahexaenoic and eicosapentaenoic omega-3 fatty acids during the gestation and lactation phases. A linear mixed-effects model including the effects of sex, supplementation, interactions, and random sow effect was used to describe the concentration of cytokines in serum.

Results

The serum concentration of the pro-inflammatory interleukin 18 was highest among pigs from non-supplemented sows. The level of the pro-inflammatory cytokine tumor necrosis factor alpha was lower in female pigs from PRRSV-inoculated sows that received supplementation relative to those from sows that were not supplemented. The concentration of interleukin 6 was higher in males from supplemented sows relative to males from sows that were not supplemented. This suggests a predominant anti-inflammatory role of interleukin 6 in the experimental conditions studied.

Conclusions

The findings from this study indicate that omega-3 fatty acids can modulate the long-lasting dysregulation of the inflammatory system elicited by viral infection during gestation. This study is supported by USDA NIFA AFRI, grant number 2018-67015-27413.

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

Notes:

V-P066 - Reduction in BRD cases and antibiotic use using NASYM and HIPRABOVIS SOMNI/Lkt in a commecial Polosh dairy farm

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Session: Physiology or Immunology

Objective

The aim of this case study was to measure the changes in BRD cases and antibiotics consumption using a combined NASYM (BRSV)– HIPRABOVIS® SOMNI Lkt (M. heamolytica & H. somni) protocol in a commercial dairy farm with known respiratory problems and to calculate the return on investment of the protocol used.

Methods

Vaccination protocol: NASYM intranasal followed by HIPRABOVIS® SOMNI/Lkt (SC), and NASYM intramuscular and HIPRABOVIS® SOMNI/Lkt (SC) as a booster vaccination. Treatment data was gathered from June 2019 up to Nov. 2020. Vaccination started in December 2019 and in January 2020 first calves completed the full protocol. The same BRD treatment protocol was used pre- and post-vaccination (5 days of antibiotics, NSAID and mucolitics). Pre- and post-vaccination amount of BRD treatments/cases were compared in order to estimate the impact of vaccination on disease. A proportion test was used to examine the impact of the vaccination using the statistical program package R v4.0. A probability level of $p < 0.05$ was chosen as the limit for statistical significance. For the calculation of the ROI the cost of vaccination, treatments and the estimated loss of future milk production were compared between a vaccinated and non-vaccinated group. For each BRD case, a loss of 233 kg of milk related to the first 305d lactation was considered (Schaffer et al, 2016) with a milk price of €0,308/l

Results

The average of BRD cases diminished significantly for all calves together from 23,3 before the start of vaccination to 3,7 per month after the completion of the first full vaccination protocol (p-value = 1.882e-05). BRD cases/treatments diminished significantly from 12,9 to 2,1/month (p-value = 0.002492), each accounting for a future milk production loss of 233 kg during the first 305 day lactation. Overall, it resulted in a ROI of 1:2,18 in favour of the vaccinated group.

Conclusions

The protocol showed a significant reduction in BRD cases, a reduction of antibiotics consumption of 84% and a ROI of 1:2,18 taking into consideration the cost of the treatments and the future losses in milk production

Notes:

V-P067 - Investigation of serum brain-derived neurotrophic factor (BDNF) levels in weaned piglets following transport

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Session: Physiology or Immunology

Objective

Describe brain-derived neurotrophic factor (BDNF) concentrations detectable in piglet sera, using a commercially available assay, following exposure to transport; and, assess if BDNF concentrations are correlated with other physiological indicators of stress and fatigue.

Methods

Eight male and eight female newly-weaned piglets (weaned 5 days prior) were randomly selected out of a subset of 21 piglets that were sampled. Blood samples were collected from the orbital sinus one day before and immediately after long commercial transport events (>30 hours). A complete blood count, serum biochemistry, and BDNF assay were conducted. The change in piglet serum BDNF, serum cortisol, and neutrophil to lymphocyte ratios (N:L) before and after transport were evaluated using paired t-tests for parametric (cortisol) and non-parametric (BDNF, N:L) data. Spearman correlation coefficients between BDNF and indicators of stress (serum cortisol, N:L, white blood cell count (WBC)) and muscle injury (creatine kinase (CK), aspartate aminotransferase (AST) and blood lactate) were calculated.

Results

Serum BDNF levels increased following transport with median values rising from 268.06 pg/mL to 496.15 pg/mL ($P<0.01$). This corresponded to decreasing average serum cortisol values from 122.06 nmol/L to 55.28 nmol/L ($P<0.01$) and median N:L values from 1.29 to 0.79 ($P=0.02$). A strong negative correlation was observed between serum BDNF and N:L at the pre-transport timepoint ($r_s(14)= -0.75$, $P<0.01$). Correlations between BDNF and the other included indicators of stress and muscle injury were not significant at either timepoint.

Conclusions

This study demonstrates that serum BDNF detection is feasible using a commercially available assay and may be a useful marker to measure piglet stress response. The change in BDNF levels was biologically logical and inversely related to changes in serum cortisol and N:L values. The results support the interpretation that piglet stress was higher at the sample timepoint before transport compared to after.

Financial Support
Swine Innovation Porc

Notes:

V-P068 - NASYM triggers both local and systemic cellular immune responses in newborn calves after intranasal administration

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Session: Physiology or Immunology

Objective

Newborn calves were studied after intranasal application of NASYM®, a modified live vaccine (MLV), and after challenge with a heterologous BRSV strain, with the aim of characterising the immune response.

Methods

Calves with <14 days of age were distributed randomly into 'Vaccinated' (NASYM®) and 'Control' (PBS) groups, including at least seven seronegative animals per group. Immunological testing characterised different immune responses: (i) specific IgA antibodies against BRSV in nasal secretions; (ii) serum antibodies against BRSV; and (iii) cellular response (IFNγ production). After vaccination, the animals were sampled and immunological parameters were monitored weekly for 3 weeks and 2 months (Trial 1 and Trial 2, respectively). Animals were challenged 3 weeks after vaccination with a European strain (Trial 1) or after 2 months with an American strain (Trial 3). Immunological testing was repeated after the challenge.

Results

Intranasal vaccination induced an increase in local IgA response in the upper airways from 2 weeks and detectable 8 weeks after vaccination, in addition to a systemic cellular (IFNγ) response 1-4 weeks after vaccination. Once the animals had been infected with either American or European challenge strains, vaccinated animals produced a statistically significant increase in IgA content in nasal secretions 1-2 weeks after challenge ($p < 0.05$). This kind of response may neutralise BRSV locally, preventing it from entering the target respiratory tissues and protecting animals against viral infection. Additionally, although no antibodies were detected in sera with intranasal vaccination, a significant serological anamnestic response appeared 2 weeks after challenge ($p < 0.05$).

Conclusions

In conclusion, intranasal vaccination (NASYM®) of newborn animals induced local IgA production and a systemic cellular response, and according to the booster response observed after challenge, it also primed lymphocytes. All of these mechanisms may contribute to protecting animals against BRSV infection.

Notes:

V-P069 - NASYM triggers both humoral and cellular immune responses in young calves after intramuscular administration

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Session: Physiology or Immunology

Objective

The main goal of this work was to characterise the immune response triggered by a live attenuated BRSV vaccine (NASYM®) after intramuscular administration in young calves.

Methods

Two studies were conducted. In the first study (OOI), animals were challenged with a heterologous BRSV strain 21 days after vaccination with NASYM®. In the second study (DOI), animals were challenged 4 months after vaccination. BRSV seronegative animals were selected and randomly distributed into two groups: vaccinated (NASYM®) and control (PBS). The vaccine or PBS were applied intramuscularly (2 mL) at about 10 weeks of age and again 4 weeks later. The immune response was studied after vaccination and challenge: BRSV-specific antibodies in serum and cellular response (IFNγ production).

Results

A serologic response was already observable one week after completion of the vaccine schedule, reaching its peak 3 and 4 weeks after vaccination in the OOI and DOI studies, respectively. However, the serological titres of BRSV antibodies decreased two months after vaccination. Nevertheless, an anamnestic response was observed in the vaccinated groups after challenge, reaching significantly higher titres of serum antibodies compared to the control groups. A cellular response was already observable after the first dose in vaccinated animals (DOI) and also seen after completion of the vaccination schedule (OOI). After challenge, a cellular response was observed in both vaccinated and control groups, but it was higher in the vaccinated groups finding a significant increase that peaked 7 to 10 days after challenge.

Conclusions

After intramuscular vaccination (NASYM®), a systemic immune response was triggered, including both humoral and cellular responses. Although the humoral response was not maintained throughout the four-month period, an anamnestic response was observed after challenge. Similarly, a cellular response was observed after vaccination and was also boosted to significant levels after challenge. Therefore, this vaccine triggers both local and systemic responses, conferring short and long-term protection against BRSV.

Notes:

V-P070 - Effects of an antiviral and ibuprofen treatment on NETs in a model of bovine respiratory syncytial virus infection

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Session: Physiology or Immunology

Objective

The function of neutrophils in viral infections has long been established and studies have been done to examine the role of neutrophil extracellular traps (NETs). Further study and analysis of NETs in viral infections may reveal a new therapeutic target. Administration of ibuprofen and GS-561937, a fusion protein inhibitor (FPI), was experimentally shown to decrease the severity of bovine respiratory syncytial virus (BRSV) infection. In this study, our aims were to determine the effect of ibuprofen and FPI on NETs in the lung after BRSV infection as a monotherapy or combined therapy.

Methods

We conducted a randomized placebo-controlled trial of ibuprofen, FPI, or as a dual therapy initiated at 3 or 5 days after experimental infection with BRSV in 36 five to six-week-old Holstein calves (Bos Taurus). Lung tissue samples were collected and stained with antibodies conjugated with fluorescence dyes to visualize and quantify the NETs in situ. We estimated the average NETs in the sample lung tissue slides and compared the areas occupied by NETs within and between the treatment groups.

Results

There were significantly fewer NETs in the lung tissue from calves that were given ibuprofen alone (p=0.012) and both ibuprofen and fusion protein inhibitor (p=0.034)when treatment was initiated on day 3 post infection compared to the placebo group. Calves treated with ibuprofen, fusion protein inhibitor or both from day 5 had visually fewer NETs than the placebo but the difference was not significant.

Conclusions

In conclusion, Ibuprofen and FPI, on average, decrease clinical severity of illness and NETosis in a bovine model of RSV but should be used together rather than as monotherapy. We also speculate that there is need for early diagnosis of diseases exacerbated by NETs for the therapy to be effective since timing of treatment initiation is critical for competing with the inflammatory response. Our work underscores the necessity for more studies on compounds that influence NETs formation.

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

Notes:

V-P071 - Immune response to influenza D virus (IDV) in calves previously inoculated with bovine viral diarrhea virus (BVDV)

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Session: Physiology or Immunology

Objective

Acute BVDV infection leads to thymus depletion in calves and possible altered immune responses to subsequent infections. In this ongoing study, we are evaluating the immune responses to IDV in calves previously inoculated with BVDV.

Methods

Twenty BVDV-IDV-naïve colostrum deprived calves about 3-week-old were allocated into 4 groups of 5 animals. Animals in G1 and G2 were inoculated intranasally with BVDV2 (10^6 $TCID_{50}$/ml), while G3 and G4 were BVDV mock-inoculated. On day 13, animals in the G1 and G3 were euthanized and necropsied. On day 21, animals in G2 and G4 were inoculated intranasally with IDV (10^6 $TCID_{50}$/ml). Animals in G2 and G4 were euthanized and necropsied on day 42. The thymus depletion was inferred based on the mass ratio of the thymus and kidney. Lymphocyte proliferation and intracellular cytokine staining assays evaluated the cellular immune responses to BVDV and IDV in peripheral blood mononuclear cells (PBMC).

Results

RT-qPCR detected BVDV and/or IDV in nasal swabs and/or serum of all infected animals. The thymus mass in the BVDV acute infected animals (G1) was about 50% decreased compared to the control animals (G3). The thymus in the IDV infected animals (G4) was comparable to the control. Two out of the 5 calves in G2 (BVDV+IDV) demonstrated thymus depletion (about 60% reduction) at day 42. The T cell proliferation and percentage of interferon-gamma (IFN-γ) producing cells in the PBMC collected at day 21 post-IDV infection (study day 42) suggest decreased number of calves responding to IDV in the BVDV+IDV animals (G2) compared to G4. Additionally, the overall IFN-γ responses are decreased in G2. Moreover, the two calves in G2 with the highest level of thymus depletion were the animals with the lowest T cell proliferation response.

Conclusions

Results suggest that BVDV-inducted thymus depletion may be transient. However, persistent thymus depletion (up to day 42) was noted in 40% of the calves. Additionally, animals with prolonged thymus depletion had the weakest Tcell proliferation and suggested the prolonged effect of BVDV on the immune system of calves.

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

Notes:

V-P072 - Effect of contaminated gloves in horizontal transmission of parvovirus in mink farming

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Session: Physiology or Immunology

Objective

Aleutian mink disease virus (ADV) can be found in bodily fluids such as saliva, blood, urine and feces of infected mink, and can persist for a long period of time in a contaminated environment (cages, soil, gloves, boots) serving as an indirect mode of viral transmission within and among mink farms. It is known that horizontal transmission via contaminated gloves occurs, but the extent of transmission has not been well characterized. It is unknown how much of a viral load remains on a farm worker's gloves after handling an infected animal, and how long before the viral load would be diluted after the handing of uninfected animals. This study utilized a parvovirus non-harmful to mink (feline panleukopenia virus) to examine and quantify the dilution of viral load on contaminated gloves after handling a series of uninfected animals.

Methods

Viral inoculum were applied to the fur coat of an animal, and workers with gloved hands handled the contaminated animal to purposely contaminate the gloves before working with animals in the farm. Sampling of gloves was done by swabbing the surface of the gloves, and swabs were immersed in viral transport media for use in PCR assay.

Results

When starting with high viral load (mimicking viral load found in infected tissue), PCR was able to pick up viral DNA even after handling 1200 animals. At a much lower dilution, PCR was consistently positive after the handling of 500 animals.

Conclusions

The preliminary data provide evidence of horizontal transmission of viral DNA via contaminated gloves. This data coupled with a better understanding of the extent of viral shedding in bodily fluids would support the feasibility of using the glove swabbing method as a surveillance tool for ADV contamination on farms.

Notes:

V-P073 - Norepinephrine influences the growth and virulence of *Clostridioides difficile*

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Session: Systemic and mucosal immunology

Objective

The United States Centers for Disease Control and Prevention (CDC) classified infections with *Clostridioides difficile* (formerly known as *Clostridium difficile*) as an urgent threat to the public. The CDC's decision was based on the considerable number of infections that exceeded 223,000 hospitalizations and 12,800 death cases in 2017, with estimated attributable healthcare costs of $1 billion in the US solely. Norepinephrine (NE), the stress-associated neuroendocrine hormone, modulates the bacterial behavior of several Gram-positive and Gram-negative bacterial species, including *Staphylococcus*, *Escherichia coli*, *Salmonella* and *Vibrio cholerae*. The current study investigates the response of *C. difficile* to norepinephrine in growth and virulence.

Methods

BHIS media with and without NE was used for constructing the growth curve of C. difficile strains. Quantitative real-time PCR analysis was performed for analysis of virulence gene expression between NE treated and untreated bacteria. Antibacterial activity of the anticlostridial drug was determined by microdilution assay.

Results

NE enhanced the growth of many strains of *C. difficile* and, the expression of virulence genes (*flagellin*, *tcdA*, *tcdB*, *spo0A* and several pilin gene such as *pilA*, *pilJ*, *pil5* etc), was upregulated in three strains of *C. difficile* in the presence of NE. Also, the presence of norepinephrine enhanced the antibacterial activity of the anticlostridial drug, fidaxomicin.

Conclusions

The response of *C. difficile* to NE (and other catecholamines) warrants further investigation. Modulating this response can reduce the virulence of *C. difficile*, yielding benign bacteria that can be controlled by normal bacterial flora or host immunity.

Notes:

V-P074 - Functional potential of *Brucella*-specific T cells following RB51 vaccination

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Session: Vaccines and vaccinology

Objective

We have previously developed an *in vitro* assay to expand *Brucella*-specific T cells and subsequently assess proliferation and interferon gamma (IFN-g) production concurrently. This method has allowed us to enhance the detection of *Brucella*-specific responses and further characterize the functional potential of T cells following vaccination of cattle with *Brucella abortus* strain RB51.

Methods

Using peripheral blood mononuclear cells (PBMC) from RB51-vaccinated cattle, we assessed RB51-specific responses *in vitro* over the course of vaccination via concurrent measure of proliferation and cytokine production using flow cytometry. Additionally, we tested how antigen availability modulates these two functional phenotypes.

Results

The data presented here demonstrate the presence of two distinct populations of RB51-specific T cells: one that proliferates in response to antigen and one that proliferates and produces IFN-g. While proliferating-only cells have the ability to produce IFN-g, this is only observed following re-stimulation with a pan-T cell stimulator. Additionally, we observed that variation in the amount of antigen availability during *in vitro* stimulation, results in the loss of functional potential starting with IFN-g production first and then proliferation.

Conclusions

The information presented here provides further insights into the T cell response following RB51 vaccination in cattle. These data suggest that there are differences in the functional protentional or RB51-specific T cells, which may be partially driven by antigen availability.

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

Notes:

V-P075 - US-UK Collab: Influence of imperfect vaccines, host genetics, and pathogen mutation rates on infectious diseases

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Session: Vaccines and vaccinology

Objective

It is important to understand how viruses are transmitted and evolve. It's been argued that imperfect vaccines or host genetic resistance may alter the balance of selection between pathogen transmission and virulence by allowing a few more divergent but still virulent strains to be transmitted at reduced cost. Our objectives are to 1) determine the influence of imperfect vaccines and host genetics on transmission and evolution to higher virulence; 2) validate viral genome polymorphisms associated with increased virulence; 3) build data-informed evolutionary-epidemiological simulation models to develop strategies to control the ecology, evolution and economic burden of Marek's disease (MD); and 4) disseminate information on Marek's disease virus (MDV) and infectious bronchitis virus (IBV), and the impact of vaccination to the public through training, workshops, online videos, etc.

Methods

This project initiated on Jan. 1, 2021, thus, only Objective 1 is described. We are using a shedder-sentinel challenge model to naturally passage MDV through 10 successive groups. Each group consists of 10 birds kept in an individual isolator and replicated at least 3 or 6 times. Viral replication and transmission are assessed by sampling shedder (donor) birds that transmit infectious virions prior to, at, and following co-housing with the contact (recipient) birds. Birds infected in Passage 1 transmit virus to recipients in Passage 2, and so on.

Results

Presently, we've completed 4 serial passages of MDV in vaccinated or unvaccinated chickens. MD incidence has been maintained at high incidence through passage of the virus through unvaccinated chickens.

Conclusions

Preliminary results suggest that MDV is being transmitted through serial passage in both vaccinated and unvaccinated chickens. We are expecting that virulence will increase as we progress to later passages. We are also preparing to begin the next stage which is identical to the experiment describe above, except groups will differ based on host genetics instead of vaccination status.

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

Notes:

V-P076 - Enhancing the production of type I interferons to create rationally-defined Marek's disease vaccines

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Session: Vaccines and vaccinology

Objective

Marek's disease (MD) is an oncogenic disease of poultry caused by Marek's disease virus (MDV). MD is controlled by vaccination with a live attenuated virus, but novel strains of MDV will likely emerge and break the vaccinal protection provided by existing vaccines. Viruses encode gene products which inhibit secretion of the type I interferons (IFN-Is). We will ablate MDV genes which frustrate the production of IFN-Is during infection to create vaccine strains with improved protection. Our collaboration with the Boeke laboratory (NYU-Langone) will give us access to rapidly-assembled MDV viral genomes. This proposal will result in new and more protective vaccine strains for MDV.

Methods

In the initial stage of this proposal we will create an HD-11 cell line which expresses nano-luciferase under control of the native IFNB/IFNW1 control elements. This cell line will be used to test MDV mutants for increased production of IFN-Is upon infection. The Boeke laboratory has started viral mutagenesis on the rMd5B40 clone provided.

Results

The Dunn laboratory has verified the infectivity of the MDV rMd5B40 BAC. We are producing HD11 cells which contain a nano-luciferase reporter driven by the native IFNB control elements. This cell line will be used to test the rMd5B40 constructs produced by the Boeke laboratory. The Boeke lab is currently resequencing the rMd5B40 BAC. To enable the editing of the BAC rMd5B40, they have focused on designing a version that is able to replicate in yeast, enabling homologous recombination-mediated cloning. Specifically, they plan to insert a fluorescent reporter in one of the *meq* sites and a CMV-driven nano-luciferase in the OTHER *meq* site. The BACs will be recovered and returned to evaluate infectivity and IFN-I production.

Conclusions

We anticipate that this project will result in several new and highly effective vaccine candidates for MDV.

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

Notes:

V-P077 - General farm performance as a tool to reveal the subclinical impact of IBD and to improve Gumboro vaccination strategy

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Session: Vaccines and vaccinology

Objective

The purpose of this study was to evaluate the best vaccination programme against Gumboro disease (IBD) in broilers based on productive parameters, in the context of the subclinical form of the disease.

Methods

Historical zootechnical data from a company that used two types of IBD vaccine (HIPRAGUMBORO® CW and HIPRAGUMBORO® G97, Hipra) from August 2017 to April 2019, were analysed. In total, 4.9 million broilers were included in the analysis. Farms were separated into 3 ranking groups according to their performance results (EEF). Within each ranking group, farms were classified according to the history of vaccine use in the same building: CW (HIPRAGUMBORO® CW only); G97 (HIPRAGUMBORO® G97 only); G97&CW (either HIPRAGUMBORO® CW or HIPRAGUMBORO® G97). Several productive parameters and economic results were compared between vaccine groups within each ranking.

Results

Statistical differences in Ranking 1 were observed in % mortality after 10 d, which was lower in the case of farms using CW, and also in antibiotic consumption (ALEA), with a decrease in farms using only G97. In Ranking 2, differences were observed in Average Daily Gain (ADG), this being higher in farms vaccinated with G97 alone. Finally, in Ranking 3, differences were found in ADG, ALEA and EEF parameters, showing better results with the use of vaccines alone (specially with G97) compared with the combination.

Conclusions

In the context of subclinical IBD, the use of intermediate plus vaccines, such as HIPRAGUMBORO® G97, may be the best approach for farms with the worst performance classification, while in better performing farms, intermediate vaccines, such as HIPRAGUMBORO CW, would be the most appropriate selection. Farms combining both options seem to be the worst option independently of the performance classification.
In the view of these results, general performance of the farms may be an important and practical criterion to take into account for decision-making on the IBD vaccination programme.

Notes:

V-P078 - Cellulose nanomaterials: A novel adjuvant and delivery system for aquaculture vaccine applications

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Session: Vaccines and vaccinology

Objective

Disease outbreaks are a major impediment to aquaculture production and are forecasted to continue as the industry grows and the climate warms. Vaccines are integral for disease management in aquaculture but they can be expensive, vary in effectiveness, and come with adjuvant-induced adverse effects causing fish welfare issues and negative economic impacts. The goal of this interdisciplinary project is to develop a new generation of safe, efficacious, and cost-effective vaccines for sustainable aquaculture. Our project will use novel nanomaterials produced from renewable wood fiber as depots/adjuvants in vaccine formulations to modulate the immune response of Atlantic salmon in a biocompatible, environmentally friendly, and cost effective manner.

Methods

Specifically, we will establish the role of CNM as a vaccine depot and mobile immunostimulant, the extent and location of CNM migration *in vivo*, and the efficacy of CNM bound antigen as a immunostimulant for protection against two serious Atlantic salmon pathogens. To accomplish this, our interdisciplinary research team will: 1) prepare and conduct *in vitro* characterization of CNM hydrogels and CNM/antigen (vaccine) formulations by using fluorescent CNM variants (CNM-FL) and i*n vivo* durability and migration using confocal FL microscopy. 2) Conduct *in vivo* studies to quantify the antibody kinetics in vaccinated fish serum using enzyme-linked immunosorbent assays, and 3) Evaluate the efficacy of the CNM vaccine(s) in protecting against *Vibrio anguillarum* in Atlantic salmon by performing a pathogen challenge study.

Results

Results will be determined by comparing the performance of CNM formulations in stimulating an antibody response and efficacy in preventing disease mortalities to that of a current commercially available vaccine and a negative vehicle control.

Conclusions

It is anticipated the CNM depot/adjuvant vaccine formulations will perform as well or better than current commercially available vaccines while being more cost-effective and sustainable for long-term aquaculture.

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

Notes:

V-P079 - Improved vaccine platforms for safe and effective control of Bovine Viral Diarrhea Virus

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Session: Vaccines and vaccinology

Objective

Determine whether cattle immunized with BVDV elicits $CD8^+$ T cells against epitopes conserved among diverse strains

Methods

Bovine Viral Diarrhea Virus (BVDV) is an important pathogen that plays a significant role in causing Bovine Respiratory Disease Complex (BRDC). The goal of this study was to evaluate BVDV-specific $CD8^+$ T cells in immunized cattle and identify cognate epitopes. Eight steers were immunized by intranasal infection with BVDV-1b strain (CA401186a) and four weeks later, four were boosted with gamma-irradiated BVDV-1b (TGAC) while the other four were boosted with BVDV-2a (A125) strain. Purified $CD8^+$ T cells from splenocytes and autologous $CD14^+$ monocytes pulsed with either irradiated BVDV-1b TGAC or BVDV-2a A125 were used to elucidate antigen-specific IFN-γ-secreting T cells by EliSpot assay. Two steers immunized with TGAC and one steer immunized with A125 had the highest number of TGAC- and A125-specific IFN-γ^+CD8^+ T cells, respectively. Thus, $CD8^+$ T cells and $CD14^+$ monocytes from these steers were used to screen twenty pools of 10 peptides by EliSpot as above. The 9-mer peptides were predicted from full-length BVDV polyprotein using *BoLA*-I alleles to select putative $CD8^+$ T cell epitopes. MHC-I peptide presentation was validated by using blocking mAbs.

Results

Eight epitopes that induced potent IFN-γ^+CD8^+ T cell responses were identified from E^{rns}, E1, and E2 glycoproteins. In addition, twenty epitopes were identified from N^{pro}, NS2-3, NS4A-B, and NS5A-B. Majority of the epitopes are highly conserved among more than two hundred BVDV-1 and -2 genotypes. The conserved epitopes were also validated as cross-reactive since they induced high recall IFN-γ^+CD8^+ T cell responses, *ex vivo*, in purified bovine $CD8^+$ T cells isolated from BVDV-1- and -2-immunized cattle. The epitopes were shown to be MHC-I-restricted by using blocking mAbs.

Conclusions

The findings support feasibility for the development of a safe, effective, and broadly protective $CD8^+$ T cell-based BVDV subunit vaccine capable of addressing virus heterogeneity more effectively than current vaccines.

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

Notes:

V-P080 - Efficacy of prototype live-vectored African swine fever virus vaccines

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Session: Vaccines and vaccinology

Objective

Evaluate protective efficacy of adenovirus-vectored ASFV antigen cocktails in pigs

Methods

The African Swine Fever Virus (ASFV) poses a serious threat to the pork industry, but there is no safe vaccine or treatment available. Emerging evidence support a role for cytotoxic T lymphocytes (CTLs) in clearance of infected cells. Thus, development of a protective subunit vaccine requires empirical identification of cognate antigens. The ASFV pp220 polyprotein, encoded by a 7.4 kb gene, is critical for virus production. The polyprotein is processed to generate p14, p34, p37, and p150 individual proteins with the last one being the largest subunit. We have shown that immunization of pigs with a cocktail of adenoviruses expressing the proteins, induced robust IgG, IFN-γ T cell, and strong cytotoxic T lymphocyte (CTL). To identify T cell epitopes, pools of predicted *SLA*-I binding 9-mer peptides were screened by IFN-γ EliSpot assay using PBMCs and splenocytes from the immunized pigs. Individual peptides from positive pools were then evaluated for inducing IFN-γ^+ PBMC and splenocyte recall responses.

Results

Four peptides, namely p34 (161-169), p37 (859-867), p150 (1363-1371), and p150 (1463-1471), recalled strong IFN-γ^+ PBMC and splenocyte responses. Peptide p34 (161-169) was recognized by PBMCs isolated from 7/10 pigs and by splenocytes isolated from 8/10 pigs. Peptides p37 (859-867) and p150 (1363-1371) stimulated recall IFN-γ responses in PBMCs and splenocytes isolated from 8/10 pigs, whereas peptide p150 (1463-1471) recalled responses in PBMCs isolated from 7/10 pigs and splenocytes isolated from 9/10 pigs, respectively.

Conclusions

The pp220 polyprotein contains multiple T cell epitopes that induced robust IFN-γ responses in commercial pigs. Notably, these epitopes are conserved among different ASFV genotypes and were predicted to bind different *SLA*-I alleles. These outcomes and our previous demonstration that pp220 induces strong IgG and CTL responses suggests that pp220 is a promising candidate antigen for inclusion in a prototype vaccine.

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

Notes:

V-P081 - PARTNERSHIP: Single-cycle replicon-based African swine fever virus subunit vaccine

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Session: Vaccines and vaccinology

Objective

Evaluate protective efficacy of a live-vectored replicons encoding multiple ASFV antigens.

Methods

African Swine Fever Virus (ASFV) is a major swine pathogen but there is no vaccine or treatment available. Protective immunity depends on antibody and probably cytotoxic T lymphocytes (CTLs). Identification of protective antigens is needed for development of subunit vaccines. Immunization of pigs with adenovirus expressing a few antigens induced CTL responses, but protective efficacy was low. To improve efficacy, putative CD8+ T cell targets were selected based on predicted peptide binding to defined *SLA*-I alleles. Recognition of the predicted peptides by T cells from pigs immunized with ASFV antigens was validated by IFN-γ EliSpot. Genes encoding FLAG-tagged multicistronic expression cassettes were synthesized and protein expression was evaluated by using anti-FLAG mAb. Antigen authenticity was validated using ASFV convalescent serum. The cassettes were then used to generate recombinant adenoviruses. Pigs were immunized with adenovirus cocktail, with and without adjuvant, and then challenged with ASFV (Georgia 2007/1).

Results

The adenovirus-expressed antigens were recognized by ASFV convalescent serum and the putative CD8+ T cell epitopes were recognized by T cells from pigs immunized with ASFV antigens. Only one out of five pigs immunized with the virus cocktail without adjuvant survived until study termination 30 days post-challenge. The pig cleared challenge virus and regained weight. All pigs in the other treatment and control groups succumbed within two weeks post-challenge.

Conclusions

The adenoviruses encoding multicistronic expression cassettes expressed ASFV antigens containing putative CD8+ T cell epitopes. This vector was used to conduct a pilot pig study while generation of replicons encoding the target antigens was being optimized. Pigs immunized with the recombinant virus cocktail, but with no adjuvant, performed better than pigs that received adjuvanted cocktail. A prototype vaccine formulated using replicons expressing the rationally selected $CD8^+$ T cell targets has potential to induce ASFV-specific CTL responses in pigs and thereby confer protection.

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

Notes:

V-P082 - Novel bovine herpes virus type 1 vectored vaccine against Rift Valley fever virus infections in cattle and sheep

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Session: Vaccines and vaccinology

Objective

Rift Valley fever virus (RVFV) is an emerging pathogen that maintains high biodefense priority based on its threat to cattle and sheep, its ability to cause human hemorrhagic fever, and its potential for aerosol spread. Currently available vaccines have either safety issues or are not desirably efficacious. We have developed a bovine herpesvirus 1 quadruple mutant virus (BoHV-1qmv) vector that lacks virulence and immunosuppressive properties. We showed that BoHV-1qmv replicates in the nasal mucosa of calves and sheep and induces BoHV-1-specific neutralizing antibody as well as cellular immune responses. Additionally, BoHV-1qmv vectored bovine viral diarrhea virus (BVDV)-specific subunit vaccine protects vaccinated calves against virulent BVDV challenge. Our objective in this project is to i) Generate and characterize *in vitro* a BHV-1qmv vectored RVFV-subunit vaccine (RVFV-sub) that expresses chimeric RVFV envelope glycoproteins, Gn ectodomain (eGn) fused with Granulocyte-macrophage colony-stimulating factor (GM-CSF) and Gc, ii) Evaluate the neutralizing and cellular immune responses induced by the BHV1qmv/RVFV-sub in cattle and sheep following immunizations, and iii) Determine nasal virus shedding property following latency-reactivation of the RVFV-sub vaccine virus in the trigeminal ganglia of the vaccinated animals.

Methods

We have constructed RVFV Gn-GMCSF and Gc chimeric genes and incorporated them into BoHV-1 gG-deletion and gE-CT/Us9-deletion plasmid vectors, respectively. Currently, BoHV-1 qmv full-length genomic DNA co-transfection with the resulting RVFV Gn-GMCSF and RVFV Gc- insertion vector DNA is in progress. Putative BoHV-1 qmv expressing the RVFV chimeric genes will be isolated, plaque purified, and tested for the chimeric Gn and Gc expression and maturation in BoHV-1qmv/RVFV-sub-infected cells *in vitro*.

Results

We successfully designed, synthesized, and validated the protein expression of chimeric RVFV-Gn-GMCSF and Gc *in vitro*.

Conclusions

This is the Year 1 of the project. We expect to generate and characterize the BoHV-1qmv/RVFV-sub vaccine virus soon.

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

Notes:

V-P083 - Bovine respiratory syncytial virus experimental calf infection – BRSV vaccine challenge model

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Session: Vaccines and vaccinology

Objective

Bovine respiratory syncytial virus BRSV is a major player in the Bovine Respiratory Disease Complex (BRDC) resulting in significant economic losses. Besides its relevance for veterinary medicine, BRSV is closely related to human respiratory syncytial virus (RSV), responsible for severe respiratory disease in infants, young children, and the elderly, to which no vaccine is currently available. Our goal was to establish a reliable infection model for BRSV in calves, allowing the test of single cycle RSV vaccines developed at OSU, as well as host-interaction studies. In the present study, calves were infected with BRSV and observed for clinical signs and pathology development for up to 10 days.

Methods

Six ~16-week-old colostrum-deprived crossbred calves were inoculated with BRSV (strain 375). Each animal received ~10^5 TCID by aerosol. Calves were monitored daily for clinical signs (including cough, respiratory movements, increased temperature, nasal/ocular discharge, body temperature, behavior, and feces). Euthanasia occurred on days 7 and 10 post-infection (3 calves each day). Extent of gross pulmonary lesions were scored as follow: 0 = free of lesions; 1 = 1–5% affected; 2 = 5–15% affected; 3 = 15–30% affected; 4 = 30–50% affected; 5 = >50% affected). Tissues were also collected for histological assessment.

Results

Infected calves developed upper respiratory tract disease and displayed mild clinical signs, including cough, nasal discharge, and increased respiratory rate, evident from day 6 post-infection to the end of the experiment on 10 dpi. Animals necropsied on day 7 demonstrated nasal congestion and inflamed nasal mucosal. Mild lung involvement was also present in 2 out of the 3 calves (scores 1-2). On day 10 post-infection, upper respiratory tract pathology and lung involvement were observed in all 3 calves (scores 2-4).

Conclusions

We were able to induce mild to moderate BRSV associated clinical disease and pathology in 12-week-old crossbreed calves. Pathological findings were more pronounced at day 10 post-infection and were present on both upper and lower respiratory tract.

Financial Support
Oklahoma State University

Notes:

V-P084 - The effects of vaccination and vaccine type on the prevalence of BVD in South African communal and commercial herds

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Session: Vaccines and vaccinology

Objective

The aim of this study is twofold, showing that there are differences in the prevalence of diseases in different farming scenarios and that the type of vaccine chosen has different efficacies.

Methods

A total of 1206 unvaccinated animals between the ages of 6mo and 3y, consisting of 230 communal animals (unvaccinated open herds) and 976 commercial animals (vaccinated with MLV, KV and unvaccinated closed herds) from throughout South Africa were sampled. The sample sizes were determined as follows: 10% of the herd if below 350 animals and 6-8% of the herd if over 350 animals in the herd. Blood was collected in red top tubes and spun down for the serum (fresh or frozen). The samples were run by an independent laboratory using the HIPRA ELISA kits. The titre levels of the individual animals were then ranked as negative, suspect positive and positive. For the purpose of these results only strong positive cases were taken as positive exposure and circulation of the viruses.

Results

Historic prevalence data from RSA, illustrate that commercial herds had a high prevalence of BVD, ranging from 79,85% to 96% with an outlier of 36,8%. We see that the commercial sector has changed over time (82,93% down to 35%) with closing the herds and implementing different vaccination strategies, seen as lower titre levels ranging from 17% to 39% depending on the type of vaccine. MLV still have a high prevalence of BVD, close to that of the unvaccinated animals

Conclusions

The difference between sectors and vaccine types has a noticeable effect on the risk from circulating viruses. This is most noticeably seen with the KV having the lowest BVD titres when compared to MLV and unvaccinated animals. In conclusion KV provide a safe and effective holistic approach to control and reduce BVD in multiple farming sectors

Notes:

V-P085 - Use of emergency vaccination with a live attenuated BRSV vaccine (NASYM) as an aid to control onfarm BRD outbreaks

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Session: Vaccines and vaccinology

Objective

Veterinary practitioners apply emergency vaccination against Bovine Respiratory Syncytial Virus (BRSV) to aid stopping BRD outbreaks. This study was meant to describe the dynamic of the disease in farms using a live attenuated BRSV vaccine (NASYM) in the face of a BRD outbreak.

Methods

Five farms which used NASYM in face of a BRD outbreak during 2020 were recruited in a retrospective observational study. Data of the vaccination, the diagnostic of the disease, the antimicrobial and anti-inflammatory drugs use which concerned the BRD outbreak period were retrospectively retrieved. An animal was considered ill when a treatment was administered for therapeutic purposes. Based on this criterion, two farms were excluded from the study because they used drugs for prophylaxis and metaphylaxis purposes. Considering the rapid spread of the disease within a herd in a BRD outbreak and no antiviral drugs are available against viral pneumonia, intramuscular vaccination with NASYM was promptly applied before knowing the laboratory results and thus just based on the clinical signs although two out of three farms performed PCR from nasal swabs and later confirmed the presence of BRSV.

Results

The disease outbreak showed a bell-shaped dynamic in all three farms and involved 19.48%, 46% and 52.50% of the animals respectively. Notably, almost all of these cases were detected during the 3-4 days before vaccination and no new cases were observed from the day after and up to a week later except for one: specifically, 2 farms showed no new case of disease and one farm showed a single new case at 6 days post vaccination.

Conclusions

The data indicated the BRD outbreaks had a rapid course and left little time to rationally decide the therapeutic approach. Vaccination was used as an emergency treatment because of the suspected involvement of BRSV in these BRD outbreaks. The present study showed a sudden concomitant decrease of new disease cases after the administration of the vaccine. Prevention with vaccination should be a recommended rational strategy to minimize the effect of BRSV on the BRD.

Notes:

V-P086 - Efficacy of novel staphylococcal surface protein vaccines against mastitis in dairy cattle

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Session: Vaccines and vaccinology

Objective

Mastitis, the most common disease in dairy cattle, is mainly caused by *Staphylococcus*, *Streptococcus,* and coliform bacteria. Staphylococcal mastitis is prominently a result of *S. aureus* and non-aureus staphylococci (NAS) and is difficult to control by antibiotic treatment due to the low cure rate and progression to chronic form as well as the lack of an effective vaccine. Therefore, sustainable staphylococcal mastitis control tools, such as an effective vaccine, are needed. Study objectives were 1) evaluation of immunological responses of dairy cows vaccinated with *S. aureus* surface protein (SASP) and *S. chromogenes* surface protein (SCSP) vaccines, 2) determine the efficacy of induced immunity against staphylococcal mastitis.

Methods

Dairy cows (n=45) in their 1st or 2nd lactation were randomized to one of three groups: SCSP (n=16), SASP (n=15) or control (n=14). Cows in the SCSP and SASP groups were vaccinated subcutaneously with 1.2 mg of SCSP or SASP vaccines mixed with Emulsigin-D® adjuvant, respectively at drying (D0), D21, and D42 after drying. Control cows were injected with PBS (pH 7.4) mixed with Emulsigin-D® at similar time points. Serum and milk antibody titers (IgG, IgG1, IgG2, and IgA) were measured by ELISA. The efficacy of induced immunity was monitored by measuring milk somatic cell count (SCC), bacterial count, mastitis status, and milk yield.

Results

Vaccination with SASP or SCSP induced significantly increased serum and milk anti-SASP and -SCSP antibodies. Vaccination did not have any effect on milk production. Clinical staphylococcal mastitis was not observed in any of the cows, but some cows developed subclinical mastitis during the follow-up period. Mean SCC was significantly (P<0.001) higher in the unvaccinated cows at calving but return to the baseline level 7 days after calving.

Conclusions

In conclusion, the SCSP and SASP vaccines are immunogenic, and vaccinated cows did not develop clinical staphylococcal mastitis but some developed subclinical mastitis. Thus, with optimization of vaccination regimen SASP and SCSP vaccines are promising effective vaccine.

Financial Support
University of Tennessee

Notes:

V-P087 - Respiratory disease and lung pathology in calves co-infected with bovine viral diarrhea virus and influenza D virus

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Session: Virology

Objective

Bovine viral diarrhea virus (BVDV) is a major contributor to bovine respiratory disease complex. Cattle are the natural reservoir of the recently discovered Influenza D virus (IDV), but little is known about its pathogenic potential. IDV pathogenesis studies report subclinical to mild respiratory disease or lung pathology in calves infected with IDV only. We aimed to evaluate the pathogenic potential of IDV in calves previously infected with BVDV.

Methods

Fifteen ~4 week-old, crossbred, colostrum deprived calves were allocated into three groups: IDV inoculated 3 days after BVDV inoculation (n=5), IDV inoculated 6 days after BVDV inoculation (n=5), and control group (n=5). Infected groups received nasal BVDV and nasal and aerosol IDV (2.5 ml/nostril, 6.0 log10 TCID/ml). Calves were monitored daily for clinical signs; and euthanized on day 7 post-infection IDV infection. Respiratory tract gross pathology was evaluated, and tissues collected in formalin for histological assessment. Gross lung lesions were scored according to the % area affected. Histological analysis was performed by a board-certified pathologist blinded to treatment groups.

Results

Infected calves developed mild clinical disease consisting of elevated rectal temperature and occasional respiratory signs (nasal discharge). At 7 dpi, mild gross lung pathology was present in 2 out of 5 calves in both infected groups. Calves infected with IDV 6 days after BVDV infection had an increased lung pathology scores (scores 2 and 4) compared to calves infected with IDV at day 3. Histological findings also pointed to increased pathology in calves infected with IDV at 6 days post BVDV infection (11.5± 2.14) as compared to (5.4±1.33) for the group inoculated with IDV on day 3.

Conclusions

We were able to reproduce respiratory disease in calves after BDVD+IDV inoculation. Lung pathology was detected in 4/10 animals, regardless of IDV time of infection, animals were more severely affected when IDV inoculation was performed 6 days after BVDV infection.

Financial Support
Oklahoma State University

Notes:

V-P088 - Viperin (RSAD2) induction in Bovine Respiratory Syncytial Virus infection: effects of drug treatment

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Session: Virology

Objective

Viperin (RSAD2) is an interferon-induced antiviral molecule produced by epithelial cells in response to virus infection. Our previous work has shown that *H. somni* stimulates viperin production by respiratory epithelial cells in vitro and in vivo. We reported (CRWAD 2020): following experimental infection in BRSV-infected calves viperin is produced in both *H.somni* inoculated and uninoculated calves with no difference in the clinical course of disease. To further evaluate the role of viperin as an innate immune defense against BRSV, we examined the effect of treatment with an antiviral and a COX inhibitor on viperin production after infection with BRSV.

Methods

We used nasal swab samples from 36 calves in a 10 day BRSV infection study. They had been treated with either ibuprofen from day 3 post infection, ibuprofen from day 5 post infection, placebo, fusion protein inhibitor from 5 days post infection, or a combination of ibuprofen and fusion protein inhibitor from either 3 or 5 days post infection. In previous work we inoculated calves intranasally with nonpathogenic H. somni or sham for 3 days before infection with BRSV and then used nasal swabs for viperin detection. Quantitative RT-PCR was performed for viperin expression. Data on clinical sign scores was available for both studies

Results

Viperin gene expression in the calves from the drug study followed a similar pattern as we previously found in H. somni inoculated and sham inoculated calves. Viperin production in nasal passages of BRSV infected calves is greater than or equal to fivefold increase over baseline by day 4 post infection, regardless of treatment with a COX inhibitor, fusion protein inhibitor, or nasal instillation of a non-pathogenic isolate of H. somni.

Conclusions

In two separate studies BRSV stimulated production of viperin in the nasal passages, too late to influence the severity of disease in our experimental model. Our further studies focus on how best to initiate viperin production prior to or at the time of exposure to virus to facilitate an anti-viral effect and improve clinical outcome.

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

Notes:

V-P089 - BVDV compromises fetal immune organ development leading to postnatal predisposition to secondary infections

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Session: Virology

Objective

Aim 1 tests the hypothesis that fetal immune gene expression in fetuses persistently (PI) and transiently (TI) infected with noncytopathic bovine viral diarrhea virus (ncpBVDV) continues to be impaired postnatally.
Aim 2 tests the hypothesis that fetal TI with ncpBVDV results in impaired postnatal immune responses to secondary infections such as bovine respiratory disease (BRD).

Methods

In Aim 2, day 175 pregnant heifers were inoculated with phosphate buffered saline (n = 12) or ncpBVDV-2 (n = 12) to generate control or TI heifer calves. Calf blood samples were collected at d 0, 7 and 4 months of age for clinical pathology, radioimmuno diffusion, serology, flow cytometry and PBMC RNA and DNA analysis. After weaning, calves will be vaccinated with modified live BRSV and blood samples will be assessed for gene expression and serum neutralizing antibodies. Calves will be challenged with a type 1b ncpBVDV and *M. Haemolytica* BRD pathogen to study clinical assessment, clearance of the infection, immune cell gene expression, select whole genome methylation analysis, and flow cytometry on PBMCs. Feedlot performance and carcass characteristics will be studied. Lung, thymus, spleen and liver will be collected for histopathology, immunohistochemistry, metabolomics (liver), flow cytometry, RNA-seq and whole genome methylation (thymus & spleen).

Results

Aim 1: Blood from PI, TI, acutely infected and control calves have been collected at 4 months of age. PBMCs are being examined for immune cell markers by flow cytometry, RNA-seq and genome methylation
Aim 2: All BVDV-inoculated heifers seroconverted. Control heifers were seronegative. Serum colostrum was adequate in all calves. All control calves were healthy. One TI fetus died (mummification) and 1 calf was weak at birth. Gestation lengths did not differ. TI calves weighed less (p=0.05) than controls. Blood neutrophil numbers were higher in controls compared to TI calves on d 0. Lymphocytes, monocytes and neutrophils did not change in TI vs control calves.

Conclusions

It is premature in these studies to comment on data further.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture; Colorado State University

Notes:

V-P090 - US-UK-China Collaboration: Predictive phylogenetics for evolutionary and transmission dynamics of newly emerging avian influenza viruses

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Session: Virology

Objective

Influenza A virus poses one of the greatest infectious disease challenges of the 21st Century. It is a fast evolving ubiquitous avian pathogen with vast antigenic diversity that hinders conventional vaccine approaches, especially in low value livestock species like poultry. It causes huge economic losses and drains public health budgets. Surveillance programs generate huge amounts of viral sequence data; surpassing 1 million entries on Genbank. Aspects of virus behavior could be predicted from these sequences, knowledge of host immune pressures, and epidemiological drivers and we think that advances in computational approaches mean that the construction of modeling tools with genuine predictive power for the future evolution and spread of avian influenza is possible. For this NIFA funded research grant we have assembled an international team of experts with interdisciplinary expertise in mathematical/phylodynamic modeling, influenza, and the infectious disease-public and animal health interface. Importantly this includes Chinese colleagues who run a surveillance program in the epicenter of viral diversity.

Methods

The prediction tool will be the sum of three separate models: one which identifies key viral sequence polymorphisms; one which models virus evolution within host under selection pressure; and one that integrates outputs from the first two along with additional inputs from surveillance programs. The primary data inputs are virus sequence information, both at quasi-species and consensus level.

Results

In this first year of the project, data are still be collected from field and laboratory studies. Models will be produced from existing data and a series of planned "wet lab" experiments that measure virus fitness.

Conclusions

The output tools will be useful to stakeholders such as the OIE and WHO as well as small and large poultry holders; development will therefore be informed by a series of knowledge-exchange exercises to get input from these groups on viral evolution risk and predictions.

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

Notes:

V-P091 - Replication of PDCoV is limited in neonatal piglets co-infected simultaneously or 16 hours prior with virulent PEDV

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Session: Virology

Objective

Porcine epidemic diarrhea virus (PEDV) and porcine deltacoronavirus (PDCoV) cause acute diarrhea/vomiting in neonatal pigs and share similar tissue or cellular tropisms in the gastrointestinal tract. We investigated if or how these two swine enteric coronaviruses interact with each other in gnotobiotic (Gn) piglets.

Methods

Seventeen 9-10-day-old Gn piglets were randomly assigned to 5 groups and inoculated with PEDV strain PC21A [9.3 $\log_{10}$ genomic equivalents (GE)/pig] and/or PDCoV strain OH-FD22 (8.6 $\log_{10}$ GE/pig) as follows: dually with PEDV and PDCoV [16 hrs later (n=4) or simultaneously (n=3)] or singly with PEDV (n=4), PDCoV (n=4), or mock (n=3). Clinical signs, fecal virus RNA shedding titers, and histopathology were evaluated in PEDV or PDCoV singly or dually inoculated pigs.

Results

No enhanced clinical disease or fecal PEDV shedding were observed in dually inoculated pigs compared with PEDV or PDCoV singly inoculated pigs, coinciding with no significant differences in jejunal VH:CD ratios and PEDV antigen-positive scores at post-inoculation days (PIDs) 3-4 among the groups. Compared with PDCoV singly inoculated pigs, low to moderate fecal PDCoV RNA titers were detected only at PID 1 in both dually inoculated pig groups. At PIDs 2-4, there was no detectable PDCoV RNA in the feces, coinciding with no or few PDCoV antigen-positive cells in the small and large intestine of the dually inoculated pigs at PIDs 3-4. There was a trend towards higher serum IFN-α in PDCoV singly inoculated pigs compared with PEDV singly or dually inoculated pigs at PIDs 3-4. The failure to establish PDCoV infection and replication in the gastrointestinal tract after co-infection with PEDV might explain lower serum IFN-α and lack of synergistic effects of PEDV and PDCoV dual infection compared with single PEDV or PDCoV infection.

Conclusions

Our data indicate: 1) no increased severity of PEDV infectivity by PDCoV co-infection; and 2) possible interference or inhibition of PDCoV replication in the gastrointestinal tract of pigs co-infected with PEDV that may influence PDCoV infection in PEDV co-infected pigs.

Notes:

V-P092 - Influenza A N1 neuraminidase diversity and evolution in North American swine

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Session: Virology

Objective

Influenza A (IAV) is one of the most prevalent respiratory pathogens in swine and leads to significant economic losses every year. H1N1 IAV have persisted in North American swine following a human-to-swine spillover associated with the 1918 flu pandemic. Additional human-to-swine transmission events in 1979 (Eurasian-avian lineage), 1998 (triple reassortant H3N2), and 2009 (H1N1 pandemic) have lead to a rapid increase in genomic diversity via reassortment between these introductions and endemic swine H1N1. Determining the mechanisms that affect reassortment and the evolution of the HA and NA in IAV and whether these dynamics are entangled are pivotal to preventing and responding to outbreaks of IAV in swine.

Methods

We conducted a comprehensive phylogenetic analysis of H1 and N1 IAV genes in swine for publicly available data collected in North America from 1930 to 2020. We phylogenetically quantified N1 diversity and explored the drivers of this diversity including geographic distribution, temporal trends, and reassortment, and quantified evolutionary rates using time-scaled Bayesian phylodynamics.

Results

We describe 14 N1 clades, 7 with evidence of onward circulation. From 2010 to present the predominant N1 clade and N1-HA pairing varies across regions and years. For contemporary N1 clades, the N1-pandemic gene evolved faster than genes from classical N1 clades. Though reassortment was prevalent, reassortment events that persisted were rare and are evidence for purifying selection.

Conclusions

These data suggest that N1 has strong preferential pairings with HA, likely due to the interaction between HA and NA and its impact on viral fitness and transmission. Our phylogenetic framework classified N1 IAV diversity in swine and demonstrates how reassortment and regional movement of viruses affects N1 and paired HA evolution. These data form a baseline from which we can identify N1 clades that may be expanding in range or diversity, or that may be associated with phenotypes that could impact the health and wellness of North American swine.

Financial Support
U.S. Department of Agriculture, Agriculture and Research Services; U.S. National Institutes of Health; Oakridge Institute for Science and Education

Notes:

V-P093 - MDV pathogenesis: The role of MDV genome integration on disruption of host chromosome architecture

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Session: Virology

Objective

Marek's disease (MD) is a highly contagious lymphoproliferative disease of chickens, causing significant economic loss in poultry industries. MDV genomic integration into host chromosomes is essential for tumor formation. An unexplored consequence of MDV integration events is effects on the host chromosome architecture. We will examine the role of somatic cell integration in MDV tumorigenesis via disruption of 3D host chromosome architecture.

Methods

Genomic studies using capture Hi-C (cHi-C), Hi-C, RNA-seq, and ChIP-seq will be used to examine the effects of MDV integration on genomic interactions and host transcriptome profiles. In aim 1, tumor cell architecture will be compared among several MDV+ chicken B-cell, chicken T-cell, and turkey B-cell lines. The role of viral Meq and LAT expression in chromatin interactions will be examined in knock-down studies and histone modifications associated with genomic loops will be surveyed by ChIP-seq. In aim 2, tumor cell architecture will be examined in tumor samples obtained from MDV infected chickens. CD4+ T cells from uninfected chickens will serve as controls. For each sample, cHi-C will be used for enrichment of rare heterotypic MDV-host genomic interactions, while conventional Hi-C will be used to examine abundant homotypic host genomic contacts.

Results

Since the establishment of this project in July 2021, cell line samples for aim 1 have been collected. Experiments to generate tumor samples for aim 2 will commence in the latter part of 2021. Upon collection of all datasets, they will be further analyzed and integrated as planned. Both aims 1 and 2 use the same genomic approaches and software/analysis pipelines, which should facilitate the completion of the proposed studies.

Conclusions

None yet as the experiments were only recently initiated. However, if successful, the results will contribute to our understanding of how integrated genomes contribute to the establishment of a unique and specific genomic architecture to support continuous cell growth and will aid in our understanding of the underlying mechanisms.

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

Notes:

V-P094 - Epitope mapping of the ASFV C-type lectin protein

D. Kolarova[1], **V. Celer**[1]. [1]University of Veterinary and Pharmaceutical Sciences Brno. celerv@vfu.cz
Session: Virology

Objective

African swine fever (ASF) is a highly contagious viral disease of swine which causes high mortality, approaching 100%, in domestic pigs. ASF is the only member of the *Asfarviridae* family, genus *Asfivirus*. Identification of the most antigenic viral proteins is highly relevant for the improvement of serological diagnostic tests. C-type lectin protein was identified as highly antigenic during infection. The goal of this work was to identify immunodominant peptides in the structure of C-type lectin protein of ASFV which could be used as antigens to detect ASFV specific antibodies following natural infection in pigs.

Methods

Translated C-type lectin gene (EP153R) of the virus was analyzed for prediction of linear B-cell epitopes. Surface accessibility, epitope prediction, hydrophilicity and antigenicity of the protein were estimated by using software modules available at the Immune Epitope Database and Analysis Resource (IEDB) web page (http://tools.iedb.org). Seven peptides covering the full length of the protein were synthesized and used as the antigens in indirect ELISA test. ASFV positive and negative sera were used to test the serological reactivity of the peptides. Positive sera were obtained from feral pigs from Poland (n = 5), Spain (n = 5) and as positive control sera from diagnostic kits (n = 2). ASFV negative sera (n = 20) were obtained from pig farms in the Czech Republic.

Results

Serological reactivity was detected only with the peptide no. 3 and 7 spanning amino acids 50-70 (peptide 3) and 124-153 (peptide 7), corresponding to highly variable region of the protein. All ASFV positive swine sera reacted with both aforementioned peptides, no reactivity was detected with ASFV negative sera. However, the two reactive peptides did not correspond in position to the immunodominant regions identified by in silico analysis.

Conclusions

The results of the project show that the C-type protein can be used as another antigen important for the identification of ASFV serologically positive animals.
This work was supported by the National agency for agricultural research (NAZV), project no. QK1920187.

Financial Support
This work was supported by the National agency for agricultural research (NAZV), project no. QK1920187.

Notes:

V-P095 - The physiological effect of EGTA infusion during prepartum on calcium metabolism postpartum in Holstein cows

L. Hernandez[1], W. Frizzarini[1], P. Monteiro[1]. [1]University of Wisconsin-Madison. llhernandez@wisc.edu
Session: Dairy cattle physiology, immunology or disease

Objective

Commonly, high-producing dairy cows undergo clinical (5-10% of US dairy herd) and subclinical (50% of US dairy herd) hypocalcemia during the periparturient period. Our goal was to induce transient hypocalcemia before calving to activate the homeostatic calcium mechanism during early postpartum.

Methods

To induce hypocalcemia, cows were infused with ethylene glycol tetraacetic acid (EGTA), a molecule that selectively binds to calcium. Thirty multiparous Holstein cows were enrolled 21 d before expected parturition date and maintained on a common dry cow diet. Approximately 7 d prepartum, cows were blocked by lactation number and randomly assigned to receive one of 2 different treatments: 5% EGTA (EGTA; n=15) or saline (CON; n=15). Cows received daily IV infusion for 6 hours per day until parturition. An infusion pump (Heska Vet IV) was used to maintain ionized calcium (iCa) concentrations at 0.7 mmol/L (equivalent to clinical hypocalcemia), saline infused cows were infused at a rate equal to that of their paired EGTA cow. iCa was monitored immediately before the infusion, hourly during the infusion, 1 h after termination of infusion, immediately after calving, 12 h, 1, 2, and 3 d after calving with a handheld cow-side biochemical analyzer (iStat system, Abbott Laboratories) using CG8+ cartridges. Data were analyzed using the GLIMMIX procedure of SAS.

Results

As expected, there was a treatment effect ($P<0.01$) on iCa concentration during the daily infusion, and 1 h after termination of the infusion, with EGTA-treated cows having decreased iCa concentrations than CON cows. There was a treatment effect ($P<0.01$) on iCa concentration 12 h, 1, and 2 d after parturition, with CON cows having decreased iCa concentrations compared to EGTA-treated cows. Before termination of infusion, blood pH was increased in EGTA-treated cows, but during the infusion, CON cows had increased blood pH ($P<0.01$).

Conclusions

In conclusion, EGTA cows responded to treatment by decreasing iCa concentrations during treatment period, leading to an improved response on iCa concentrations postpartum.

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

Notes:

V-P096 - Evidence of *Campylobacter* species shed in chickens and pigs in Uganda

P. Pithua[1], T. Odoch[1]. [1]Virginia-Maryland College of Veterinary Medicine, Virginia Tech. ppithua@vt.edu
Session: Epidemiology and/or public health

Objective

To estimate the prevalence of Campylobacter species isolated from wet poultry markets, poultry farms and pig abattoirs in Uganda.

Methods

Fecal samples were gathered from 175 live chickens from backyard farms, 132 chickens destined for slaughter at wet poultry markets and 265 pigs at a slaughter plant in Uganda. Samples were cultured for *Campylobacter* of any species using standard protocols followed by speciation. Positive samples from initial culture were sub-cultured for purification and DNA extraction. A multiplex PCR targeting genes consistent with six *Campylobacter* species *(C, hyointestinalis subp hypointestinalis, C. coli, C. fetus, C. lari, C. jejuni,* and *C. upsaliensis)* was performed and sequenced for confirmation of the species in the isolates.

Results

Twenty eight percent of samples collected from live chickens from backyard farms were positive for any *Campylobacter* species. *Campylobacter jejuni, Campylobacter coli*, and *Campylobacter lari* accounted for 59%, 53% and 2% of these isolates. For samples collected from chickens at wet markets, 37% were positive for any Campylobacter species. Speciation yielded 22%, 79% and 1% of *Campylobacter jejuni, Campylobacter coli*, and *Campylobacter lari* respectively among the culture positive isolates. *Campylobacter* of any species was detected in 31% of the samples collected from pigs at slaughter and of these 37%, and 81% of these were *Campylobacter jejuni* and *Campylobacter coli,* respectively. Evidence of concurrent colonization by *Campylobacter jejuni* and *Campylobacter coli* in samples collected from chicken in backyard farms, wet poultry markets and Pig slaughter abattoir was noted suggesting the possibility of colonization with multiple species of *Campylobacter* in chickens and pigs in Uganda.

Conclusions

This is the first study to quantify shedding of *Campylobacter* in chickens and pigs in Uganda confirming that poultry and pigs are potential reservoir for human Campylobacteriosis in Uganda.

Notes:

V-P097 - Changes in bovine leukemia virus diagnostic parameters in naturally infected dairy cattle over a lactation cycle

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Session: Dairy cattle physiology, immunology or disease

Objective

Bovine leukemia virus (BLV) is a delta-retrovirus which infects the B lymphocytes of cattle. 46% of all U.S dairy cattle are estimated to be infected with BLV and ~30% of infected animals develop a persistent lymphocytosis. Our objectives are to: 1) determine if common parameters used to diagnose BLV infection change over time and 2) identify important time points for new BLV infections over a lactation period in dairy cattle.

Methods

Two cohorts of 44 animals each were enrolled at 150±4 days prior to calving and included animals about to enter their first or greater lactation. 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 in milk (DIM) and once at ~120 DIM. Additional animal data will be added when available.

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. Initial disease data will be added when collected.

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. The periparturient period may be important for viral transmission with most new sero-conversions occurring early in a lactation cycle.

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

Notes:

V-P098 - Detection of African swine fever virus (ASFV) antibodies in pigs slaughtered from abattoirs in central Uganda

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Session: Epidemiology and/or public health, 6:00 - 8:00 PM

Objective

African Swine fever (ASF), is a highly fatal and contagious disease of pigs, caused by ASFV a DNA arbovirus of the family Asfarviridae. The morbidity and mortality due to ASFV can approach 100%, constraining the pig industry, and leading to poverty, food insecurity. Yet, mortality can range in endemic areas and there are reports of chronic disease and carrier states. To mitigate the risks associated with ASF it requires a robust surveillance program for early detection of the potential sources of infection. Therefore, in this study, we set out to analyze serum from domestic pigs slaughtered at abattoirs in central Uganda to ascertain the use of serology in detection.

Methods

A total of 1200 pigs will be systematically sampled from six abattoirs (Wambizzi, Lusanja, Budo, Katabi, Buwate and Kyetume) over a period of 13 months (May 2021- June 2022). To date, serum has been collected from at least 276 pigs, and analyzed by the Ingenasa ASF indirect ELISA to detect ASFV antibodies. The ELISA positivity rate and 95% confidence interval was calculated.

Results

Of the 276 serum (pigs) samples analyzed, 3 (1.1%, 95%CI: 0.22%, 3.3%) pigs from the abattoirs studied were shown to contain ASFV antibodies. One of these animals had clinical signs and lesions that include hemorrhages in the skin of ears, legs and flanks, markedly enlarged and darkened spleen, enlarged and diffusely hemorrhagic gastro-hepatic and renal lymph nodes. The low sero-prevalence observed in these preliminary results could be reflective of the virulence of the ASFV isolates circulating in market pigs in Uganda since pigs infected with virulent strains are known to die before a specific antibody immune response is mounted.

Conclusions

Early data suggest ASFV sero-prevalence of 1.1% among pigs slaughtered at the six abattoirs, an indication that some pigs have been exposed to ASFV and survived; and most mortalities occur before induction of detectable antibody response. . Analysis of the samples using qRT-PCR and sequencing will establish disease status and delineate the infecting ASFV genotype.

Financial Support
U.S. Defense Threat Reduction Agency; U.S. Department of Defense, Defense Threat Reduction Agency

Notes:

V-P099 - Detection of antibodies against *Ornithodoros moubata* saliva antigen in pigs slaughtered in Central Uganda

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Session: Epidemiology and/or public health, 6:00 - 8:00 PM

Objective

African swine fever (ASF) is an important disease of pigs in sub-Saharan Africa and is threatening the pig population and the agricultural economy of other continents. ASF is caused by a DNA virus in the family *Asfarviridae* and can be transmitted from wild suis to domestic pigs through soft ticks of the *Ornithodoros* species. The role of *Ornithodoros moubata* in the transmission of the African swine fever virus to domestic pigs has been inadequately studied in Uganda. The objective of this study is to detect the presence of antibodies against *O. moubata* saliva antigen in pigs slaughtered in the Kampala metropolitan area to provide an understanding of their exposure to this vector.

Methods

A total of 276 serum samples collected from market pigs slaughtered at Wambizi, Lusanja, Budo, Katabi, Buwate, and Kyetume pig slaughterhouses will be tested for the presence of antibodies against *O. moubata* using an indirect rtTSGP1 ELISA developed at IRNASA, CSIC in Madrid, Spain. Two negative controls and two positive controls as well as duplicate sera samples will be used to calculate the sample to positive ratio (SP ratio). Summary statistics (frequency and proportion) of pigs with an exposure to the *O. moubata* tick will be calculated across slaughterhouses and geographic origins.

Results

This study is ongoing, and we expect to identify sampled pigs originating from areas with negligible, medium, high, or very high probability of *Ornithodoros moubata* presence.

Conclusions

The study will provide evidence on the potential role *of O. moubata* in the current outbreaks of ASF in Uganda and will be useful in better understanding the transmission dynamics of ASF.

Financial Support
U.S. Defense Threat Reduction Agency

Notes:

V-P101 - Immune responses and efficacy of a Brucella dry dart vaccine in bison

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Session: Vaccines and vaccinology

Objective

To determine the immunogenicity and efficacy of a novel Brucella vaccine delivery vehicle in bison.

Methods

Bison (*Bison bison*) heifer calves from a brucellosis-free herd were randomly assigned to 4 treatments: control, single or boostered parenteral vaccination with 10^{10} CFU of *Brucella abortus* strain RB51, or surgical implantation of a dry dart containing approximately 10^{10} CFU of RB51 (n=8/trt). Serum and peripheral blood mononuclear cells (PBMC) were obtained at 0, 4, 8, 13, 16, 21, and 24 wk after initial vaccination and at 0, 4, 8, 12, 15, 22, and 27 wks after booster vaccination to characterize humoral and cellular immune responses. Pregnant bison were challenged in midgestation with 10^7 CFU of *B. abortus* strain 2308. Samples were obtained at necropsy with 72 hr of parturition and evaluated for recovery of the *Brucella* challenge strain.

Results

Bison in RB51 vaccination treatments demonstrated greater (P<0.0001) serum humoral responses when compared to nonvaccinates, with parenteral vaccinates demonstrating greater (P<0.01) responses when compared to mean responses of bison inoculated with the dry dart. Only the booster vaccinated treatment demonstrated greater (P<0.001) humoral responses than control bison in samples collected after booster vaccination. At 4, 8, 12, 16, and 24 wks after initial vaccination, PBMC from parenteral RB51 vaccinates demonstrated greater proliferative responses to RB51 when compared to responses of control animals. Bison innoculated with the RB51 dry dart did not demonstrate greater (P>0l05) proliferative responses when compared to non-vaccinates. Bison in parenteral vaccination treatments had reduced (P<0.05) abortions and infection in uterine and fetal samples as compared to non-vaccintates, with booster vaccinates tending to have the lowest colonization (CFU/gm) in tissues. The dry dart formulation reduced (P<0.05) but not infection in most tissues when compared to non-vaccinated bison.

Conclusions

Data from this study reaffirms the efficacy of a boostered parenteral vaccination strategy for bison in preventing brucellosis abortion and infection. Our data suggests that the dry dart RB51 formulation does not induce sufficient efficacy in bison after a single inoculation.

Notes:

V-P102 - Optimization of *Bacillus thuringiensis* crystal protein 5B for control of gastrointestinal parasites in livestock

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Session: Parasitology and/or ticks

Objective

The studies objectives were two-fold: 1) Test a single dose treatment of *Bacillus thuringiensis* (Bt) crystal (Cry) protein Cry5B for its anthelmintic efficacy against an experimental infection of *Haemonchus contortus* in sheep and 2) increase the stability of Cry5B IBaCC for passage through the monogastric and ruminant digestive tract.

Methods

Objective 1: A paraprobiotic form of Bt Cry5B called IBaCC, that cannot reproduce in the environment, was used for this study. Dorset lambs were infected with 10,000 *Haemonchus contortus* infective larvae. Once the infection was patent, lambs were stratified by fecal egg count (FEC) into one of three treatment groups (n=5) and orally administered: 1) Cry5B IBaCC (40 mg/kg BW), 2) Cry5B IBaCC (10 mg/kg BW), or 3) untreated control (C). FEC were measured daily for one week, lambs were then euthanized and total abomasal worm burden was quantified. *Objective 2:* The stability of Cry5B IBaCC encapsulated with enteric coated gelatin capsules or varying formulations of polymer-coated granules (Eudragit® L 100, Eudragit® E) will be tested in monogastric and ruminant gastrointestinal tracts.

Results

Objective 1: Within 48 hours of dosing there was a 58% and 89% reduction in FEC and 67% and 92% FEC reduction by one week for lambs in the 10mg/kg and 40mg/kg BW treatment groups respectively. There was a 40% reduction in worm burden in lambs dosed with 10 mg/kg (976 ± 249 worm, mean ± SEM, $p = 0.014$ versus C) and an 82% reduction in worm burden in lambs dosed with 40 mg/kg (296 ± 115 worms, $p < 0.0001$ versus C). *Objective 2:* The stability of Eudragit L 100 coated Cry5B IBaCC to simulated gastric fluid is significantly increased *in vitro*. Future studies will test this formulation in horses. The Eudragit E coated formulations will be tested for rumen fluid stability *in vitro* prior to testing in lambs.

Conclusions

A single dose of Cry5B IBaCC at 40mg/kg BW was highly effective at reducing the FEC and associated worm burden in lambs. Further studies into the effectiveness and optimization of Cry5B IBaCC and other nematode-active Cry proteins is ongoing.

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

Notes: