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Presentation Abstracts

101st Conference of Research Workers in Animal Diseases

Live Streaming of Featured Presentations:

- **Dec 5th-8th, 2020**

On-Demand Streaming of Recorded Presentations:

- **Dec 5th 2020 – July 1st 2021**
- **Available at www.CRWAD.org**

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CRWAD 2020 President's Message

December 2020

Welcome to the 101st meeting of the Conference for Research Workers in Animal Diseases. This year's meeting couldn't be more different from the 100th meeting in 2019, when we were together in Chicago for a conference that included special events marking that remarkable milestone. For the first time in the history of the organization, the 2020 conference is being delivered entirely online, with a combination of live streamed and pre-recorded content, because of the COVID-19 pandemic. We have no doubt benefitted from the experiences of other organizations that held online meetings earlier in 2020, identifying pitfalls we want to avoid. The CRWAD Council and Program Committee have endeavored to give attendees easy-to-use access to the latest developments in animal health research, in spite of the pandemic. Most important to us was the fact that graduate students are still moving through their training, and still need a forum to present their work to the scientific community. I hope you'll agree that we've made this possible for the many students and their advisors who would not have been able to travel to Chicago.

Any success of this novel CRWAD meeting will be due in large part to the really quite tireless work of our Executive Director Paul Morley, and the support of Jennifer Stalley and the rest of our management team at Midwest Solutions. Thanks also to Lorraine Sordillo, Brandy Burgess, and the rest of the Program Committee, who spend hours organizing the program. We are indebted to Dag Lerdal, our consultant who developed Preseria, the program we use to upload and deliver content. Dag also custom built the version of the CRWAD website that will allow us to deliver the meeting virtually, and he will be working throughout the meeting, remotely from his office in Norway, to provide IT support for presenters and moderators. Much appreciation goes to Bob Gordon of D.E. Systems, who has been invaluable in helping us use their software to manage abstracts, our proceedings, and registration. And many thanks to the current CRWAD Council members, who have been actively engaged in discussions regarding the frequently changing plan as it evolved over the past 12 months.

If every cloud has a silver lining, the silver lining in the massive thunderhead of the COVID-19 pandemic may be that concepts from microbiology, immunology, and epidemiology, which form the foundation of much CRWAD programming, are now recognized as relevant to non-scientists around the world. Thus, the importance of work done by many CRWAD participants is clearer than ever. Perhaps some children currently trying hard to learn, in spite of the limits of online education, will grow up to pursue careers in microbiology, immunology, or epidemiology, to make the next pandemic more manageable. And perhaps some of them will organize the 2040 CRWAD meeting.

For those of you who have been personally and directly impacted by COVID-19, we send wishes for speedy recovery when possible, and heartfelt condolences when not.

Thank you for your support of CRWAD.

Amelia R Woolums, DVM, MVSc, PhD, DACVIM, DACVM
CRWAD President

CRWAD 2020 Featured Speakers

"T Cell Memory and Exhaustion: Implications for Immunotherapy"
Rafi Ahmed, PhD – CRWAD Keynote Speaker
Director, Emory Vaccine Center
Livestream Presentation
Saturday, 12/5/2020 10:00 AM

"The Characteristics of Pandemic Pathogens"
Amesh Adalja, MD, FIDSA – CRWAD Centennial Featured Speaker
Johns Hopkins University Center for Health Security
Livestream Presentation
Saturday 12/5/2020, 11:00 AM

"*Converting a pathogen to become a servant****"***
"Vaccine Discovery and Development -From Lab to Market Place"
Roy Curtiss, PhD - ACVM Distinguished Microbiologist
University of Florida Gainesville, Department of Infectious Diseases and Immunology
Livestream Presentations
Monday, 12/7/2020, 10:00 AM
Monday, 12/7/2020, 3:00 PM

"A Journey Through Vaccine Valley"
Scott McVey, DVM, PhD, DACVM – AAVI Distinguished Veterinary Immunologist
Center Director, USDA ARS Center for Grain and Animal Health Research
Livestream Presentation
Monday, 12/7/2020, 1:30 PM

“Utilizing big data in veterinary medicine”
Larry Glickman, VMD, DrPH – Calvin Schwabe Award
Professor Emeritus, Purdue University
Livestream Presentation
Monday, 12.7.2020, 11:00 AM

“ASF: US prevention, preparedness and response”
Paul Sundberg, DVM, PhD, DACVPM – African Swine Fever Symposium
Executive Director, Swine Health Information Center
Livestream Presentation
Saturday, 12/5/2020, 12:30 PM

“Novel tools for ASF control and prevention: 7 years’ research in Kansas Biosecurity Research Institute”
Raymond (Bob) R. R. Rowland, PhD, Professor and Head, Department of Pathobiology, University of Illinois – African Swine Fever Symposium
Livestream Presentation
Saturday, 12/5/2020 1:15 PM

“The risk of feed for viral transport and transmission: What do we know and what can we do”
Scott Dee, DVM, MS, PhD. Director of Pipestone Applied Research, Pipestone Veterinary Services – Africa Swine Fever Symposium
Livestream Presentation
Saturday, 12/5/2020 2:15 PM

“Prospects for an African Swine Fever Virus Subunit DIVA Vaccine”
Waithaka Mwangi, PhD, College of Veterinary Medicine, Kansas State University – African Swine Fever Symposium
Livestream Presentation
Saturday, 12/5/2020 3:00 PM

"DIVA vaccine cross-protects against Salmonella serovars in food animals"
Shawn Bearson, Research Microbiologist, USDA ARS
Livestream Presentation
Monday, 12/7/2020, 4:00 PM

"The Biology of the Human-Animal Bond"
Alan Beck, ScD, College of Veterinary Medicine, Purdue University
Pre-Recorded Presentation

"From Scientific Discovery to Commercial Product in 10 Easy Steps: The Case of CD163 and PRRS Vaccines"
Jay Calvert, PhD, Associate Research Fellow, Zoetis
Livestream Presentation
Monday, 12/7/2020, 2:30 PM

"Small Animals – Big Data!"
Charlene Edinboro, DVM, PhD, Senior Scientist in Health Sciences, Exponent
Pre-Recorded Presentation

"Host factors controlling susceptibility to coronavirus infections"
Thomas Gallagher, PhD, Loyola University
Pre-Recorded Presentation

"The U.S. National Biodefense Strategy for developing veterinary countermeasures to prepare and respond to biological threat agents"
Cyril Gay, DVM, PhD, Senior National Program Leader, Animal Production and Protection, USDA ARS
Livestream Presentation
Tuesday, 12/8/2020, 9:15 AM

"Advances in African swine fever live-attenuated vaccines"
Doug Gladue, Senior Scientist, USDA ARS
Livestream Presentation
Monday, 12/7/2020, 3:30 PM

"Big Data (Big Benefits or Big Obstacles?)"
George Moore, DVM, PhD, DACVIM, Director, Department of Veterinary Administration, Purdue University
Pre-Recorded Presentation

"Iron regulation in Mycobacterium paratuberculosis – A tale of two regulators"
Srinand Sreevatsan, BVSC, MVSC, MPH, PhD, Associate Dean for Graduate Studies, Michigan State University
Pre-Recorded Presentation

"From Discovery to Corporate Development; Challenges between Academia and Commercialization"
John H. Wyckoff, III, PhD, Director of BioMARC, Colorado State University
Livestream Presentation
Tuesday, 12/8/2020, 10:30 AM

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101 - T cell memory and exhaustion: implications for immunotherapy

R. Ahmed Emory University. rahmed@emory.edu
Session: FEATURED SPEAKER - CRWAD KEYNOTE

In this talk I will first contrast the distinct differentiation pathways that CD8 T cells undergo during acute versus chronic viral infections. The main part of my talk will then focus on the lifestyle of CD8 T cells during chronic viral infection and cancer. I will then end on potential implications for immunotherapy.

Notes:

102 - Characteristics of microbes most likely to cause pandemics and global catastrophes

A. Adalja Johns Hopkins University Bloomberg School of Public Health. aadalja1@jhu.edu
Session: FEATURED SPEAKER - CRWAD KEYNOTE

Predicting which pathogen will confer the highest global catastrophic biological risk (GCBR) of a pandemic is a difficult task. Many approaches are retrospective and premised on prior pandemics; however, such an approach may fail to appreciate novel threats that do not have exact historical precedent. In this paper, based on a study and project we undertook, a new paradigm for pandemic preparedness is presented. This paradigm seeks to root pandemic risk in actual attributes possessed by specific classes of microbial organisms and leads to specific recommendations to augment preparedness activities. Predicting which pathogen will confer the highest global catastrophic biological risk (GCBR) of a pandemic is a difficult task. Many approaches are retrospective and premised on prior pandemics; however, such an approach may fail to appreciate novel threats that do not have exact historical precedent. In this paper, based on a study and project we undertook, a new paradigm for pandemic preparedness is presented. This paradigm seeks to root pandemic risk in actual attributes possessed by specific classes of microbial organisms and leads to specific recommendations to augment preparedness activities.

Financial Support

Notes:

103 - ASF: US prevention, preparedness, and response

P. Sundberg Swine Health Information Center. psundberg@swinehealth.org
Session: FEATURED SPEAKER - ASF SYMPOSIUM

Prevention: The pork industry is funding a project to identify gaps in US pork industry national biosecurity. The goal is to identify and act on biosecurity gaps to prevent entry of foreign animal disease (FAD) into the country. Swine Innovation Porc facilitates research in the Canadian swine sector. A Coordinated African Swine Fever (ASF) Research Working Group helped create an ASF-related research priorities document. Ongoing feed transmission research caused updated and revised information for feed holding times to mitigate virus transmission, one tool to use in conjunction with other mitigations to enhance feed safety. Preparedness and Response: A USDA-Foreign Ag Service grant supports enhancing ASF response in Vietnam. The first section focuses on strengthening Vietnamese veterinary services capacity. The second section will help with industry response in Vietnam and the U.S. through field projects. Titles of the projects are listed below. “Full validation of two commercial ELISA assays for the detection of antibodies to African swine fever.” “Evaluation of the diagnostic performance of a ASFV serum/oral fluid antibody ELISAs under field conditions in Vietnam.” “Potential of rodents to be a vector in the transmission of African Swine Fever in two commercial farms in Vietnam with differing biosecurity levels.” “Evaluate the diagnostic performance of pen-side tests for ASF detection.” “Time and temperature required for complete inactivation of African swine fever virus.” “Identifying pathways of entry of ASF virus onto farms to enhance information for improving biosecurity in Vietnam.” “Validating the Composting Process for ASF Virus Inactivation.” Funded by the National Pork Board: “Validating protocols for the targeted removal of individually housed sows when infected to move the herd to negative status.” “Field evaluation of oral fluids as a convenient, aggregate sample for early detection of African swine fever.” “Determining the pathways for ASF introduction into boar studs and risk of ASF transmission via semen movements during an ASF outbreak.”

Notes:

104 - Novel tools for ASF control and prevention: 7 years of research at the Kansas Biosecurity Research Institute

R.R. Rowland Department of Pathobiology College of Veterinary Medicine University of Illinois at Urbana-Champaign.
rowland7@illinois.edu
Session: FEATURED SPEAKER - ASF SYMPOSIUM

Objective

The prevention and control of African swine fever (ASF) incorporates an integrated approach. For the past seven years we have been utilizing collaborative approaches for exploring new tools for the control of the virus, including 1) understanding the receptor, 2) exploring the genetics of the host response, 3) conducting epitope analysis of viral antigens, and 4) understanding risks related to virus introduction.

Methods

Methods included the infection of genetically edited pigs to understand the role of the macrophage CD163 receptor. Exploring the host response incorporated the analysis of the transcriptome following infection with virulent and attenuated viruses. The analysis of antigens included the mAb epitope mapping of p72, p54 and p30 antigens. The stability of virus in feed components was used to understand the risk for introducing ASF through feed.

Results

CD163 is not a biologically relevant receptor for ASFV. There are some unique gene expression patterns in the host associated with ASFV infection. Monoclonal and pig polyclonal antibodies recognize conserved and variable regions of p30, p54 and p72.

Conclusions

These new tools, including mAbs, create new opportunities to develop molecular and immunological tests for the detection of virus and for predicting protection following vaccination.

Financial Support
Kansas Bioscience Authority; Kansas State University; National Pork Board; U.S. Department of Homeland Security

Notes:

105 - Risk of feed for viral transport and transmission: What do we know and what can we do?

S. Dee[1], M. Niederwerder[2], G. Patterson, G. Spronk[3], E. Nelson[4]. [1]Pipestone Applied Research, Pipestone Veterinary Services, Pipestone, MN, USA, [2]Department of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS, [3]Pipestone Veterinary Services, [4]South Dakota State University. scott.dee@pipestone.com
Session: FEATURED SPEAKER - ASF SYMPOSIUM

Objective

In 2014, feed was proposed as a risk factor for the domestic and transboundary movement of PEDV. Since that time, the concept of feed and feed ingredients as a vehicle for viral transport and transmission has been expanded across multiple viruses of foreign animal disease significance, including ASFV, CSFV, and PRV. The North American swine industry has responded in several ways to manage this risk; therefore, the purpose of this paper is to review the science regarding the risk of feed and the subsequent industry response.

Methods

To evaluate the risk of feed, transboundary models were developed to simulate the transport of feed ingredients from Asia and Eastern Europe to the US to evaluate survivability and calculate T ½ life. Transmission studies were conducted to evaluate the ability of ASFV and PEDV to spread to pigs following consumption of contaminated feed. High risk ports of entry for the importation of soy-based products to the US from ASFV-positive countries were identified. Finally, mitigation efforts involving feed additives and extended storage protocols (Responsible Imports) were tested for their ability to reduce viral load.

Results

These collective studies have consistently demonstrated the transport and transmission of viral pathogens in feed. Of particular interest has been the protective effect of soy-based ingredients and the identification of specific seaports for entry of these products to the US. Industry response to this information has been proactive using effective feed additives, along with the adoption of Responsible Imports protocols for the introduction of essential feed ingredients from high-risk countries.

Conclusions

The science of feed risk has been a highly collaborative effort. These studies have raised the awareness of a new risk factor for the domestic and transboundary spread of diseases of animals. They have introduced the concept of “feed biosecurity” and have changed human behavior regarding the management of feed and feed ingredients, resulting in improved food and feed safety for the benefit of global agriculture.

Notes:

106 - Prospects for an African Swine Fever Virus subunit DIVA vaccine

W. Mwangi Kansas State University. wmwangi@vet.k-state.edu
Session: FEATURED SPEAKER - ASF SYMPOSIUM

The project goal is to identify protective African Swine Fever Virus (ASFV) antigens and develop a safe and efficacious subunit vaccine. ASFV is causing devastating loses in pork production in affected regions and heightened concerns in other regions. Since there is no treatment or vaccine, disease control is difficult. Given that attenuated virus can confer protection against related virulent isolates, development of a viable vaccine is possible. Several mutants have been tested for their protective efficacy, but safety concerns and scalability hinder deployment. Current knowledge suggests that effective immunity depends on antibody and T cell responses, particularly cytotoxic T lymphocytes (CTLs). Thus, subunit vaccine development will require empirical identification of protective antigens, preferably those conserved among ASFV strains. We have evaluated adenovirus-vectored prototype vaccines in pigs and one formulation elicited CTL responses and 5/9 vaccinees were protected upon challenge. To improve protective efficacy, we have identified novel conserved antigens containing putative ASFV [Georgia 2007/1] CD8+ T cell epitopes based on computational prediction of peptide motifs that bind strongly to characterized SLA I alleles. Lymphocytes from pigs immunized with antigen cocktails were used to confirm the predicted CD8+ T cell epitopes by CTL killing assays and by IFN-g EliSpot assays using peptides generated using the predicted peptide motifs. These data were used to select novel ASFV polypeptide sequences that were used to generate multicistronic expression cassettes to allow efficient in vivo delivery of multiple antigens. The multicistronic expression cassettes were used to generate adenoviruses encoding the novel ASFV antigens containing putative CD8+ T cell epitopes. The encoded antigens are authentic as confirmed using ASFV convalescent serum. A prototype vaccine formulated using the adenoviruses expressing the rationally selected putative CD8+ T cell targets has potential to induce ASFV-specific CTL responses in vivo and thereby confer protection.

Notes:

107 - Converting a pathogen to become a servant

R. Curtiss III Department of Infectious Diseases & Immunology, University of Florida. rcurtiss@ufl.edu
Session: FEATURED SPEAKER - ACVM

I was introduced to Salmonella in 1948 to screen chickens for antibodies to S. Pullorum. In 1956, I began research on genetics of S. Typhimurium phage P22 and in 1980 selected S. Typhimurium attenuated mutants to generate a recombinant vaccine against Streptococcus mutans. Success was modest and this necessitated better understanding of Salmonella pathogenicity. Discovery of Cya and Crp as global virulence regulators led to the currently marketed Megan Vac vaccine to control Salmonella in poultry. This was followed by discovery of how Salmonella invaded cells/tissues that led to the definition of Salmonella Pathogenicity Island 1 and type 3 secretion. Characterization of the Salmonella virulence plasmid identified the spv operon as responsible for conferring internal tissue invasiveness. A balanced-lethal vector-host system using genes for peptidoglycan precursor synthesis eliminated use of antibiotic-resistance markers to maintain plasmid vectors in Recombinant Attenuated Salmonella Vaccines (RASVs). By the 1990s our research largely shifted to design RASVs to protect against a diversity of pathogens. We soon realized that Salmonella has multiple means to modulate (suppress) induction of immunity by the infected host. We thus eliminated these means as discovered and also multiple means by which Salmonella produces polymers to facilitate biofilm formation. We then established means for regulated delayed expression of attenuation and synthesis or encoded protective antigens that enables recombinant vaccine vectors to efficiently colonize internal lymphoid tissues almost as well as wild-type virulent Salmonella. We also perfected means for regulated delayed lysis in vivo to release a bolus of synthesized protective antigens and confer biological containment with no persistence of vaccine cells in vivo or survival if shed into the environment. These Protective Immunity Enhanced attenuated Salmonella Vaccine (PIESV) vector strains are thus some of the most extensively genetically modified organisms to be our friends and servants in combating infectious diseases.

Notes:

108 - Host factors controlling susceptibility to coronavirus infections

T. Gallagher n/a. tgallag@luc.edu
Session: FEATURED SPEAKER - ACVM

Coronavirus infection requires several host susceptibility factors controlling virus attachment to cells, priming for successful entry via viral protein conformational transitions, and proteolytic activation of virus-cell membrane fusion. These factors influence virus tropism and zoonotic transmission, and they also are favored targets for therapeutic antiviral agents. This presentation will review coronavirus – cell interactions involving host entry factors and will highlight new findings in the field.

Notes:

109 - Iron regulation in *Mycobacterium paratuberculosis* – A tale of two regulators

S. Sreevatsan Michigan State University. sreevats@msu.edu
Session: FEATURED SPEAKER - ACVM

Objective

Johne's disease is a chronic infectious disease of ruminants leading to chronic enteric inflammation, progressive wasting, and death. A prolonged (2-5 year) subclinical phase of infection complicates diagnosis and control measures. There are no cost-effective therapies for Johne's disease and vaccination, currently not used in the U.S., is only marginally effective. MAP is truly a global pathogen, causing significant losses to dairy, beef, deer, and sheep producers from Europe to New Zealand to North America, South America, and Africa. Sreevatsan lab has researched MAP molecular biology, pathobiology, and molecular genetics all aimed at improving our understanding of this extremely important global pathogen. One of the unique characteristics of *Mycobacterium avium* subsp. *paratuberculosis* (*MAP*), the causative agent of Johne's disease (JD), is that it requires supplementation of a siderophore, mycobactin, for optimal growth in laboratory media – a functional characteristic that has been implicated for its fastidious nature and slow growth in culture media. Prior studies have characterized the function of a *mycobacterium*-specific transcriptional regulator, IdeR, in iron regulation by *MAP* strains. A second ferric uptake regulator (Fur)-like element (*MAP3773c*), discovered on a *MAP*-specific genomic island (LSP15), is upregulated in-vivo in intestinal lesions. This gene is present in a 5 gene cluster (*MAP3776c-MAP3772c*) that has been proposed to function as an operon. The presence of a second MAP-specific iron regulator is intriguing and has been the object of deeper investigations in my laboratory. In this presentation, I will summarize the evolution of work in our laboratory starting from genomes to enhanced understanding of iron regulation in MAP.

Methods

Genomics, Genetic diversity, Ex-vivo models, Cell invasion, Iron regulation, diagnostics

Results

The findings support the idea that IdeR is the primary iron regulatory element in MAP.

Conclusions

I will summarize the salient findings of our research over the last 20 years.

Financial Support
U.S. Department of Agriculture

Notes:

110 - Big data to the rescue of ProHeart6: how a drug once declared unsafe by FDA returns to the market

L.T. Glickman[1], L.T. Glickman[1], G. Moore[1], R. Cobb[2], H.B. Lewis[3]. [1]Purdue University, [2]Fort Dodge Animal Health, [3]Banfield Pet Hospital. ltg@purdue.edu

Session: FEATURED SPEAKER - SCHWABE SYMPOSIUM

Heartworm infection is a life-threatening parasitic infection of dogs caused by Dirofilaria immitis and transmitted by mosquitoes. In June 2001 moxidectin in the form of ProHeat6 was approved and launched in the United States by Fort Dodge Animal Health to prevent heartworm infection for 6 months. A major advantage of this product is that it does not depend on dog owners to administer it on a monthly basis. Shortly after the US launch of ProHeart6 the FDA expressed concern about reports of allergic type reactions after administration. When Fort Dodge announced that it was recalling ProHeart6 until resolution of FDA's safety concerns, regulatory agencies in other countries also reviewed the safety record of ProHeart6 in dogs. None of these countries found ProHeart6 to be a danger to dogs and did not recall the product despite the fact that all ProHeart worldwide is manufactured at one site by the same method. Following voluntary recall, Fort Dodge sponsored an epidemiological study to determine the incidence of potential adverse everts associated with ProHeart 6 using the medical records of Banfield Pet hospitals in 40 states with approximately 1.4 million active patients and 60,000 patient visits per week. The incidence of adverse events was compared for dogs receiving ProHeart 6 or one of two oral heartworm preventives. The major conclusions of the Banfield study were that: 1) The safety profile of ProHeart6 was similar to that of the two commonly used oral monthly heartworm preventives; 2) Vaccines explained most of the allergic events when given with ProHeart 6; 3) An Internet frenzy probably played a causal in the apparent epidemic of owner reported adverse events associated with ProHeart 6 in the United States. This epidemiologic approach has several advantages. It enabled us to adjust for the effects of concurrent vaccination and other potential confounding factors such as age, breed, and gender when measuring the risk of adverse events. Utilizing recorded medical events are preferable to use of unfiltered and potentially biased reports from dog owners.

Notes:

111 - The biology of the human-animal bond

A.M. Beck Purdue University. abeck@purdue.edu
Session: FEATURED SPEAKER - SCHWABE SYMPOSIUM

Domestication is a relationship between people and animals that has behavioral, psychological, physiological, and evolutionary bases. Humans are a social species that has evolved to be attentive to nature and animals, especially animals that have juvenile features, which evoke nurturing behaviors. In addition to the comfort we derive from our domestic animals, they are social species as well and find comfort from us. These behaviors provide the social support we need for us to thrive. Therefore, it should not be surprising that our bonding with animals has many positive health benefits; indeed, it would be difficult to explain if they did not. All indications are that companion animals play the role of a family member, often, a member with the most desired attributes. Ordinary interactions with animals can reduce blood pressure and alter survival after a heart attack. Pets, for some, afford increased opportunities to meet people, while for others; pets permit people to be alone without being lonely.

Notes:

112 - Small animals - big data!

C. Edinboro Exponent Inc Health Sciences. cedinboro@exponent.com
Session: FEATURED SPEAKER - SCHWABE SYMOSIUM

Let's recall that the early work of veterinary epidemiology began with the detection and prevention of disease in large, farmed animals and in parasitic, zoonotic diseases. Dr. Glickman's work began in public health and moved into small animal epidemiology. He expanded this field, training veterinary clinicians, pathologists, and epidemiologists, as well as statisticians, nurses, and physicians. He has used datasets both small and "Big." Much can be learned from well-designed small studies to quantify well-defined measurable effect differences. Field trials at humane shelters of intranasal vaccines in cats and dogs to prevent upper respiratory infections yielded important results. A case-control study of feline hyperthyroidism identified host, environmental, and dietary risk factors, shedding light on this epidemic and potential public health concerns. The feline hyperthyroid research used the Veterinary Medical Database. This veterinary school hospital database, created in 1964 to study cancer in animals, has been used for studies of numerous animal diseases and treatments. It is limited in age and other characteristics, and incomplete data, yet remains a valuable data source. With greater computational resources, it will be possible to interrogate larger sources of data faster using more complex and flexible algorithms; this is the basis of Big Data. Developers and users of large datasets must address the potential for biases in data collection and predictive models. Caution must attend to avoid spurious associations; significant differences may exist that are not clinically impactful. Sources of Big Data in small animal medicine include databases developed by regional or national corporate veterinary practices and by shelter organizations. Access to privately-held datasets can be challenging, and funding and time to use them may be limited. Epidemiologists should look for opportunities to use available large datasets and to encourage further development of readily accessible Big Data that includes small animal data to enhance our "One Health" understanding.

Notes:

113 - Big data (big benefits or big obstacles?)

G. Moore Purdue University. gemoore@purdue.edu
Session: FEATURED SPEAKER - SCHWABE SYMPOSIUM

Veterinary epidemiologists seek to investigate determinants and distribution of diseases in populations, but where does population information come from? In "the old days" populations of interest were typically herds or flocks, and we depended on herd records generated by the producer or owner. While populations of production animals still exist and remain quite important, the overall population of companion animals seeking veterinary care has grown immensely in recent decades. These animals however may be the sole animal of their species in their local "herd" or home environment, and so veterinarians must be the collectors of information or data on companion animals. University teaching hospitals were the principal location historically for both large sets of medical records and large numbers of clinician-investigators inclined to research and publish information from these records. In recent decades the increasing size and number of large, multi-location private and corporate practices have resulted in large databases of EHRs/EMRs. These databases however remain the property and purview of these practice owners. Can "big [veterinary] data" be leveraged? For what purpose? Will proprietary leverage have a public benefit? If so, how can that benefit be maximized? If not, why not? This presentation will look closer at the challenges in tapping into the potential benefits of veterinary medicine's big data.

Notes:

114 - A journey through vaccine valley

D. McVey University of Nebraska-Lincoln. dmcvey2@unl.edu
Session: FEATURED SPEAKER - AAVI

The seminar will include summaries and reviews of a 40-year career that includes private practice, traditional academia, the biologicals development industry, diagnostic laboratory service, and federal service (USDA ARS). These experiences included basic research, commercial biologicals research and development, state diagnostic laboratories, teaching in veterinary colleges and administrative services. A common theme through all of this has been research and development of countermeasures and diagnostics for infectious diseases of animals. Much of the work has focused on respiratory diseases of livestock. This presentation will include examples relevant to respiratory disease, arbovirology, arthropod vector biology, virus/vector ecology as well as vaccinology. As infectious diseases threaten human and animal health, there is great pressure to develop new countermeasures including vaccines. In the marketplace, technology may sometimes drive sales. But the principles of efficacy and effectiveness have not changed. This seminar will discuss these principles and also reflect on critical questions for future research and development.

Notes:

115 - From scientific discovery to commercial product in 10 easy steps: The case of CD163 and PRRS vaccines

J.G. Calvert Zoetis. jay.calvert@zoetis.com
Session: FEATURED SPEAKER - AAVI

At the interface of immunology and microbiology lies the discipline of vaccinology. Modern veterinary vaccinology has given rise to many hundreds of USDA licensed vaccine products conceived by clever research scientists in academic, industrial, and government labs. But the road from discovery to commercial vaccine is littered with the remains of thousands of equally clever ideas that were determined to be inappropriate for commercial development. In order to help bridge the gap between discovery and commercial vaccine product, I have compiled a list of 10 potential roadblocks for consideration by scientists at the early stages of a new research project. These include: (1) Product Profile, (2) Safety, (3) Efficacy, (4) Purity, (5) Stability, (6) Cost and Profit, (7) Regulatory Hurdles, (8) Patentability, (9) Freedom to Operate, and (10) Public Perception. As an example of how these roadblocks can be anticipated and successfully navigated, the extensive research and subsequent development of two commercial PRRS vaccines are reviewed here. Included are the first reverse genetics system for PRRSV-2, the first use of PRRSV-2 as a vector, the discovery of CD163 as a primary PRRSV receptor, and the attenuation of PRRS viruses on novel cell lines.

Financial Support
Zoetis

Notes:

116 - Vaccine discovery and development – from lab to market place

R. Curtiss III Department of Infectious Diseases & Immunology, University of Florida. rcurtiss@ufl.edu
Session: FEATURED SPEAKER - AAVI

In 2002 papers linked elimination of using subtherapeutic antibiotics to enhance feed conversion and growth in poultry with an increased incidence of necrotic enteritis caused by Clostridium perfringens (Cp), causing elevation in antibiotic use. I proposed to use attenuated Salmonella vaccine vectors to deliver modified Cp toxin antigens to chickens to control this disease. I thus applied successfully for an Ellison Medical Foundation Global Infectious Disease Senior Scholar award. Bereket Zekarias initiated the effort aided by Hua Mo to deliver non-toxic alpha and NetB toxins to vaccinated broiler chickens. Yanlong Jiang, Kenneth Roland and Shifeng Wang made significant improvements in the delivery of these antigens using Salmonella vaccine vectors with improved type 2 secretion system for delivery of protective antigens. The vaccine also regulated delayed attenuation and protective antigen synthesis to enhance colonization of effector lymphoid tissues and regulated delayed lysis to provide biological containment as perfected by Wei Kong and Shifeng Wang. Most recently, Shifeng Wang has designed Salmonella vaccine vector strains in which key traits are dependent on three sugars that can be provided during fermenter growth of the vaccines and are totally absent in the vaccinated animal host. We refer to these improved vaccine vectors as Protective Immunity Enhanced Salmonella Vaccine (PIESV) vector strains. This PIESV-Cp construct has advanced through all required evaluations and field trials with results accepted by the Center for Veterinary Biologics. The vaccine is designed for spray application to newly hatched chicks in the hatchery with a second vaccination in drinking water ten days later. In the last two years, we have defined two additional protective Cp antigens that decrease ability of Cp to colonize the chicken intestinal tract. The use of a regulated lysis plasmid for delivery of multiple secreted protective Cp antigens and further improved PIESV vector delivery strain will hopefully lead to more efficacious second generation PIESV-Cp vaccine.

Financial Support
U.S. Department of Agriculture; U.S. National Institutes of Health

Notes:

117 - Advances in African swine fever live-attenuated vaccines

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Session: FEATURED SPEAKER - AAVI

Objective

African Swine Fever (ASF) is a disease of domestic and wild swine that produces a spectrum of disease, from sub-clinical to highly lethal, depending on the acting virus strain. Currently a highly lethal strain is causing outbreaks throughout Asia and Europe, recently spreading to the wild boar population in Germany. There are no commercially available vaccines for ASF, so disease outbreaks are controlled by removing infected animals. The rapid spread of ASF combined with its high lethality make it a significant threat to global protein availability. The causative agent, ASF virus (ASFV), is a large, highly structured, enveloped DNA virus with a double-stranded DNA genome (180-190 Kb) encoding for at least 160 open reading frames (ORFs). Most of these ORFs has been predicted using functional genomics without experimental characterization, limiting application to development of therapeutics. The identification of viral proteins involved in virus replication and virus virulence in swine is critical to developing any control measures. Interestingly, only a few virus genes have been successfully deleted from the ASFV Georgia genome that did not attenuate the virus (e.g.: TK, NL, CD2, MGF360-16R, L83L, C962R, X69R) and only a small number of genes were shown to be essential for virus replication (e.g.: EP152R, p30, p54, p72,).

Methods

Until recently the production of a recombinant ASFV lacking a gene was troublesome and very time consuming to make. Although several advances in ASF have allowed for faster production of recombinant ASFV, however it still largely remains a difficult labor and time-consuming process that is necessary to expand the knowledge gained experimentally towards the role of uncharacterized viral proteins in virus virulence and replication. Understanding the role of specific individual genes in virus virulence is a critical step in the development of novel ASF vaccines.

Results

Several experimental live-attenuated vaccines have been developed that effectively produce protection against the current strain circulating in Europe and Asia. These experimental vaccines are recombinant live attenuated viruses derived from the virus isolate that initiated the outbreak in the Republic of Georgia in 2007. These recombinant viruses are attenuated by deleting one or more genes from the field isolate genome by genetic manipulation.

Conclusions

The most promising experimental candidate has a single deletion of gene I177L. This experimental vaccine offers 100% protection at a low dose, sterile immunity, and no viral shedding to unvaccinated animals. Importantly even at higher doses there are no clinical symptoms. These characteristics make ASFV-G-ΔI177L have the most promising profile for any live-attenuated vaccine that protects against ASFV-G.

Notes:

118 - DIVA vaccine cross-protects against *Salmonella* serovars in food animals

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Session: FEATURED SPEAKER - AAVI

Objective

The human foodborne pathogen *Salmonella* can reside in the gut of food-animals for extended periods of time without causing clinical signs of disease, posing long term contamination threats throughout the food chain. Advancing targeted mitigations to control *Salmonella* on the farm is important in enhancing food safety. Vaccination against *Salmonella* is an effective pre-harvest intervention strategy administered during the production of food-animals such as poultry, swine and cattle. Potential limitations of current vaccine strategies are the lack of cross-protection against the >2,600 serovars of *Salmonella* as well as interference with surveillance programs that monitor the *Salmonella* status of herds and flocks.

Methods

To overcome *Salmonella* vaccine limitations, we designed and constructed a live, attenuated *Salmonella enterica* serovar Typhimurium vaccine (BBS 866) with genetic mutations in the *rfaH* gene as well as several small RNA (sRNA) genes.

Results

The BBS 866 vaccine not only protected swine from *Salmonella enterica* serovar Choleraesuis that causes systemic disease in pigs but also reduced colonization and fecal shedding of *Salmonella enterica* serovar Typhimurium that causes foodborne disease in humans. Vaccination with BBS 866 did not interfere with herd level monitoring for *Salmonella* spp., thereby allowing for differentiation of infected from vaccinated animals (DIVA). In addition to designing the vaccine to protect against multiple *Salmonella* serovars, our intent was also for application in multiple food-producing animal species. BBS 866-vaccination of turkeys resulted in a significant reduction in systemic and intestinal colonization following challenge with multi-drug resistant *Salmonella enterica* serovar Heidelberg associated with the 2011 ground turkey outbreak.

Conclusions

The dual-purpose, *Salmonella* BBS 866 DIVA vaccine reduced serovar specificity to provide cross-protection against diverse *Salmonella* serovars, thereby broadening applicability for multiple animal species to support food safety and public health by reducing the spread of human foodborne *Salmonella*.

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

Notes:

119 - The significance of veterinary vaccines for the U.S National Biodefense Strategy

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Session: FEATURED SPEAKER - AVRN SYMPOSIUM

The National Biodefense Strategy was established in 2018 to prepare and protect the U.S. from biological threats. The Strategy recognizes that a collaborative, multi-sectoral, and trans-disciplinary approach to the national biodefense enterprise is necessary to counter biological threats effectively and efficiently. Biological threats include animal diseases that present complex problems with multiple facets that require specific fit-for-purpose detection, control, and eradication strategies. These strategies typically include surveillance, biosecurity, and the use of countermeasures to contain a disease outbreak. Vaccines represent the single most cost-effective countermeasure to respond and mitigate disease outbreaks. Their effective use in disease control programs is paramount to global food security and the safe production of livestock, poultry, and the rapidly expanding aquaculture sectors. Moreover, the threat of emerging zoonotic diseases has renewed interest in the use of animal vaccines as an integral component of sustainable animal production. But are available vaccines up to the challenge? Have tangible advancements been made in the field of veterinary vaccinology? Are there new technologies driving the discovery of new vaccines that will fundamentally change the way we approach the stockpile of veterinary vaccines, prepare for disease outbreaks, and implement disease control programs? This presentation will outline key challenges that need to be addressed, examples of new technologies in the research pipeline, and provide specific examples of new vaccines under development for some important diseases that threaten animal agriculture and the livelihood of people worldwide.

Notes:

120 - From discovery to corporate development; challenges between academia and commercialization

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Session: FEATURED SPEAKER - AVRN SYMPOSIUM

This presentation will identify and discuss major hurdles in the translation of scientific discoveries made in academia to commercialization as pertains to the veterinary vaccine industry. Many different technologies originate in the academic sector with high potential for commercial product development. However, a number of milestones are necessary to enable an economically feasible product development path that is also in compliance with regulatory agency standards in order to achieve market authorization. Major factors including corporate research and development pipeline portfolio, market demand and scientific milestones will be discussed with the background of why these are critical path aspects in moving from research into a product development work stream that yields a product that is highly viable in the market.

Notes:

121 - United States Animal Vaccine Research Coordination Network (USAVRCN)

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Session: FEATURED SPEAKER - AVRN SYMPOSIUM

Significant progress was made in the past year in implementing the USAVRCN (http://www.usanimalvaccinenetwork.org/). The primary goal of the USAVRCN is to act collectively to improve strategies and streamline efforts for the timely development of efficacious next generation vaccines and flexible diagnostic platforms important for monitoring and mitigation of infectious diseases with potential for catastrophic impact on U.S. agriculture. The USAVRCN seeks to leverage current and future vaccinology research at multidisciplinary member institutions, and in doing so consolidate technologies and quickly gather and disseminate information from within and across the network. USAVRCN leadership is provided by a management team at the University of Connecticut, a five-member external Board of Directors, and six working group Team Leaders from across the research community and representing Aquaculture, Equine, Poultry, Ruminants, Swine, and Vaccine Technology groups. The USAVRCN has formalized a collaborative relationship with the International Veterinary Vaccine Network based in the UK, which will expand our international contacts and access to further technologies. The USAVRCN is implementing an Early Career Vaccinologist program with the specific goal of assisting talented young vaccinology researchers from academia, government, and industry in career development and mentorship. In the next year, we anticipate implementing our Advanced Vaccines Fellowship to promote vaccine research that fosters collaboration and crosses disciplines in search of novel solutions, specifically by assisting placement of pre- and postdoctoral candidates in different laboratories. As the leadership and infrastructural groundwork have been laid, USAVRCN now looks forward to growing its membership, implementing its programs, and broadening its reach.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture; USDA-NIFA Food Animal Residue Avoidance Databank

Notes:

125 - Develop a risk-free in situ non-animal (RISNA) megavirus surrogate assay for ASFV

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Session: AFRICAN SWINE FEVER

Objective

African swine fever (ASF) causes high mortality in swine and is a significant and urgent threat to the U.S. pork industry if ASFV, the etiological viral agent of ASF, were to be introduced. The ASFV has been shown to survive in soybean meal for extended periods of time and be capable of infecting pigs at very low concentrations. However, there is very limited information on ASFV survival in feed ingredients, inactivation kinetics, and the effectiveness of mitigation strategies to inactivate ASFV due to regulatory restrictions for using ASFV in research laboratories, and the inappropriateness of using other RNA-based viral surrogates. Our objective is to develop a risk-free *in situ* non-animal (RISNA) megavirus surrogate assay for ASFV for use in implementing effective biosecurity to prevent transmission of ASFV under real-world scenarios.

Methods

The RISNA will be developed using *Emiliania huxleyi* virus (EhV), which shares a common origin and genetic similarity with ASFV. Moreover, ASFV and EhV enter and exit their cellular hosts by similar mechanisms. These similarities make EhV a strong candidate for studying ASFV inactivation in feed ingredients such as soybean meal. Furthermore, use of EhV as a surrogate is safe and poses no animal, plant or human health risk. Time and temperature were used as an inactivation method to validate whether EhV is a suitable surrogate for ASFV.

Results

We evaluated several different time and temperature inactivation combinations to determine effects of exposure on EhV inactivation which included replicating the conditions known to inactivate ASFV. We showed that EhV is similarly sensitive to temperatures greater than 50˚C. EhV is still alive if treated at 37˚C and inactivation at either 50˚C or 60˚C occurs as quickly as 5 min.

Conclusions

EhV is a suitable surrogate for ASFV. Subsequent studies with industry partners will involve using RISNA to evaluate the effectiveness of chemical mitigants to eliminate ASFV if present in feed ingredients and complete feed. Therefore, this research benefits multiple segments of the global swine and feed industry.

Notes:

127 - The current situation of African swine fever in Cameroon

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Session: AFRICAN SWINE FEVER

Objective

Cameroon is located in central Africa with an estimated population of 25.8 million inhabitants as of 2019 wherein 43.5% of them live below the poverty line of less than 3.1US dollars/day/capita. The agricultural sector contributes 20% of the gross domestic product with 13% from the Pig industry. Cameroon is the highest pork producer in central Africa with an estimated 2,02kg/person production which is far lower than the expected 5kg/person due to the endemic nature of African swine fever in the country after its first entry in 1982 from an unknown source. The virus causes a lethal hemorrhagic disease with mortality ranging from 30 to 100 % depending on the virulence of the circulating strain. The focus is to look at the situation of the African swine virus in Cameroon through available literature

Methods

A thorough review of available articles shows that

Results

there are 24 genotypes of the virus of which only the genotype I is implicated in the recurrent outbreaks in Cameroon with subgroups A, B, and C as opposed to only subgroup A during the 1982 outbreak. The absence of the soft tick and the warthog in the major pig production Regions reveals that the virus is circulating principally in the domestic cycle. A joint project by the FAO and the Cameroon Government has helped to reduce the incidence of the disease from 11% in 2000 to 1% in 2014 through the provision of diagnostics tools and effective control of African swine fever in the country

Conclusions

The fact that only genotype I is present in Cameroon with all isolates being serotypically similar is of importance in vaccine development though very little research has so far been effected into the search of a vaccine or antiviral drug for the field situation of Cameroon.

Notes:

129 - Discovery of stable cell lines for both African swine fever vaccine production and ASFV diagnostics

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Session: AFRICAN SWINE FEVER

Objective

African swine fever virus (ASFV) outbreaks are currently spreading rapidly across Europe and Asia both in domestic pig farms and in the wild boar population. Since the first introduction into china in 2018, ASFV has spread to all provinces in China and to other countries throughout southeast Asia, and most recently to India. Currently the only cell line that supports ASFV is primary swine macrophages, which require resources, expertise, time to produce, and a stable supply of donor swine. Primary swine macrophage cultures are infrequently available in most regional veterinary diagnostic laboratories. There is a desperate need for cell lines both for diagnostic purposes and for vaccine production.

Methods

A systematic review of stable cell lines to determine cell lines with characteristics for diagnostics and for vaccine production was performed.

Results

We identified a commercially available stable cell line that can be widely used as a diagnostic tool for detecting live ASFV in clinical samples, with detection limits just below swine macrophages, and higher detection than current PCR diagnostics for ASFV. This cell line is capable of seeing both CPE and rosette formation (for hemeadsorbing ASFV), similar to primary swine macrophages, and for non hemeadsorbing strains of ASFV, virus can be visualized also by immunohistochemistry for the presence of ASFV without background staining. For vaccine production we also identified a different cell line that is suitable to grow ASFV at high titer for vaccine production of experimental live-attenuated vaccine candidates, We report on the production of ASFV-G-ΔI177L in this cell line.

Conclusions

Two seperate immortalized cell lines were identified one with the characteristics for ASFV vaccine production and the other with charactistics for ASFV diagnostics.

Financial Support
U.S. Department of Homeland Security

Notes:

130 - Predicting global risk of introduction of African Swine Fever (ASF) using spatial neighborhood

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Session: AFRICAN SWINE FEVER

Objective

African Swine Fever (ASF) is an emerging disease that is threatening the global pig population. Although wild African pigs are resistant, due to 100% mortality in commercial pigs, the introduction of ASF can be detrimental to the pig industry in ASF-free countries. The objective of this study was to explore reported outreaks in order to create a predictive model that could help identify the risk of ASF introduction to countries that are currently free from African Swine Fever on a monthly basis.

Methods

Data sources included the ASF outbreak data from 1996-2020 reported to the World Organization for Animal Health, pig production and trade obtained from Food and Agriculture Organization of the United Nations (FAOSTAT), and the spatial polygons at the country level obtained from Esri/ArcGIS Hub. Original data were processed to create monthly time-series data with the outcome of interest (i.e. month and year of the first introduction of ASF to a country), and the relevant time-variant predictors. Eight risk factors were identified including: number of ASF positive neighbors in a given time period, proportion of ASF positive neighbors in a given time period, length of land borders, number of animals at risk in a given year and pig product import in a given year. Initial model was built and internally validated using Random Forest using 3-fold cross validation.

Results

Using lift charts, this model, on average, was able to identify approximately 70% of positive cases within approximately 10% of the highest ranked predicted probabilities. Preliminary investigations of apparently false negative probabilities revealed that their substantial proportion was linked with months leading to introduction of ASF to specific countries.

Conclusions

Investigations of the predictive probability of the model through lift charts suggested that the current model offers a reasonable platform for risk-based surveillance at the global level.

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:

131 - Immunogenic T cell-directed African Swine Fever vaccine designed with immunoinformatic tools

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Session: AFRICAN SWINE FEVER

Objective

There is no licensed vaccine against African Swine Fever (ASF), a lethal viral disease that has spread across Asia and Europe resulting in severe economic losses. We set out to develop a T cell-directed ASF vaccine that focuses immune responses on conserved virus sequences in genotypes I and II.

Methods

The iVAX T cell epitope identification and vaccine design software platform was used to identify, rank, and select conserved, ASF virus-specific class I and class II SLA epitopes. Multi-epitope genes encoding class I and class II epitopes separately were each subcloned into plasmids to produce a DNA vaccine. A pilot vaccine study was performed to assess epitope immunogenicity. Four-week old pigs were primed and boosted three weeks later with plasmid DNA vaccine delivered intradermally using Pulse NeedleFree technology. A matching group of pigs received empty plasmid (no epitopes) as a control for epitope-specific immune responses. One week later, blood was collected, peripheral blood leukocytes isolated and recall responses to vaccine epitopes measured by interferon-gamma ELISpot assay.

Results

A total of 40 class I and 26 class II peptides were selected for inclusion in the ASF vaccine constructs following immunoinformatic predictions. Selection was based on high conservation among 21 European, Asian and African isolates covering genotypes I, II, IX, and X, high binding likelihood to 13 SLA class I and 8 class II alleles, and low tolerogenicity potential. Immunization stimulated strong epitope-specific interferon-gamma responses in all pigs that received the ASF DNA vaccine. No control pigs responded.

Conclusions

The immunogenic ASF plasmid DNA vaccine may be effective against ASF virus challenge when used in combination with an inactivated or live attenuated virus vaccine in a heterologous prime-boost regimen for robust cellular and humoral responses, as we observed previously for swine influenza.

Notes:

132 - PARTNERSHIP: Single-cycle Replicon-based African Swine Fever Virus Subunit Vaccine

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Session: AFRICAN SWINE FEVER

Objective

Evaluate efficacy of a prototype ASFV subunit vaccine in pigs and wild boars.

Methods

Attenuated African Swine Fever Virus [ASFV] can induce protection, but safety concerns hinder deployment. Subunit vaccine development requires identification of protective antigens. Preliminary studies demonstrated that an adenovirus-vectored prototype vaccine induced CTL responses and conferred protection in 56% of vaccinees. Antigens containing putative CD8+ T cell epitopes were identified, *in silico*, by screening ASFV proteome for peptides that bind strongly to *SLA* I alleles. Binding affinities of the peptides and the number of predicted CTL epitopes was used to select novel vaccine candidates. Synthetic peptides were used to authenticate the predicted CD8+ T cell epitopes by IFN-g EliSpot using T cells from previously immunized pigs. Selected protein sequences were used to design multicistronic expression cassettes that were modified to add FLAG-tag at the C-termini. Synthetic genes encoding the multicistronic expression cassettes, codon-optimized for protein expression in swine cells, were cloned into pCDNA3 vector to generate protein expression constructs. Immunocytometric assays were used to evaluate protein expression using anti-FLAG mAb and antigen was authenticated using ASFV convalescent serum. Protein expression was used to select the best clone for each construct for generation of recombinant single-cycle adenovirus plasmid constructs to be used for virus rescue. Protective efficacy of the replicon-based prototype vaccine will be evaluated in pigs and wild boars.

Results

The constructs are expressing the antigens containing putative CD8+ T cell epitopes. The expressed antigens are authentic as confirmed using ASFV convalescent serum and the putative CD8+ T cell epitopes are recognized by T cells from pigs immunized with ASFV antigens as judged by IFN-γ EliSpot.

Conclusions

The replicons expressing ASFV antigens containing putative CD8+ T cell epitopes have potential to induce protective CTL responses.

Financial Support
USDA National Institute for Food and Agriculture

Notes:

133 - Efficacy of prototype live-vectored African swine fever virus vaccines

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Session: AFRICAN SWINE FEVER

Objective

Evaluate protective efficacy of adenovirus-vectored ASFV antigen cocktails in pigs

Methods

We have previously evaluated protective efficacy of three adenovirus-vectored prototype vaccines containing African Swine Fever Virus [ASFV Georgia 2007/1] antigens in pigs using two different adjuvants (*Lokhandwala, S., et al., 2019. Vet. Micro*). We also evaluated protective efficacy of one cocktail in European wild boars (*Cadenas-Fernández, E., et al., 2020. Pathogens J.*). However, the best performance achieved so far was 56% protection of pigs following immunization with one formulation and mucosal challenge. Knowledge gained from the previous studies was used to develop a next generation prototype vaccine using conserved antigens selected based on the presence of putative CD8+ T cell epitopes. Peptides were used to confirm the putative CD8+ T cell epitopes by IFN-g EliSpot assays using T cells from previously immunized pigs. Codon-optimized genes encoding FLAG-tagged multicistronic antigen expression cassettes were synthesized and used to generate recombinant replication-incompetent adenoviruses. A negative control construct encoding luciferase, was similarly generated. Anti-FLAG tag monoclonal antibody was used to evaluate protein expression and ASFV-specific convalescent serum was used to validate authenticity of the expressed antigen.

Results

The recombinant adenoviruses encoding the ASFV expression cassettes containing putative CD8+ T cell epitopes are expressing the antigens and the expressed antigens are authentic as confirmed using ASFV convalescent serum. A peptide library and recombinant proteins for *in vitro* immune readouts have also been generated.

Conclusions

We expect that a prototype vaccine containing a cocktail of the recombinant adenoviruses expressing the ASFV expression cassettes containing putative CD8+ T cell epitopes will significantly improve protective efficacy upon challenge and thereby allow identification of the protective antigens needed for development of an efficacious subunit vaccine.

Notes:

134 - Tracing African swine fever: viral evolution and disease transmission in the Southern African Development Community

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Session: AFRICAN SWINE FEVER

Objective

Here we present an overview of our recently awarded project that aims to 1) assess the pig contact networks, pig management and socio-economic factors, tick involvement in African Swine Fever virus (ASFV) transmission and viral diversity in the sylvatic and domestic cycles, 2) model ASFV transmission dynamics, economic impact and risk of introduction into free areas in different eco-epidemiological settings using multi-scale simulation models, 3) integrate genomic-to population level data and modeling methods into an open-access analytical platform and develop interactive educational and training materials.

Methods

We will use a combination of field work (sampling and surveys), diagnostic methods, full genome sequencing and experimental infections to gather necessary data. Data will be then analyzed using value chain analysis, network analysis and spatial-explicit stochastic disease spread and economic models to assess the transmission dynamics of ASF and evaluate the risk of ASF introduction/spread into new territories. Finally, we will integrate data and modeling methods into a user-friendly dedicated site referred to as ASF-BioPortal to facilitate data access, analysis and visualization by stakeholders, policy makers and the general public.

Results

We expect to provide a better understanding of the ASF genetic diversity among different susceptible hosts and the main ASF transmission pathways within and between the domestic and sylvatic cycles in the different study regions. We will also identify the areas at high risk for ASF introduction and spread and will provide estimates for the magnitude, duration and economic impact of ASF epidemics under diverse epidemiological settings.

Conclusions

Results of this project aim to support, inform and engage researchers, livestock producers, policy makers and the general public to participate in the collaborative effort of ASF prevention, control and eradication and contribute to more coordinated, synergistic and cost-effective prevention and control of ASF (and other TADs) at a local, regional and global scale.

Financial Support
USDA National Institute of Food and Agriculture

Notes:

135 - Surveillance and detection of African Swine Fever Virus in pork products using the MatMaCorp Solas 8 platform

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Session: AFRICAN SWINE FEVER

Objective

African Swine Fever Virus is found throughout Africa, Asia, and parts of Europe. As of writing this abstract, there is no commercial vaccine available and mass culling is the main method available for control. A comprehensive ASF surveillance program is the optimal strategy for preventing the virus from entering unaffected countries. Such a program requires rapid testing, with a robust portable platform for on-site use, and the flexibility to test multiple sample types including biological and environmental samples along with feed ingredients and meat products. Our objective was to evaluate the MatMaCorp Solas 8 platform and compare it with traditional laboratory methods for the detection of ASFV.

Methods

Three international sites were established for testing of samples from naturally and experimentally infected pigs. The first reported study was performed by the US Department of Homeland Security Science and Technology at Plum Island Animal Disease Center. DNA from various biological samples was extracted using MagicTip DNA isolation kits for blood, tissue, and oral fluid. Extracted DNA was tested using the ASFV C-SAND assay, a padlock probe-based isothermal method, and analyzed on the Solas 8 fluorescence detection device. Results were compared to Ct values from established ASFV diagnostic real-time PCR assay.

Results

For inclusivity of the assay, all eight tested ASFV strains were detected from cell culture, including direct detection from the supernatant without DNA extraction. ASFV was detected in blood (fresh and frozen), oral fluid, spleen, raw muscle tissue, and bone marrow. Virus was detectable to a sensitivity of 33 Ct compared to real-time PCR. No false-positives were detected in negative control samples.

Conclusions

The MagicTip DNA isolation kits, ASFV C-SAND assay, and Solas 8 fluorescent detection device from MatMaCorp were able to quickly detect virus from various strains and sample types, including potential food products. The assay meets inclusivity, sensitivity, and specificity criteria needed for a field-deployable surveillance platform.

Notes:

136 - Partitioning© A framework to design cost-effective on-farm ASF surveillance in endemic areas

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Session: AFRICAN SWINE FEVER

Objective

African Swine Fever (ASF) has established in SE Asia with devastating consequences to the pig industry. In high endemic areas such as Vietnam where culling entire premises is unsustainable and underreporting is hindering control efforts, we propose Partitioning©, a system for on-farm surveillance of animal units as a strategy for early detection and prevention of disease spread, aiming at maintaining farm production while culling infected units in case of disease outbreaks.

Methods

We created a framework to quantify the efficacy and cost-benefits of various on-farm surveillance regimens in partitioned units[SC1] , using different testing and monitoring methods. ASF introduction, spread, and progression were stochastically modeled along with efficacy and cost of diagnostic tests. Farm management and economics e.g., animals' value, time and cost to reach production stages, and direction and frequency of animal movements between units were modeled. While the model is applicable to different production systems, here we modeled a large-scale modern farrow-to-finish farm in Vietnam.

Results

The value of partitioning surveillance schemes is highly dependent on test timing prior to animal movements, disease spread dynamics, and the value of animals. Sensitivity and specificity of the test and clinical surveillance are both highly influential, as although false negatives can result in spread, false positives can increase costs of confirmatory tests and delay animal movements between units causing a cascading disruption in the production flow of farrow-to-finish farms.

Conclusions

Partitioning on-farm surveillance can be used as a strategy for early outbreak detection and prevention of spread, and thus limit loss and maintain production in disease-free units. In endemic areas where strict controls have proven hard to enforce, we argue that it may be an alternative approach to disease control. The framework and model developed in this project can contribute to the design of programs to reduce ASF spread and losses to swine producers.

Financial Support
U.S. Department of Agriculture

Notes:

137 - Develop a risk-free *in situ* non-animal (RISNA) megavirus surrogate assay for ASFV

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Session: AFRICAN SWINE FEVER

Objective

African swine fever causes high mortality in swine and is currently a significant and urgent threat to the U.S. pork industry if ASFV were to be introduced. ASFV and other viruses have been shown to survive in soybean meal for extended periods of time. However, there is very limited information on ASFV inactivation kinetics required for conducting risk assessments and the effectiveness of various mitigation strategies to inactivate ASFV. Therefore, it is imperative that a megavirus surrogate assay for ASFV be developed and validated for use in implementing effective biosecurity and mitigation procedures to prevent transmission of ASFV under real-world scenarios. Our overarching objective is to develop a risk-free *in situ* non-animal (RISNA) megavirus surrogate assay for ASFV.

Methods

The RISNA will be developed using *Emiliania huxleyi* virus (EhV) a megavrus that is distantly related to ASFV. ASFV and EhV enter and exit their cellular hosts by similar mechanisms. These similarities make EhV a strong candidate for studying ASFV inactivation in complete feed and feed ingredients such as soybean meal. Furthermore, use of EhV as a surrogate is safe and poses no swine, animal, plant or human health risk. Time and temperature were used as an inactivation method to validate whether EhV is a suitable surrogate for ASFV.

Results

We evaluated several different time and temperature inactivation combinations to determine effects of exposure on EhV inactivation which included replicating the conditions known to inactivate ASFV (60ºC for 20 min). We showed that EhV is similarly sensitive to temperatures greater than 50ºC. EhV is still alive if treated at 37ºC and inactivation occurs at either 50ºC or 60ºC occurs as quickly as 5 min.

Conclusions

EhV is a suitable surrogate for ASFV. Subsequent studies will involve using RISNA to evaluate the effectiveness of chemical mitigants to eliminate ASFV if present in feed ingredients and complete feed. Therefore, this research benefits multiple segments of the global swine and feed industry including the U.S. soybean industry (a funder of this reaerch).

Notes:

138 - Tracking visit to pig farms of livestock vehicles after passing through affected areas of ASF in wild boars

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Session: AFRICAN SWINE FEVER

Objective

To estimate potential extent of virus spread from the affected areas of ASF through livestock vehicles

Methods

We tracked down the pig farm visits of livestock vehicles having passed through 3 km radius from the point where ASF was confirmed in wild boars, during 46 weeks from the first case of wild boar in October 2019 to the 721st in August. The movement of livestock vehicles tracked with GPS (geographical positioning system) and recorded in the KAHIS (Korea Animal Health Integrated System) were extracted. List of visits pig farms by these livestock vehicles were analyzed in the NDAP (National Data Analysis Platform), a big data platform of the APQA (Animal Plant and Quarantine Agency).

Results

A total of 1,646 livestock vehicles visited pig farms after passing 3km radius of the 721 points of ASF cases in wild boars. Carriers of live animals (422, 25.6%), feed lorries (378, 23.0%), and consultants' car (206, 12.5%) were the most common vehicle types. The vehicles visited 1,308 farms across Korea, located in 81 cities/counties of 11 metropolotan cities/provinces. The total number of visits pig farms by livestock vehicles was 24,422. Most (85.7%) of the vehicles visited only one or two cities/counties, while 1.8% visited 6 or more cities/counties.

Conclusions

Most livestock vehicles run in nearby areas, but some vehicles have been found to travel long distances. In Europe, ASF cases in wild boars were identified jumped a long distance by human activities. So that there would be no more such cases, a thorough biosecurity is needed to prevent spread of virus through vehicles, a human-mediated transmission.

Financial Support
Animal and Plant Quarantine Agency, South Korea

Notes:

139 - Development of a blocking enzyme-linked immunosorbent assay for detection of antibodies against ASFV

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Session: AFRICAN SWINE FEVER

Objective

Recent outbreaks of African Swine Fever virus (ASFV) in China and some European countries pose the potential pandemic threat to global swine industry. Highly sensitive and specific diagnostic assays are urgently needed for rapid detection and implementation in quarantine and elimination of infected animals. Monoclonal antibody (mAb) is a key reagent for detecting of viral infection. In our previous study (Petrovan et al., 2019, Virus Research, 269:197632), we produced a panel of mAbs against ASFV p30 protein. One of the mAbs showed specific blocking activity against ASFV specific serum antibodies in a blocking ELISA (bELISA) format. The objective of this study is to develop a mAb-based bELISA for detecting host antibody response against ASFV infection.

Methods

The ASFV specific bELISA was developed using recombinant p30 as the antigen and a biotinylated mAb against p30 as the secondary antibody for detection. Test conditions were optimized using standard serum samples generated from pigs immunized with Alphavirus replicon particles expressing p30 antigen. Validation of the bELISA was performed using serum samples from pigs immunized with experimental vector vaccines as well as serum samples from ASFV-infected and non-infected pigs. Diagnostic sensitivity and specificity were determined by receiver operating characteristic (ROC) analysis.

Results

Serum standards were established, which includes high positive, low positive and negative standard. Analytical sensitivity analysis showed that the 1:64 dilution of high positive standard serum is the limit of detection. ROC analysis calculated a diagnostic sensitivity of 98.1% and a diagnostic specificity of 98.9%. A cut off value of the assay was determined as percentage of inhibition (PI) at 53.7%. The coefficient of variation of an internal quality control serum was 6.81% for between-runs, 6.71% for within-run, and 6.14% for within-plate.

Conclusions

These results indicate that the bELISA can be used as a serological test for ASFV with high levels of sensitivity and specificity. This assay provides a valuable tool in ASFV epidemiological surveys and outbreak investigations.

Financial Support
National Pork Board

Notes:

140 - African swine fever virus distribution in a feed mill after manufacture of virus-inoculated feed

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Session: AFRICAN SWINE FEVER

Objective

The objective of this study was to understand African swine fever virus (ASFV) distribution on feed mill surfaces after manufacturing ASFV-contaminated swine feed.

Methods

A pilot-scale feed mill was constructed in a biosafety level three facility, including a mixer and bucket elevator. Initially, a batch of swine feed was primed through the system. Then, swine feed contaminated with ASFV was added into the mixer, mixed, and discharged through the bucket elevator. Subsequently, four additional batches of feed free of ASFV were processed through the same equipment. Environmental swabs from 18 locations were collected after each batch of feed was discharged and categorized into four zones: A) feed contact surface, B) non-feed contact surface < 1m away from feed contact surface, C) non-feed contact surface > 1m from feed contact surface, and D) transient surfaces including the soles of researcher's boots. Environmental swabs were analyzed using qPCR analysis specific for the P72 gene to detect ASFV DNA.

Results

There was evidence of a sampling zone × batch interaction for the number of ASFV genomic copies/mL (P=0.002). After producing the initial batch of ASFV-contaminated feed, zone C had fewer genomic copies/mL compared to zone A surfaces (P<0.05), with no evidence of a difference among other zones. Following batch sequence 1, 2, and 3, zone D had a greater number of genomic copies/mL detected compared to other zones (P<0.05). After sequence 4, there was no evidence of a difference in the number of detected ASFV genomic copies/mL between zones (P>0.05). There was no evidence of a zone × batch interaction for ASFV qPCR cycle threshold (Ct) values, but zone D had lower Ct values compared to all other zones (P<0.05), and samples collected after sequence 3 had lower Ct values compared to samples collected immediately following the inoculated batch of feed (P<0.05).

Conclusions

Once ASFV was introduced into a feed mill, the virus became widely distributed. Key areas of contamination included transient surfaces, illustrating the importance that biosecurity measures must include movement of people.

Financial Support
National Pork Board

Notes:

141 - Peptides affecting membrane phospholipid transport as novel therapeutics against avian pathogenic *E. coli* (APEC)

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Session: ANTIMICROBIAL ALTERNATIVES

Objective

Avian pathogenic *E. coli* (APEC), an extra-intestinal pathogenic *E. coli* (ExPEC), is one of the most common bacterial pathogens affecting poultry. It causes colibacillosis in chickens which results in high morbidity and mortality, decreased meat and egg production, and increased condemnation of carcasses during slaughter. Antibiotics are commonly used to control APEC infections; however, the emergence of antibiotic-resistant APEC strains and increasing restrictions on use of antibiotics in food animals necessitate the development of new therapeutics.

Methods

Here, we measured the efficacy of newly identified probiotic derived peptides against APEC *in vitro* and in chickens and uncovered their target/mode of action.

Results

We have identified probiotic *Lactobacillus* effective in reducing APEC colonization (up to 1.6 logs) in chickens. Further investigation revealed small peptides in the *Lactobacillus* culture supernatant. Subsequent synthesis and testing identified three peptides (P-1, P-2, P-3) displaying bactericidal activity against multiple APEC serotypes, including antibiotic-resistant strains, at concentrations ranging 12 to 18 mM. Microscopy studies revealed that these peptides disrupt APEC membrane either by causing membrane shedding, rupturing, or flaccidity. Further, gene expression studies showed that peptides downregulated the expression of *mla*A (>4.9 folds), a gene responsible for transport of phospholipid from outer leaflet of bacterial outer membrane to inner leaflet. These peptides were effective against APEC in pre-formed biofilm, cultured chicken macrophage (HD11) cells, and wax moth (*Galleria mellonella*) larva model. Further, two peptides (P-2 and P-3) when administered orally (50 mg/kg body wt.) to one-week-old broiler chickens significantly ($P<0.05$) reduced the APEC load in cecum and internal organs.

Conclusions

These results demonstrate that peptides can be developed as novel therapeutics to mitigate APEC infections in poultry. Further, the identification of novel antibacterial target can facilitate the future therapeutic development against APEC and other related ExPECs.

Financial Support
Ohio State University; U.S. Department of Agriculture

Notes:

143 - Evaluating the effectiveness of organic therapies on the treatment and management of bovine digital dermatitis

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Session: ANTIMICROBIAL ALTERNATIVES

Objective

Digital dermatitis, DD, is an infectious disease that causes acute and chronic lameness in dairy cattle. Common topical therapies contain antibiotics, which are prohibited for use on organic dairy farms. The objective of this prospective randomized clinical trial was to identify organic-compliant products with improved treatment outcomes for DD.

Methods

Dairy cattle (n=385) with DD were enrolled at Day 0 on two commercial organic dairy farms. Cows were assigned to one of four randomized topical treatments: copper sulfate, iodine, honey, or hydrogen peroxide. Treatment was applied on D 0, 7, and 14. Cows were re-evaluated in a chute on D 7, 14, 28, 56, and 112. Treatment outcomes included: Locomotion score (LOCOSCORE), an adapted DD scoring system (DDSCORE), and an algometer pressure reading (ALGOSCORE). DDSCORE and LOCOSCORE were evaluated using ordinal regression, with the relative odds of a score 1 to 4 as the outcome of interest. ALGOSCORE was evaluated using linear regression.

Results

This study proved copper sulfate rapidly and significantly improved all treatment outcomes. It showed a significant ($P<0.01$) decrease in proportional odds resulting in the lowest DDSCORE by D28. Lesions treated with copper sulfate were predicted to have an ALGOSCORE of 7.1 (95% CI 6.41, 7.69) at D28 compared to 3.4 (95% CI 2.47, 4.26), 4.0 (95% CI 3.16, 4.75), and 2.7 (95% CI 1.91, 3.49) for honey, hydrogen peroxide, and iodine, respectively. The LOCOSCORE analysis resulted in copper sulfate producing the most rapid recovery and highest probability for a score 1. All statistical analyses showed global significance for treatment and period main effects, and their interactions ($p<0.05$).

Conclusions

In this prospective randomized clinical trial, copper sulfate was the most effective treatment for decreasing DDSCORE and LOCOSCORE, and increasing ALGOSCORE. All cows remaining in the study by D112 showed improved outcome measurements compared to D0. However, the effects of culling and change of treatment due to treatment failure confound those findings. Copper sulfate is the superior topical treatment to manage DD.

Financial Support
USDA National Institute of Food and Agriculture

Notes:

144 - Pre-clinical studies on an enzyme therapeutic for *S. uberis*-associated bovine mastitis

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Session: ANTIMICROBIAL ALTERNATIVES

Objective

Bovine mastitis is the most common and economically significant disease affecting dairy cattle and is the leading cause of antimicrobial use on dairy farms. *Streptococcus uberis* is currently the most prevalent Gram-positive pathogen causing this infection. Recent concerns among consumers regarding the potential for antimicrobial resistance have led to the examination of alternative strategies for controlling mastitis. A streptococcal bacteriophage endolysin, PlyC, is a cell wall hydrolase that rapidly lyses *S. uberis* and other susceptible streptococci on contact. Therefore, it represents an alternative to conventional antibiotic therapy. The goal of this study is to evaluate PlyC as a novel antimicrobial enzyme against *S. uberis* mastitis.

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.

Results

Our results show that PlyC possesses potent lytic activity against all *S. uberis* strains tested. To date, no endolysins successfully functioned in raw cow's milk, presumably due to inactivation by native proteins and lipids. However, PlyC attained three logs of killing at a dose of only two times the minimal inhibitory concentration when administered to raw, mastitic milk derived from clinically affected cows. Due to the absence of neutralizing antibodies that specifically target PlyC, the potential of this enzyme as a novel antimicrobial treatment is further bolstered. PlyC was found to be non-toxic as observed on a bovine mammary cell line and non-irritating as observed on rabbit epidermis and mucous membrane models. Preliminary 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.

Conclusions

The *in vitro* and *in vivo* findings support advancement of PlyC to *S. uberis*-associated bovine mastitis clinical trials, which were scheduled for Spring 2020, but are temporarily halted due to COVID-19.

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

Notes:

145 - The multi-domain PlySs9 cell wall hydrolase is a potential therapeutic against *Streptococcus suis*

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Session: ANTIMICROBIAL ALTERNATIVES

Objective

Increasing resistance to antibiotics amongst livestock has forced the discovery of alternative therapeutics to treat bacterial infections successfully. Bacteriophage-encoded peptidoglycan hydrolases, also known as endolysins, are able to lyse the bacterial cell wall and offer possible applications in food safety, human health and veterinary science. *Streptococcus suis*, which infects pigs, is zoonotic in nature is a potential human health threat. The economic loss of $100 million per year on the swine industry is devastating. Preventing on-farm disease outbreaks is extremely difficult and current approaches are often ineffective to eradicate *S. suis* from herds. There is a pressing need to identify and evaluate *S. suis*-specific endolysins.

Methods

A bioinformatic approach was conducted to identify proteins in bacteriophage genomes with similar homology to catalytic domains of known endolysins. We chose five candidates for synthesis, expression, purification and characterization. Lytic activity of the endolysins was assayed by turbidity reduction, plate lysis, and log-fold killing assays. The binding capacity was evaluated by fluorescent microscopy.

Results

PlySs9 represents our lead candidate and is predicted to contain an N-terminal amidase catalytic domain, a central LysM-based cell wall binding domain, and a C-terminal CHAP catalytic domain. We have determined the optimal conditions for the lytic activity of PlySs9, characterized its broad activity spectrum, and investigated its ability to disrupt streptococcal biofilms. Active-site residues were identified by site-directed mutagenesis. We also assessed the contribution of each individual domain to activity or binding. Lastly, a triple-acting enzyme of PlySs9 was engineered using three unique, potentially synergistic lytic domains to reduce the risk of resistance development.

Conclusions

These results indicate that the broad lytic spectrum of PlySs9 and its catalytic domains have the potential to be used as therapeutic agents against *S. suis* infections.

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

Notes:

146 - Non-antimicrobial options for prevention or treatment of infectious bovine keratoconjunctivitis: A Scoping Review

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Session: ANTIMICROBIAL ALTERNATIVES

Objective

Infectious Bovine Keratoconjunctivitis (IBK) is the most common ocular disease of cattle, typified by corneal ulceration, decreased weight gain, and often requiring antimicrobial treatment. Our objective was to systematically describe the available literature for clinically relevant outcomes on non-antimicrobial prevention and treatment options through a scoping review and to identify topics with knowledge gaps.

Methods

The guidelines on Preferred Reporting Items for Systematic Review and Meta-Analysis extension for Scoping Reviews (PRISMA-ScR) were followed. A protocol was registered with Systematic Reviews for Animals and Food (SYREAF) available through escholarship (https://escholarship.org/uc/item/3p7072n6). Three databases were searched; two reviewers screened 1226 titles/abstracts for topic relevance followed by full-text screening of 131 articles to identify 81 articles eligible for data charting, performed by a single reviewer.

Results

Described study designs were 83% experimental, of which 60% were challenge trials and 40% allowed natural exposure.
Topics identified were vaccine trials (49%), genetic/breed/physical characteristics of cattle (22%), fly abatement (7%) and alternative treatments (6%). Pre-1995 vaccine trials were often challenge trials (80.5%) and lacked placebo-controls (7.5%), random allocation of treatment (19.5%), or investigator blinding (5%). Vaccine trials post 2003 were 90% field trials, 90% used placebo controls, all randomly allocated and 80% blinded. Autogenous (30%) and *Moraxella bovoculi* (40%) vaccine trials increased in prevalence post 2003, however, most field trials failed to show vaccine efficacy.
The non-vaccine studies related to IBK prevention noted protective effects of periocular pigmentation and fly control as reducing the risk of IBK. Nearly all (4/5) alternative treatment studies claimed efficacy against IBK, although study design rigor varied.

Conclusions

Aside from vaccine trials, there is a shortage of data on applied non-antimicrobial interventions for prevention or treatment of IBK, and further research in this area is warranted.

Notes:

147 - Phage Endolysins As Alternative Antibiotics To Control Clostridia In Poultry

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Session: ANTIMICROBIAL ALTERNATIVES

Objective

Clostridium perfringens (Cp) is a Gram-positive, spore-forming anaerobe and causative agent of necrotic enteritis (NE) in poultry. Cp control was via long-term, low-dose antibiotics in feed. There are regulatory moves reducing the use of antibiotics in animal feed. Alternative antimicrobials to fight this pathogen are needed. Bacteriophage lytic enzymes (Ply) (peptidoglycan hydrolases; PGHs) degrade the major structural component of the bacterial cell wall (peptidoglycan; PG) and can kill Gram positive bacteria (Cp). The objective of this project is to express, characterize and quantify *Clostridium perfringens* phage lytic enzyme (PlyCP41) expression in plants and yeast, and find the optimal plant and yeast formulation to deliver the antimicrobial to the lower gut. The second objective is to develop an animal model for Cp colonization in poultry to test the efficacy of plant or yeast formulations harboring PlyCP41 to reduce Cp in poultry gut.

Methods

A prelimary study was conducted to evaluate the endolysin actitivy from yeast when it is stored in a cold environment. The yeast strains were induced with galatose/raffinose and after overnight growth were harvested in 8 x 6ml aliquots. Media was removed from cells and cells were resuspended in 50mM NaPO4 pH7.5, 100mM NaCl. Four tubes were processed immediately for proteins and the remaining four were frozen -80 after adding glycerol to 30%. Fresh cells were prepped via Fast Prep (speed 6/40sec) with 0.5mm zirconium/silica beads in 1ml buffer described above. The frozen cells were removed from -80 after 24hr, thawed on ice, and centrifuged 5000G/5min/4C. Glycerol buffer was removed and replaced with 1ml 50mM NaPO4 pH7.5, 100mM NaCl. Cells were then processed via Fast prep as described above. Equal amounts of protein were serially diluted for plating in the same 50mM NaPO4 pH7.5, 100mM NaCl buffer.

Results

There was not any detectable difference in yeast endolysin activity with protein extracted from fresh and frozen cells.

Conclusions

Preliminary data has indicated the the enzyme in the yeast is stable even after long time freezing.

Financial Support
USDA National Institute for Food and Agriculture

Notes:

148 - Equine mesenchymal stromal cells display antimicrobial properties in an *ex vivo* skin biofilm explant model

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Session: ANTIMICROBIAL ALTERNATIVES

Objective

Bacterial infections associated with biofilm formation are often unaffected by conventional antibiotic treatments. Additionally, if microorganisms develop resistance, previously successful drugs are no longer effective, creating a need for alternative approaches. Mesenchymal stromal cells (MSC) are adult multipotent progenitor cells that can be isolated, expanded in culture, and used therapeutically. We found that MSC secrete factors which directly kill bacteria and inhibit biofilms *in vitro*. We now hypothesize that MSC secreted factors (i) inhibit biofilms in a physiologically relevant *ex vivo* model and (ii) indirectly promote antibacterial defense mechanisms by stimulating the host´s innate immune response. To study this, we use cutaneous wound infections in horses as proof-of-concept.

Methods

We assessed the efficacy of MSC secreted factors, collected as conditioned medium (CM), in an *ex vivo* skin biofilm explant model. Direct effects of MSC CM on bacterial growth in biofilms was evaluated using fluorescent live/dead staining. Indirect effects of MSC CM on the innate immune responses mediated by resident skin cells, *ex vivo* and *in vitro*, was evaluated using fluorescent labeling of antimicrobial peptides (AMPs) and bacterial growth inhibition assays.

Results

We found that MSC CM reduced live bacteria in biofilms established by methicillin-resistant *Staphylococcus aureus* (*MRSA*) in our *ex vivo* skin biofilm explant model. We also found that keratinocytes in the explant model were the predominant skin cells producing the AMPs cathelicidin, lipocalin-2, and elafin, and that MSC CM increased this expression. Similar results were obtained with primary equine keratinocyte cultures *in vitro*, and moreover, CM from MSC CM-stimulated keratinocytes showed a significantly increased bacterial growth inhibitory effect when compared to unstimulated keratinocytes.

Conclusions

Based on these results, we conclude that in addition to direct antimicrobial effects, MSC secreted factors can affect biofilms indirectly by stimulating antimicrobial responses from resident skin cells.

Financial Support
USDA National Institute of Food and Agriculture

Notes:

149 - A scoping review of the evidence for the medicinal use of natural honey in animals

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Session: ANTIMICROBIAL ALTERNATIVES

Objective

Honey has a history of medicinal use that predates written records. The objective of this scoping review was to determine the nature and extent of the current body of evidence concerning the medicinal use of natural honey and its derivatives in animals.

Methods

Although the focus of the review was the veterinary literature, all animal species except insects were eligible, including animals used in translational research. The following electronic databases were searched: MEDLINE, CAB Abstracts, AGRICOLA, Web of Science Core collection, and Web of Science SciELO citation index.

Results

A total of 398 articles reporting 437 studies were included. The majority of these articles were biomedical research articles (n=350); fewer veterinary research articles were identified (n=48). Aside from one systematic review, all biomedical studies were challenge trials. Most veterinary studies were case reports/series (n=23), followed by challenge trials (n=19), and controlled trials (n=8). The animal species examined within veterinary studies consisted primarily of dogs, horses, cats, and cattle, whereas the majority of biomedical research studies examined rats and mice. Wound healing was the most common indication examined among all studies; other indications examined included the prevention and/or treatment of gastric ulcers, bacterial and parasitic infections, toxic exposures, metabolic conditions (e.g., diabetes), and cancer. The majority of studies examined non-medicinal honey (n=412/437), followed by commercially available medicinal honey (n=29/437), and derivatives of natural honey (n=9/437).

Conclusions

With much of the current veterinary literature in this area consisting of case reports/series, high quality primary veterinary research in the form of controlled trials and/or challenge trials is needed to advance this field, as well as to provide sound data for research synthesis that will form the basis for evidence-based assessments of the efficacy of honey in clinical veterinary practice.

Notes:

150 - Antimicrobial resistance of fecal *Escherichia coli* and *Enterococci* from California dairy cows by region and season

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Session: ANTIMICROBIAL RESISTANCE - CATTLE

Objective

The use of antimicrobial drugs (AMD) comes with the risk of antimicrobial resistance (AMR) affecting both dairy cattle and human populations. The objective of this study was to describe AMR patterns in *Escherichia coli* and *Enterococcus* spp. isolated from dairy cows, following the implementation of AMD regulations on California dairies.

Methods

The 10 study dairies were distributed across California's 3 milk sheds: Northern CA (NCA), Northern San Joaquin Valley (NSJV) and Greater Southern California (GSCA) from winter 2018 to summer 2019. Individual cow fecal samples were collected monthly from the 2 seasonal cohorts, each consisting of 12 cows from prepartum to 120 d in milk enrolled on each dairy. A total of 2,171 *E. coli* and 2,158 *Enterococcus* spp. isolates were tested for AMD susceptibility.

Results

Results showed that 13.19% of *E. coli* isolates and 37.3% of *Enterococcus* isolates were pan-susceptible to all drug classes tested. The *E. coli* had high AMR to florfenicol (83.3%), and sulphadimethoxine (32.45%), but low AMR to ampicillin (1.1%), ceftiofur (1.9%), danofloxacin (4.0%), enrofloxacin (3.3%), gentamicin (0.3%), and neomycin (1.6%). *Enterococcus* spp. were highly resistant to tildipirosin (50%), tilmicosin (48%), tiamulin (42%), and florfenicol (46%), but were minimally resistant to ampicillin (0.23%) and penicillin (0.20%). Multidrug resistance (MDR) (resistance to at least 1 drug in ≥3 antimicrobial classes) was observed in 14.14% of *E. coli* and 39% of *Enterococcus* spp. isolates. *Escherichia coli* isolates recovered during winter showed higher MDR prevalence compared to summer isolates (20.33% vs 8.04%). A higher prevalence of MDR was observed in NSJV (17.29%) and GSCA (15.34%) compared with NCA (10.10%).

Conclusions

Our findings showed high rates of AMR to several drugs that are not labeled for use in lactating dairy cattle 20 months of age or older. Conversely, very low resistance was observed for drugs used in adult dairy cows, such as cephalosporins and penicillin. Overall, our findings identified important differences in AMR by antimicrobial class, region, and season.

Notes:

151 - Geographical distribution and abundance of multidrug-resistant *Staphylococcus aureus* in milk and beef production

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Session: ANTIMICROBIAL RESISTANCE - CATTLE

Objective

Staphylococcus aureus is an eminent problem in medical and veterinary practices. However, its spatiotemporal population heterogeneity, antimicrobial resistance (AMR) dynamics, and geographical distribution are poorly studied in Ethiopia. This cross-sectional study evaluated the spatial distribution of multidrug-resistant (MDR) *S. aureus* in five locations of central Oromia, Ethiopia.

Methods

We collected 1001 samples of 10 different sample types from abattoir and dairy farms in 5 locations. 14 antimicrobials of 9 different classes were used for sensitivity test by disk diffusion. A resistance of an isolate to 3 or more antimicrobial classes and a multiple antimicrobial resistance index (MARI) cut off-vale of 0.2 was taken as MDR and 'high-risk' source of AMR contamination, respectively. Generalized linear model was used for risk factors analysis of *S. aureus* positivity.

Results

The prevalence of *S. aureus* in central Oromia was 17.2%, ranging from 16.1-18.3% across the 5 locations ($p>0.05$) but significantly ($p<0.001$) higher in dairy farm than abattoir. It varied among the 10 sample types ($p<0.05$). Penicillin resistance (94.6%) was the most extensively distributed problem followed by nalidixic acid (76.1%), among others whereas majority of the isolates were susceptible to gentamycin (97.8%) and ciprofloxacin (89.1%). All isolates had AMR to a range of antimicrobial classes (range=1-9; median=3). All isolates had MARI of > 0.2 showing the isolates were from a region of a 'high-risk' source of AMR contamination. MARI value had a significant positive relationship ($r^2=1$; $p=0.000$) with number of antimicrobial classes lost their efficacy. Of 10 sample types of abattoir and dairy farms, the highest and lowest MARI were displayed in slaughter line swabs (MARI=0.67) and butcher's hand swabs (MARI=0.25), respectively, although the overall MARI value in dairy farms (MARI=0.44) was higher than that of abattoirs i.e. 0.39.

Conclusions

MDR *S. aureus* is widespread in multiple fronts in central Oromia; therefore, we recommend personnel safety, proper hygiene practices, and comprehensive investigation to control.

Notes:

152 - Estimating the rate of acquisition and loss of ceftiofur resistance in preweaned dairy calves

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Session: ANTIMICROBIAL RESISTANCE - CATTLE

Objective

Our primary objective was to investigate the rates at which antimicrobial resistance (AMR) s acquired and lost under routine management in antimicrobial drug (AMD) treated and untreated preweaned dairy calves using *Enterobacteriaceae* as an indicator organism. A total of 55 calves ages 1 to 3 days were enrolled on a calf nursery located in the Southern San Joaquin Valley in December 2017 and followed for 64 days. Fecal samples were collected 3 times every week and information on antibiotic treatments recorded daily on a hutch card.

Methods

Fecal samples from 20 untreated and 13 AMD treated randomly selected study calves were cultured on MacConkey Agar (MCA) with and without ceftiofur (CEF) 30 µl/ml to quantify resistance. The concentrations of CEF were validated using internal quality control strains of *E.coli*, plating known concentrations of susceptible and AMR *E. coli* strains on MCA supplemented with 10 fold dilutions of CEF, the primary AMD used to treat calves on this ranch. Quantification of *E. coli* was performed by spiral plating serial dilutions of the fecal samples on plain and CEF supplemented MacConkey agar plates 30,3,0.003 µl/ml.

Results

The results showed an initial concentration of 4.7 $\log_{10}$ CFU/ml *E. coli* for both treated and non-treated calves. Subsequently, and specifically 3 days later there was an increase of 5.8 $\log_{10}$ CFU/ml in the treated group and 6.18 on the non-treated group on MCA infused with CEF 30 µl/ml. After a slight decrease in growth on MCA infused with CEF on day 8th, on day 11th the $\log_{10}$ CFU/ml increased to 6.32 in the treated while in the controls it decreased. Peak AMR occurred approximately 4 days post-treatment. On the following days until the end of the study the CFU/ml were ranging between 2.61 and no growth on MCA infused with CEF 30 µl/ml.

Conclusions

The use of AMD in preweaned dairy calves increased the rate of acquisition of AMR in commensal *E. coli* compared to untreated calves. In particular, the increase in AMR was about 2 log point higher compared to the control group and the acquisition of AMR takes approximately 4 to 5 days post-treatment.

Notes:

153 - The effect of tulathromycin on morbidity and prevalence of *Mannheimia haemolytica* Genotype 2 in 84 stocker heifers

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Session: ANTIMICROBIAL RESISTANCE - CATTLE

Objective

Antimicrobial administration at arrival (metaphylaxis) effectively controls bovine respiratory disease (BRD) but may increase antimicrobial resistance (AMR). The objective was to determine the effect of tulathromycin metaphylaxis on AMR in *Mannheimia haemolytica* (Mh), and Mh genotype prevalence.

Methods

Beef cross heifers (n = 84, mean weight 229 ± 18.2 kg) were purchased from local auctions and randomly assigned to receive tulathromycin at arrival (META, n=42) or no antibiotics (NO META, n=42). Two deep nasopharyngeal swabs (DNPS) were obtained from each animal at arrival and 20 days later for culture and identification, Mh genotyping by MALD-TOF-MS, and antimicrobial susceptibility testing. Groups were separated with no contact. Calves were monitored daily; calves requiring antimicrobials were sampled by DNPS then moved into separate pastures. Associations between group and treatment, Mh isolation, and Mh genotype were assessed by Chi-square test.

Results

At arrival, 12 (14 %) calves were positive for Mh (META, n=11; NO META, n=2; p = 0.0158); 10 (83 %) of these isolates were Genotype 1. By day 20, 17 calves required antimicrobials for BRD (META=9, NO META=8). Of treated calves, 4 (META=1, NO META=3) were positive for Mh with 1 isolate being Genotype 1 (NO META=1). On day 20, 32 (40%) calves were positive for Mh (META=20; NO META=12); all were Genotype 2. There was no association between metaphylaxis and treatment rate, Mh isolation, or Mh genotype (p>0.05).

Conclusions

There was no significant association between metaphylaxis and morbidity, Mh isolation after arrival, and Mh genotype; however, the groups were not identical regarding Day 0 isolation of Mh, despite efforts to randomly assign animals to groups. Antimicrobial susceptibility testing is in progress. Future trials are planned to determine reproducibility of these findings.

Financial Support
USDA National Institute of Food and Agriculture

Notes:

154 - Prevalence and detection of antimicrobial-resistant bacteria in dairy cattle farm environments in East Tennessee

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Session: ANTIMICROBIAL RESISTANCE - CATTLE

Objective

The extensive use of antimicrobials in dairy farms could lead to the emergence and spread of resistant bacteria in the farm environments. Monitoring of antimicrobial-resistant (AMR) bacteria in dairy farms is helpful to implement appropriate control and mitigation measures. The objective of this study was to determine the prevalence of AMR representative Gram-positive (*Enterococcus* and *Staphylococcus* species) and Gram-negative bacteria (*Escherichia coli)* and *Salmonella* spp) in four dairy cattle farms in East Tennessee.

Methods

Four different sample types (bulk tank milk, pooled feces from farm floor, dairy manure fertilized soil, and prairie soil; n=40 for each sample type) were collected from dairy cattle farms. The bacteria were isolated and tested for resistance against tetracycline, cefotaxime, nalidixic acid, ceftiofur, and erythromycin. A subset of phenotypically resistant and susceptible isolates was tested for the presence of a resistance gene using each gene primer pairs by PCR.

Results

All four bacterial isolates displayed the highest prevalence of resistance to tetracycline (ranges from 17.1 - 79.5%) than to other antimicrobials. Almost half (49%) of the bacterial isolates were resistant to at least one of the antimicrobials. Most of the resistant bacterial isolates 74% (70/94) were isolated from fecal samples. *E. coli* showed the highest prevalence of resistance (79.5%) compared to the other three bacteria. Both *E. coli* and *Salmonella* spp showed the highest resistance to tetracycline, followed by cefotaxime. PCR result showed the presence of extended-spectrum beta-lactamase ($bla_{CTX\text{-}M}$), *tetA*, and *tetB* resistance genes in *E. coli*; and *tetM* and *ermB* in *Enterococcus* spp.

Conclusions

The results of this study indicated that antimicrobial-resistant bacteria are widespread in dairy cattle farm environments. Thus, further investigation to determine the extent of AMR and factors driving the emergence and spread of resistance in dairy farms is of paramount importance.

Financial Support
University of Tennessee

Notes:

155 - The influence of enrofloxacin and danofloxacin on fluoroquinolone resistance in *Campylobacter jejuni* in calves

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Session: ANTIMICROBIAL RESISTANCE - CATTLE

Objective

Enrofloxacin and danofloxacin are fluoroquinolone (FQ) antibiotics used in the treatment of bovine respiratory disease (BRD) associated with *Mannheimia haemolytica*. Recent studies revealed a sharp increase in FQ-resistant (FQ-R) *Campylobacter* in cattle, but it is unclear if this is related to antibiotic use in cattle. The aim of this study was to investigate the effect of FQ treatments on the development of FQ resistance in *C. jejuni* in healthy and BRD-induced calves.

Methods

Calves sourced from commercial farms (Trial 1: 2 groups, 5/group; Trial 2: 3 groups, 10/group) were inoculated orally with a mixture of FQ-susceptible (FQ-S) strains of *C. jejuni*. Once the colonization was verified, calves in one group in each trial were administered intratracheally with a *M. haemolytica* strain to induce BRD. After 7 days, calves in both groups of Trial 1 and two groups of Trial 2 were injected subcutaneously with enrofloxacin and danofloxacin, respectively. Occurrence of FQ-R *C. jejuni* in feces were monitored before, during, and after treatment. At necropsy, lung lesions were cultured for *M. haemolytica*.

Results

Most calves were naturally colonized by FQ-R *C. jejuni* sequence type (ST)-982 prior to the oral inoculation with FQ-S *C. jejuni* ST-61 and ST-21. After the inoculation, the level of FQ-R *C. jejuni* populations dropped substantially. Most of the calves were colonized by FQ-S *C. jejuni* ST-61 and ST-929. Soon after the antibiotic treatment, the calves were recolonized by FQ-R ST-982. Molecular typing indicated a predominance by FQ-R strains, especially following the antibiotic treatment, and no evidence for colonization by FQ-R strains originating from the FQ-S inoculum. Genotyping indicated a high genetic diversity in *C. jejuni* isolates.

Conclusions

These findings suggest that treatment with FQs promotes the growth of pre-existing FQ-R *C. jejuni* by wiping out the FQ-susceptible populations and had little or no effect on the development of de novo selection of FQ-R *C. jejuni* from the inoculated susceptible strains. Commercial cattle might acquire FQ-R *C. jejuni* from the environment or other animals.

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

Notes:

156 - Antimicrobial resistance in *Enterococcus* spp. recovered from feedlot cattle and associations with antimicrobial use

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Session: ANTIMICROBIAL RESISTANCE - CATTLE

Objective

Antimicrobial drug use (AMU) in agricultural settings has become widely scrutinized as it is considered a strong promoter of AMR. The primary objective of this study was to investigate pen-level AMU as a predictor of pen-level AMR among *Enterococcus* spp. isolates.

Methods

This study leveraged a robust dataset provided by four Western Canadian feedlot cattle operations. Data were collected longitudinally over three years and represent typical practices for large-scale feedlots (>5,000 head), which comprise 87% of feedlot bunk capacity in the region. Pen-level composite fecal samples yielded 1,200 *Enterococcus* isolates. Approximately half (n=599) underwent disk susceptibility testing and half (n=600) were regrown on selective media to detect AMR; analyses were conducted separately by media type. To investigate the relationship between AMU and AMR, pen-level AMU was summarized by route of administration and analyzed as a predictor of isolate resistance. Generalized estimating equations accounted for clustering effects at the feedlot and pen levels while adjusting for other factors.

Results

Among isolates recovered on selective and non-selective media, 68.3% and 58.4% were resistant to doxycycline, 83.2% and 32.7% were resistant to erythromycin, 9.3% and 16.0% were resistant to nitrofurantoin, 10.3% and 11.5% were resistant to tigecycline, and 5.3% and 6.0% were resistant to linezolid, respectively. Overall, few associations between AMU and AMR were identified. For isolates recovered on selective media, total in-feed AMU was associated with doxycycline (OR=2.67; 95% CI=1.28-5.58) and tigecycline resistance (OR=2.54, 95% CI=1.09-5.92). No significant associations were observed among isolates placed on non-selective media.

Conclusions

These findings emphasize the sporadic and often null associations between AMU and AMR in agricultural settings that have been previously reported Likewise, our data support the growing consensus that AMR emergence is influenced by myriad unmeasured factors related to animal production practices.

Financial Support
Beef Cattle Research Council

Notes:

157 - Antimicrobial susceptibility in *E. coli* isolated from cattle specimens submitted to diagnostic laboratory, 2010-19

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Session: ANTIMICROBIAL RESISTANCE - CATTLE

Objective

Antimicrobial resistance (AMR) is a global health crisis threatening both human and animal health. Currently, we do not know the extent of AMR in *Escherichia coli* causing disease in cattle in Indiana, nor its implications for public health in the state. The objective of this study is to calculate the proportion of susceptible isolates identified in specimens collected from cattle and submitted to the Indiana Animal Disease Diagnostic Laboratory and to identify temporal patterns of susceptibility among these isolates.

Methods

Retrospective data of 831 *Escherichia coli* isolates from cattle phenotypically assessed for AMR from 2010 through 2019 were utilized in this study. Overall, 12 antimicrobials from 10 antimicrobial classes were examined in this study. The Clinical and Laboratory Standards Institute (CLSI) guidelines were used in the analysis of the antimicrobial susceptibility test results. All statistical analyses were performed in a commercial statistical software. Proportions of isolates susceptible and not susceptible to various antimicrobials were calculated and the Cochran-Armitage trend test with mosaic plots were used to investigate the temporal trends in susceptibility.

Results

Overall, 39/831 (4.69%) of the isolates were susceptible to nine of the 12 antimicrobials examined. Susceptibility for individual drugs ranged from 1.22% (tiamulin) to 73.31.5% (enrofloxacin) and no significant trends in susceptibility over time were identified for the majority of antimicrobials. However, a significant upward (increasing) trend in susceptibility was observed for sulphadimethoxine ($p<.0001$), spectinomycin ($p=0.0025$), neomycin ($p=0.0113$), and oxytetracycline ($p= 0.0345$).

Conclusions

The findings suggest that large proportions of *Escherichia coli* isolates from cattle in Indiana could be resistant to some medically important antimicrobials. The increases in susceptibility observed for some antimicrobials could be due to reduced use of those drugs over time following changes in antimicrobial prescribing habits of veterinary clinicians.

Notes:

158 - Effect of antimicrobial treatment on rate of gain and loss of antimicrobial resistance in California dairy cattle

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Session: ANTIMICROBIAL RESISTANCE - CATTLE

Objective

Dairy farm use of antimicrobial drugs (AMD) is a known risk factor for the selection and propagation of antimicrobial resistance (AMR); however, the dynamics of this relationship are poorly understood on commercial dairies. Our objective was to investigate the rate of change in counts of enteric ceftiofur-resistant *Enterobacteriaceae* in early lactation dairy cows, mainly in response to AMD treatment. Understanding the dynamics of AMR in fecal commensal bacteria will help guide future policy regarding AMD use on commercial dairies for advancing antimicrobial stewardship.

Methods

A prospective cohort study was performed on two commercial dairies in California's San Joaquin Valley over two seasons, enrolling a total of 96 cows. Individual cow fecal samples were collected from pre-parturition to 60 days in milk, three times weekly. Lactose fermenting *Enterobacteriaceae* were enumerated by spiral plating fecal samples on MacConkey agar impregnated with 0, 1, 8, 16 and 30 µg/ml ceftiofur. Analysis was performed using mixed effect negative binomial regression models for AMR bacteria counts over time and mixed effect interval regression models for mean inhibitory concentration (MIC) over time.

Results

One farm treated zero enrolled cows with systemic AMD while the other treated 40%. There was no growth of *Enterobacteriaceae* at 8 µg/ml ceftiofur in 88% of samples collected from non-AMD treated cows. Samples from AMD treated cows had peak counts resistant *Enterobacteriaceae* during treatment and returned to pre-treatment levels 5-6 days post treatment. Population mean MIC peaked 1-2 days post AMD treatment and returned to baseline levels by 7-8 days.

Conclusions

Our study showed that detectable levels of ceftiofur resistance were very low in early lactation dairy cows. The proportion of ceftiofur-resistant fecal commensal *Enterobacteriaceae* rapidly increased and decreased following systemic ceftiofur treatment. Our results suggest that the effect of systemic ceftiofur therapy on the resistance level of enteric *Enterobacteriaceae* in early lactation dairy cows was limited in extent and duration.

Notes:

159 - Antimicrobial resistant enterococci in the beef production system: a cross-sectoral scoping review

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Session: ANTIMICROBIAL RESISTANCE - CATTLE

Objective

The objective of this scoping review was to identify measurable factors that influence the prevalence of antimicrobial-resistant enterococci within the Canadian beef production system. The findings will support stochastic model development across Canadian beef operational sectors.

Methods

We conducted a double-blinded scoping review to examine published factors influencing antimicrobial resistance (AMR) specific to enterococci in cow-calf operations, feedlot, abattoir, meat processing, and retail environments. Quantitative study results for each factor at each stage were extracted and entered into a database to support meta-analysis; data were restricted to studies conducted in Canada and the United States. Available information was extracted specific to the cattle production stage, antimicrobial class, AMR odds or prevalence, size of the study, and 95% confidence range. Additional variables, including geography, breed, and year of study, were included for reference.

Results

We screened 1432 articles across five databases and selected 442 articles for further review. Antimicrobial resistance in enterococci was highly related to the timing of antimicrobial treatment. Differences in study design made comparisons between articles difficult. There was a lack of assessment specific to environmental and non-therapeutic interventions.

Conclusions

Data extracted from the scoping review will be used to seed an antimicrobial-resistant enterococci beef production integrated assessment (IAM) model, part of broader interconnected IAM on antimicrobial resistance (the "iAM.AMR"). The model will provide a holistic quantitative view of AMR within the Canadian beef industry by evaluating independent influences and their accumulative impact. The outcomes will support a multidisciplinary One Health approach to interpreting AMR risk from diverse sources and modelling its intersection between human and animal health.

Financial Support
Government of Alberta

Notes:

160 - Impact of chlortetracyline on the selection of antimicrobial resistance in *E. coli* and *Enterococcus* in beef cattle

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Session: ANTIMICROBIAL RESISTANCE - CATTLE

Objective

Antimicrobial resistance (AMR) has developed into one of the largest global public health threats of our time. AMR (AMR) happens when microorganisms mutate arewhen exposed to antimicrobial drugs and develop the ability to defeat them. As a result, medical intervention becomes ineffective at fighting off the pathogen, leading to persistent infections in the body and increasing the risk of spread to others (WHO). Widespread use of antibiotics in agriculture is one of the greatest contributors to accelerating the process of AMR.
The specific aim of my project is to determine the impact of *Anaplasma marginale* treatment regimens on the selection of AMR in off-target bovine zoonotic commensals of bacteria of public health concern in the bovine gastrointestinal tract. My study is examining bacteria common to the gut of cattle, specifically *Escherichia coli* and *Enterococcus*. I am evaluating the incidence of AMR development response to the difference CTC-treatment regimens using *Escherichia coli* and *Enterococcus* susceptibly profiles as an indicator. The primary objective is to reduce the economic impacts of anaplasmosis and the emergence of AMR in cattle production thereby supporting sustainable livestock production and antimicrobial stewardship.

Methods

Fecal samples were collected on a monthly basis. The Spot Indole and Esculin Hydrolysis tests were performed for the detection of *E.coli* and *Enterococcus* species. Once isolation and detection were complete a micro-broth dilution method was used to determine the minimum inhibitory concentration of tetracycline. Following susceptibility testing, evaluation of the bovine gastrointestinal microbiome diversity was done by subjecting a subset of samples for DNA isolation and PCR. We expect to find differences in microbiome diversity and incidence of AMR *E. coli* and *Enterococcus* among cattle in the different treatment groups.

Results

Results will be completed by the time of CRWAD conference 2020.

Conclusions

Conclusions will be completed by the time of CRWAD conference 2020.

Financial Support
U.S. Food and Drug Administration

Notes:

161 - Dogs as reservoirs of ESBL-producing *Enterobacteriaceae*

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Session: ANTIMICROBIAL RESISTANCE - COMPANION ANIMAL

Objective

Dogs are found in up to 38% of the households in the USA as per USDA and NIH reports. Tracking and monitoring of antimicrobial resistant pathogens in different companion animal populations is limited compared to production animal populations. We surveyed for the presence of carbapenemase and ESBL-*E.coli* in a population of dogs, awaiting adoption, in an animal shelter in Long Island, New York.

Methods

We screened fecal samples of 92 dogs for ESBL-*E.coli*. We used cefotaxime (CTX) and ceftazidime (CFZ) alone or with clavulanic acid (CA) discs for ESBL-*E.coli* testing besides CHROMAgar ESBL. We also tested for carbapenemase using meropenem (MEM), MEM + Dipicolinic acid, MEM + Cloxacillin, MEM + Boronic acid, and Temocillin discs. We did PCR and sequenced the ESBL. We assessed risk factors (age, sex, intact or neutered, and antimicrobial treatment in the last 14 days) for ESBL-*E.coli* positivity.

Results

Of 92 dogs, 21 (22.8%) had ESBL-*E.coli* in their feces. We grouped dogs into < 6 months (n=56) and over 6 months of age (n=36). The ESBL-*E.coli* prevalence did not significantly vary between the former (28.6%; 16/56) and the latter age groups (13.9%; 5/36). ESBL-*E.coli* was present significantly ($p=0.012$) in males (35.7%; 15/42) than female dogs (12%; 6/50) but with no significant variation between intact (33.3%; 8/24) and neutered dogs (19.1%; 13/68). Antimicrobial treated dogs in the last 14 days (80.0%, 4/5) had significantly more ESBL-*E.coli* than non-treated dogs (19.5%, 17/87). Of all risk factors, only sex and antimicrobial treatment status were significant ($p<0.05$) by a multivariable logistic regression. No isolates produced carbapenemase. Of the 21 CHROMAgar ESBL positive *E.coli* isolates, CTX, CTX+CA, CFZ, and CFZ+CA based disk diffusion detected 100, 85.7% (18/21), 90.4% (19/21), 28.6% (6/21), respectively. Of 21 ESBL-*E.coli*, 10, 5, 4, 1, and 1 had CTX-M, CTX-M+ESC, CMY-2, CTX-M+CMY, and SHV-12+TEM-1, respectively, by sequencing.

Conclusions

Over 1 out of 5 dogs carried ESBL-*E.coli.* This should alert shelter managers about the potential source and risk of ESBL in a shelter population of dogs.

Notes:

162 - Antimicrobial-resistant bacteria transmission between humans and companion animals: A scoping review

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Session: ANTIMICROBIAL RESISTANCE - COMPANION ANIMAL

Objective

The transmission of antimicrobial resistant bacteria is a bidirectional pathway between humans and pets, and the household transmission of antimicrobial resistance (AMR) between humans and pets may be a significant public health problem. The objective of this scoping review is to identify the existing evidence of antimicrobial resistant bacteria transmission between people and pets, specifically dogs and cats, globally.

Methods

A scoping review was used to determine the breadth of existing knowledge of the topic and map the body of literature. The searches were conducted through PubMed, Scopus, Web of Science, CABI Global Health, Google Scholar, and Networked Digital Library of Theses and Dissertations. The inclusion and exclusion criteria were made to assess and review the studies. All studies published in English and Mandarin that concentrated on AMR transmission between humans and pets (cats and dogs) are included in this review.

Results

This review captured 2,846 studies via PubMed, 2,583 via Scopus, 1,520 via Web of Science, 1,264 via CABI Global Health, 1,080 via Google Scholar, and 163 theses, totaling 6,274 studies after de-duplication. After title/abstract screening, 518 papers are undergoing full text review. The data extracted from the eligible AMR transmission studies will be charted to identify information of pet species, isolated bacteria species, the use of antibiotic, the means of transmission (direct contact or indirect contact), the sites of infection, the types of testing used in research, the locations of cases, and year of publication.

Conclusions

This scoping review seeks to identify existing evidence and knowledge gaps of AMR bacteria transmission between pets and humans. It provides an overview of this topic for future scientific studies, policies, and public health interventions.

Notes:

163 - Posters have limited utility in conveying a message of antimicrobial stewardship to pet owners

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Session: ANTIMICROBIAL RESISTANCE - COMPANION ANIMAL

Objective

Pet owners frequently administer antimicrobials to their pets and therefore have an important role to play in promoting antimicrobial stewardship in veterinary medicine. However, best methods of educating pet owners about antimicrobial stewardship have yet to be defined. While visual materials such as brochures and posters are often used in health promotion campaigns, their effectiveness in veterinary medicine is unknown. The objective of this study was to determine whether pet owners noticed and retained the message of a poster with an antimicrobial stewardship message placed in veterinary clinic exam rooms.

Methods

A total of 111 pet owners from 5 veterinary clinics in the greater Philadelphia area participated in the study. Participants completed a survey asking whether they noticed the poster and if they could paraphrase its message. In a follow-up survey, an antibiotic knowledge score was calculated from answers to questions assessing their knowledge of the poster message. Baseline knowledge was assessed by asking participants to define antibiotic resistance. At the end of the study, veterinarians at participating clinics were interviewed about their experiences with the poster.

Results

Only 51 (46.4%) participants noticed the poster, and only 11 (9.9%) could partially or completely reproduce its message. No demographic or clinic-level factors were significantly associated with noticing the poster or recalling its message. Antibiotic knowledge scores were highly correlated ($\rho=0.87$, $p<0.001$) with baseline knowledge and not affected by viewing the poster ($p=0.955$). Veterinarians expressed skepticism that the poster was effective in conveying a message of judicious antibiotic use to clients and noted no difference in the frequency with which they discussed antibiotic resistance or felt pressured to prescribe antibiotics by their clients.

Conclusions

Posters alone will likely have limited impact in conveying a message of judicious antibiotic use to pet owners. However, they might be useful as part of an active, multi-modal education strategy, especially if complemented by veterinarian actions.

Notes:

164 - Veterinarian perceptions of antimicrobial use metrics for hospital-based stewardship in the United States

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Session: ANTIMICROBIAL RESISTANCE - COMPANION ANIMAL

Objective

Robust measurement of antimicrobial use (AMU) is a fundamental component of stewardship interventions. Feeding back AMU metrics to individual clinicians is a common approach to changing prescribing behavior. Metrics must be meaningful and comprehensible to clinicians. Little is known about how veterinary clinicians working in the hospital setting think about AMU metrics for antimicrobial stewardship. The objective of this study was to identify hospital-based veterinary clinicians' attitudes towards audit and feedback of AMU metrics, their perceptions of different AMU metrics and their response to receiving an individualized prescribing report.

Methods

Semi-structured interviews were conducted with veterinarians working at two hospitals in the Eastern US. Interviews elicited perceptions of antimicrobial stewardship in veterinary medicine. Respondents were shown a personalized AMU Report characterizing their prescribing patterns relative to their peers, and were asked to respond. Interviews were recorded, transcribed and analyzed using the framework method with matrices.

Results

Semi-structured interviews were conducted with 34 veterinary clinicians. Respondents generally felt positively about the reports and were interested in seeing how their prescribing compared to that of their peers. Many respondents expressed doubt that the reports accurately captured the complexities of their prescribing decisions and found metrics associated with animal daily doses (ADDs) confusing. Only 13 (38.2%) respondents felt the reports would change how they used antimicrobials. When asked how the impact of the reports could be optimized, respondents recommended providing a more detailed explanation of how the AMU metrics were derived, education prior to report roll-out, guidance on how to interpret the metrics, and the development of meaningful benchmarks for goal-setting.

Conclusions

These findings provide important insight that can be used to design veterinary-specific AMU metrics as part of a stewardship intervention that are meaningful to clinicians and more likely to promote judicious prescribing.

Financial Support
University of Pennsylvania

Notes:

165 - Antimicrobial susceptibility of *E. coli* isolated from diagnostic canine specimens, 2010-2019

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Session: ANTIMICROBIAL RESISTANCE - COMPANION ANIMAL

Objective

Escherichia coli, is the most common gram-negative pathogen isolated in humans infections. Antimicrobial resistant (AMR) *Escherichia coli* originating from dogs may directly or indirectly cause disease in humans. Currently, we do not know the extent of AMR in *E. coli* causing disease in dogs in Indiana, and its implications for public health in the state. The objective of this study is to calculate the proportion of antimicrobial susceptible E. coli isolates identified in canine specimens submitted to the Indiana Animal Disease Diagnostic Laboratory and to identify temporal patterns of susceptibility among these isolates.

Methods

Retrospective data of 2738 *Escherichia coli* isolates from dogs assessed for AMR from 2010 through 2019 were utilized in this study. Overall, 27 antimicrobials from 11 antimicrobial classes were examined. The Clinical and Laboratory Standards Institute (CLSI) guidelines were used in the analysis of the antimicrobial susceptibility test results. Proportions of isolates susceptible to the various antimicrobials were calculated using commercially available statistical software and the Cochran-Armitage trend test with mosaic plots were used to investigate the temporal trends in susceptibility.

Results

Overall, 553/2738 (20.2%)of the isolates were susceptible to 17 of the 27 antimicrobials examined. Of the 2638 isolates examined for amikacin susceptibility, 2706 (97.5%) were susceptible, 2657/2673 (99.4%) isolates were susceptible to imipenem, and 2099/2670 (78.6%) were susceptible to marbofloxacin A significant downward (decreasing) trend in susceptibility was observed for amoxicillin-clavulanic acid ($p<.0001$), ampicillin ($p<.0001$), Cefazolin ($p<.0001$), ceftazidime ($p=0.0067$), chloramphenicol ($p<.0001$), and orbifloxacin ($p=0.008$).

Conclusions

The decreasing trend in the proportion of isolates susceptible to several beta lactam antimicrobials suggests that resistance of *Escherichia coli* in dogs to beta lactam antimicrobials could be increasing in Indiana. The decreasing trend in susceptibility to these drugs could be due to selection pressure from over-use.

Notes:

166 - Antimicrobial susceptibility of *Staphylococcus pseudintermedius isolated* from dog specimens, 2010-2019

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Session: ANTIMICROBIAL RESISTANCE - COMPANION ANIMAL

Objective

Staphylococcus pseudintermedius is an emerging zoonotic opportunistic pathogen that has been detected in humans primarily in contact with dogs. Understanding the extent of antimicrobial resistance (AMR) in *Staphylococcus pseudintermedius* in dogs is important for appropriate antimicrobial stewardship and prevention and control of zoonotic spread of resistant strains. This study calculated the proportion of *Staphylococcus pseudintermedius* isolated from dog specimens submitted to the Indiana Animal Disease Diagnostic Laboratory that were susceptible to antimicrobials and identified temporal patterns in susceptibility.

Methods

Retrospective data of 1963 *Staphylococcus pseudintermedius* isolates from dogs assessed for AMR from 2013 through 2019 were utilized. A total of 27 drugs from 14 antimicrobial classes were examined. The Clinical and Laboratory Standards Institute (CLSI) guidelines were used in the analysis of the antimicrobial susceptibility test results. Proportions of isolates susceptible isolates were calculated using a commercially available statistical software and the Cochran-Armitage trend test with mosaic plots was used to investigate the temporal trends in susceptibility.

Results

Overall, 138/1963 (7%)of the isolates were susceptible to 21 of the 27 antimicrobials. Of the 1776 isolates tested for amoxicillin-clavulanic acid susceptibility, 1279 (72%) were susceptible while 809/820 (98.7%) were susceptible to vancomycin. For clindamycin, erythromycin, and imipenem, 1110/1803 (61.6%), 1188/1961(60.6%) and 1318/1805 (73%) of the isolates were susceptible, respectively. A significant downward (decreasing) trend in susceptibility was observed for amoxicillin-clavulanic acid ($p<.0001$), ampicillin ($p<.0001$), oxacillin ($p <.0001$), Cefazolin ($p<.0001$), doxycycline ($p<.0001$), enrofloxacin ($p= 0.0002$), marbofloxacin ($p= 0.0017$).

Conclusions

The decreasing trend in susceptibility to the beta lactam and fluoroquinolone antimicrobials could be indicative of selection pressure on the bacteria from persistent use of these antimicrobials in the management of bacterial infections in dogs.

Notes:

167 - Uncovering *Escherichia coli* multidrug resistance patterns among dogs with association rule mining

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Session: ANTIMICROBIAL RESISTANCE - COMPANION ANIMAL

Objective

Multidrug resistance (MDR) among pets may put humans at a higher risk for MDR infections and also threatens the welfare of animals. The risk in non-foodborne sources calls for more attention and unique approaches to reveal and predict resistance profiles. This study uncovers MDR patterns in *Escherichia* coli isolated from dog urinary tract infections and shows whether the MDR patterns have changed over time.

Methods

A veterinary diagnostic lab isolated 2,963 *E. coli* from dog urine between 2007 and 2017. We extracted one isolate per sample (n=2,493) that was tested by broth microdilution against a standard urine panel, which includes amoxicillin, ampicillin, ceftiofur, cefalexin, enrofloxacin, tetracycline, and trimethoprim-sulfamethoxazole. We analyzed MDR relationships in this dataset with a machine learning method called association rule mining, which extracts associations hidden among binary variables in large, sparse datasets.

Results

Except for trimethoprim-sulfamethoxazole, the resistance prevalence trends for the other antimicrobials are similar over the ten years. Ampicillin has the highest overall resistance prevalence (32.2%), while trimethoprim-sulfamethoxazole has the lowest overall resistance prevalence (12.2%). Approximately 21% of samples (N=525) are resistant to at least two antimicrobial classes, and 12.2% of samples (N=305) are resistant to three or more antimicrobial classes and therefore are considered MDR. After selecting association sets to maintain a ≤5% false discovery rate, strong beta-lactam resistance associations exist each year and there are co-resistances with quinolones, tetracyclines, and sulfonamide. Association sets selected with a P-value ≤0.05 are consistent with the sets selected with the resampling procedure to estimate the false discovery rate.

Conclusions

Association rule mining is effective in uncovering potential MDR patterns and the change of the associations over time. There are consistent positive associations between the resistance traits identified in the study, likely the result of consistent selective pressures from antimicrobial use in dogs.

Notes:

168 - Resistome characterization of extended spectrum β-lactamase *E. coli* from sheep and abattoir environment

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Session: ANTIMICROBIAL RESISTANCE - GENOMICS

Objective

Extended spectrum beta-lactamase (ESBL)-producing *Enterobacteriaceae* are serious public health threat and are in increasing trend in the United States. The objective of this study is genotypic characterization of ESBL *Escherichia coli (E. coli)* recovered from animal and environmental samples at an abattoir in North Carolina.

Methods

A total of 113 ESBL producing *E. coli* isolates recovered from animal (n=65) and abattoir environment (n=48) were sequenced using Illumina MiSeq whole genome sequencing.

Results

Seventy-eight (95.6%) of the isolates had CTX-M-type ESBL genes (28 from sheep feces, 20 from cecal contents, 10 from sheep carcass swabs, 21 from lairage swabs, 10 from soil samples, eight from feed and nine from water and seven from resting area feces). The most common CTX-M-type genes detected were *bla*$_{\text{CTX-M-1}}$ (n=32, 28.3%) and *bla*$_{\text{CTX-M-32}}$ (n=29, 25.7%) followed by *bla*$_{\text{CTX-M-55}}$ (n=15, 13.3%), *bla*$_{\text{CTX-M-65}}$ (n=14, 12.4%), *bla*$_{\text{CTX-M-15}}$ (n=13, 11.5%), *bla*$_{\text{CTX-M-27}}$ (n=3, 2.7%) and *bla*$_{\text{CTX-M-14}}$ (n=2, 1.8%). Other beta-lactamase genes detected were *bla*$_{\text{TEM-1}}$ (n=55, 48.7%), *bla*$_{\text{CARB-2}}$ (n=16, 14.2%), and *bla*$_{\text{CMY-2}}$ (n=11, 9.7%). Among genes that confer quinolone resistance, we detected *qnrA1*(n=16, 14.2%), *qnrS1* (n=10, 8.8%) and *qnrB19* (n=22, 19.5%). Other commonly detected genes included *mph(A), aadA2, aadA5, aph(3")-Ib, aph(6)-Id*, *dfrA1, floR*, *Sul1*, *Sul2 and tet(A).* Plasmids detected include IncR, IncFIA, IncFII, IncHI2, IncFIB, Col156, IncHI1, IncI1-I(Gamma), IncX and Col(pHAD28). Mutations that confer resistance to Nalidixic acid and Ciprofloxacin were detected including gyrA_D87N, gyrA_S83L and parC_S80I. Identical sequence types and antimicrobial resistance determinants were detected in ESBL *E. coli isolates from* both animal and environmental samples.

Conclusions

The detection of multiple genotypes of CTX-M-type and CARB-type ESBLs as well as other antimicrobial resistance genes, mutations and plasmids in ESBL *E. coli from* animal samples and abattoir environment warrants vigilant surveillance of products from small ruminants and further study to determine public health risk.

Financial Support
North Carolina State University

Notes:

169 - Examination of the horizontal gene transfer dynamics of an integrative and conjugative element in *Histophilus somni*

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Session: ANTIMICROBIAL RESISTANCE - GENOMICS

Objective

Histophilus somni is a gram-negative bacterium that causes *histophilosis* in cattle and contributes to bovine respiratory disease (BRD). These diseases are significant causes of morbidity and mortality in feedlot cattle. Integrative and conjugative elements (*ICEs*) are autonomous-transferred mobile genetic elements that play a significant role in disseminating antimicrobials between bacteria via horizontal gene transfer (HGT). One recently sequenced ICE from *H.somni* isolated from feedlot cattle Alberta named *ICEHs02* is 72,914 base pairs in length and comprises 79 genes, including the tetracycline, aminoglycosides, florfenicol, sulfonamide, and multicopper oxidase resistance genes. This study aimed to investigate the host range of *ICEHs02* and to assess the effect of antimicrobial stressors on the frequency of the transfer of the ICE.

Methods

In vitro conjugation assays were conducted to examine the frequency of transfer of *ICEHs02* into other bacteria. PCR and sequence analysis was used to confirm the presence of *ICEHs02* and its circular intermediate in the recipient strains. Susceptibility testing of the *ICEHs02*-carrying recipients was conducted by broth microdilution. The effect of the antimicrobials on the excision rates and transfer frequency was investigated by mating assays and qPCR.

Results

ICEHs02 was shown to transfer into *H.somni* and *Pasteurella multocida strains*. PCR assays confirmed the ICE-associated core genes, accessory genes, and the excised circular form in the transconjugants. Susceptibility testing confirmed the functional activity of the *ICEHs02*-associated resistance in the recipient strains. The copy numbers of *ICEHs02* per chromosome exhibited a significant increase of ~37 fold after tetracycline exposure, and ~ 4 folds increase after ciprofloxacin treatment. The transfer rates were increased significantly upon tetracycline and ciprofloxacin induction.

Conclusions

This study emphasized the importance of ICEs in the dissemination of antimicrobial resistance (AMR) between bacteria and demonstrated the effect of antimicrobial stressors on the transfer rates of ICEs.

Financial Support
Alberta Agriculture and Forestry

Notes:

170 - Single nucleotide polymorphism analysis of bovine *Mannheimia haemolytica* isolates

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Session: ANTIMICROBIAL RESISTANCE - GENOMICS

Objective

Molecular epidemiology is rapidly becoming a tool for tracing infectious disease transmission throughout animal populations. *Mannheimia haemolytica*, an important cause of respiratory disease in cattle, also exists as a commensal organism. Understanding of the genetic markers associated with virulent strains of *M. haemolytica*, including those carrying antimicrobial resistance genes (ARGs), will help guide management practices and therapy for sick animals. The vSNP tool developed by USDA can provide refined trees of *M. haemolytica* isolates and groupings associated with ARGs.

Methods

In 2018, USDA APHIS' National Animal Health Laboratory Network (NAHLN) initiated the NAHLN AMR pilot project, with the objective of developing a sampling stream to monitor antimicrobial resistance (AMR) profiles in animal pathogens isolated by veterinary clinics and diagnostic laboratories across the U.S. *M. haemolytica* isolates were obtained from cattle across 29 states. Of these, 90% of samples had a final diagnosis of pneumonia or bovine respiratory disease. A subset of 88 *M. haemolytica* isolates from these laboratories were whole genome sequenced at the National Veterinary Services Laboratories.
Sequenced isolates were evaluated using kSNP to characterize overall diversity and identify general isolate positions on the *M. haemolytica* tree. vSNP produce more refined trees based on high quality single nucleotide polymorphism (SNP) positions in the genome. The SNPs were used to identify potential fluoroquinolone resistance.

Results

Evaluation of isolates revealed common clusters of predicted ARGs occurring in genetically related isolates. SNPs conferring fluoroquinolone resistance were commonly seen in a single subclade of genotype 2. The SNP scheme established in this study allows for quick assignment of isolates to this and other subclades, quickly revealing potential inherited resistance.

Conclusions

Validation of vSNP can group *M. haemolytica* isolates into clades associated with ARGs and integrative conjugative elements, providing a better understanding of circulating, disease-causing isolates of cattle in the U.S.

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

Notes:

171 - Genomic analysis of an Integrative Conjugative Element conveying antimicrobial resistance in *Histophilus somni*

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Session: ANTIMICROBIAL RESISTANCE - GENOMICS

Objective

Histophilus somni is a gram-negative inhabitant of the upper-respiratory microbiome of cattle. An opportunistic bacterial pathogen, *H. somni* contributes to bovine respiratory disease (BRD) in feedlot cattle. Antimicrobials are used for prevention, control and treatment of BRD. To identify mechanisms of resistance in *H. somni*, whole genome sequences (WGS) were examined for mobile genetic elements and antimicrobial resistance genes.

Methods

The WGS of 12 isolates from feedlot cattle were obtained from Vaccine and Infectious Disease Organization-International Vaccine Centre (VIDO-InterVac). Short-read WGS were generated using the Illumina MiSeq 500 platform. The following methods were used for quality control, assembly, scaffolding and annotation. FastQC v 1.0.0 and QUAST v 5.0.2 were used for quality assessment of sequences.. Trimmomatic v 0.38 was used for sequence trimming and adapter clipping. These reads were removed during trimming: low-quality reads (quality [Q] score <20), reads <50 nucleotides or >10% N, and reads with >15-bp overlap with Illumina adaptors. Final FASTQ files had 98% reads with Q score ≥30. Trimmed WGS files were assembled *de novo*, then scaffolded using SPAdes v 3.13.0 and MeDuSa. WGS was annotated using the NCBI prokaryotic genome annotation pipeline. The CARD database was used to identify ARGs.

Results

A novel Integrative Conjugative Element (ICE) of 72,914 bp length was identified. The ICE a member of the ICEHin1056 family, and encodes 79 proteins including a heavy metal tolerance gene (*mco*) and six ARGs: *tet(H), floR, Sul2/folP, APH (3")-Ib, APH (6)-Id, and APH (3')-Ia*. The ARGs confer resistance to tetracycline, phenicol, sulphonamide, and aminoglycoside classes. The integration site is a tRNA-leu gene (CAA motif). The ICE has identical 21-nucleotide direct repeat sequences. The ICE encodes for a completeType IV secretion system (T4SS).

Conclusions

The discovery of a novel Integrative Conjugative Element carrying multi-drug resistance in *H. somni* has important implications for the feedlot cattle industry.

Financial Support
Alberta Agriculture and Forestry

Notes:

172 - Evaluating the discriminative capacity of resistome variants

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Session: ANTIMICROBIAL RESISTANCE - GENOMICS

Objective

The objective of this three-year project is to develop and validate a method for generating resistome profiles from metagenomic data. Sub-objectives include: benchmarking existing bioinformatic tools for variant detection in metagenomic data; estimating the discriminative limit of the most promising tools; and generating a public dataset for future benchmarking and standardization efforts.

Methods

To initiate this project, we conducted a scoping review of existing bioinformatic tools and are currently testing these tools on several benchmarking datasets. These datasets include short-read sequence data generated from fecal and retail meat samples; short-read sequence data obtained from mock microbial communities; and *in silico* short-read metagenomic datasets with known resistome composition. These datasets are being used to compare the performance of bioinformatic tools identified in the scoping review.

Results

Over a dozen bioinformatic tools were identified in our scoping review. However, only a couple of these tools were specifically developed for metagenomic datasets. Initial results suggest that many tools are not feasible for use with large metagenomic datasets due to scalability issues; or do not produce biologically plausible results. For those that do, results suggest that the nucleotide variation contained within the resistome of a metagenomic dataset is likely to provide superior discriminative capability compared to a typical class-, mechanism- or gene-level resistome analysis.

Conclusions

This initial work supports the overall objectives of the project and helps guide selection of a usable and accurate computational approach for resistome fingerprinting. Next steps will be to continue benchmarking analysis using additional performance metrics. Once benchmarking is complete, we will select an optimal tool and then formally evaluate the discriminative limit of the resistome using pre-selected datasets with varying levels of sample relatedness.

Financial Support
USDA National Institute of Food and Agriculture

Notes:

173 - Bioinformatic analysis of microbiome composition and associated antimicrobial resistance genes in rumen data

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Session: ANTIMICROBIAL RESISTANCE - GENOMICS

Objective

The rumen microbiome is essential to ruminant health and production, and therefore to human food security. Metagenomic sequencing has been used to understand the microbial structure of the rumen, but few studies have evaluated the relationship between antimicrobial resistance (AMR) and the rumen microbiome. This represents a critical gap in our knowledge of AMR in ruminant production, especially because protozoa are dominant players in rumen ecology and can also impact the abundance of AMR-carrying bacteria. Therefore, the objective of this study was to identify potential associations between microbiome composition, protozoa and AMR genes in the rumen of farmed cattle and sheep.

Methods

Using 4 existing datasets of shotgun metagenomic sequences from cattle and sheep rumen samples (N=88 samples total), we generated descriptions of the microbiome and resistome profiles in each sample. Sequences were aligned to microbial taxonomic and AMR databases to identify and count microbial taxa and AMR genes. Bayesian network analysis was performed to identify associations (edges) between microbes and AMR genes (nodes).

Results

Overall, we identified 8 classes of AMR representing 6 unique mechanisms and 10 separate gene groups. The resistome and microbiome profiles differed significantly by dataset, and the sheep rumen samples contained fewer sequences that aligned to AMR genes than the bovine rumen samples. At a bootstrap threshold of 0.7, several significant interactions were identified between microbial taxa and AMR classes ; and some of these edges were identified in all 4 datasets. The subset of these edges that demonstrate a negative association with AMR can be used as the basis for more directed, hypothesis-driven studies of AMR within the rumen.

Conclusions

Our results demonstrate the existence of a diverse rumen resistome, and suggest that rumen microbes influence the presence and abundance of AMR genes. Studies such as this one, have applications in the targeting and manipulating of microbial populations as a strategy to reduce AMR in the rumen and other environments.

Financial Support
National Cattlemen's Beef Association - Beef Checkoff

Notes:

174 - Fecal microbiome-resistome dynamics of PRRSV-challenged pigs under varying antimicrobial drug exposures

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Session: ANTIMICROBIAL RESISTANCE - GENOMICS

Objective

Antimicrobial resistance (AMR) presents a growing animal health challenge, and the association between antimicrobial use and AMR is complex. Changes in the AMR profile (the "resistome") are often impacted by animal health status, herd management factors, and time. The purpose of this project was to compare resistome profiles in wean to market swine as they experienced different health challenges and antibiotic protocols.

Methods

To investigate this, 108 piglets were randomly assigned to one of three treatment groups. Two of the three groups were challenged with PRRSV, while the third group remained unexposed to the virus. The PRRSV challenged pigs were then administered a "moderate" or an "intensive" antibiotic treatment protocol. The pigs were grown to market weight and sent to slaughter, and composite fecal samples were collected at six different time points between arrival and slaughter. Samples underwent total DNA extraction and metagenomic sequencing before further analysis was conducted.

Results

Using these data, antimicrobial resistance genes (ARGs) were identified and counted in each sample (N=216), and the resistome was compared over time and between treatment groups. Across all samples, 1,067 unique ARGs were identified. Findings indicate that weaning, transport, and commingling corresponded to an appreciable shift in the resistome, whereas shifts during PRRSV challenge and subsequent antibiotic exposures were much less pronounced. However, the treatment group was a significant source of resistome variation before and during PRRSV challenge and antibiotic exposures, though it did not significantly explain resistome variation at other time points.

Conclusions

These findings demonstrate the complexity of AMR and suggest that changes in the resistome are not solely dependent on antimicrobial use. Microbiome analysis illustrates the fluctuating nature of microbial communities over a given time, and in response to different stimuli. Ultimately, this dynamic behaviour may represent opportunities for both AMR mitigation and promotion of swine health and performance.

Financial Support
National Pork Board

Notes:

175 - Antimicrobial resistance in *Streptococcus suis* isolates from USDA's Antimicrobial Resistance Pilot Project, 2019

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Session: ANTIMICROBIAL RESISTANCE - MODELING AND SURVEILLANCE

Objective

Antimicrobial resistance (AMR) is widely considered to be one of the greatest threats to human and animal health. The USDA-APHIS-National Animal Health Laboratory Network (NAHLN) launched an AMR Pilot Project in 2018, with the primary objective of developing a sampling stream to monitor AMR trends in pathogens routinely isolated by veterinary diagnostic laboratories and clinics across the U.S. from clinically ill animals. As part of this pilot we evaluated antimicrobial susceptibility phenotypes and genetic determinants associated with AMR in *Streptococcus suis* from swine.

Methods

Conventional cultivation methods and antimicrobial susceptibility testing (AST) were used by the 24 laboratories enrolled in this pilot during 2019. Isolates were tested for 16 antibiotics, six of which have breakpoints established for *S. suis*. AMR genes were detected using AMRFinder and ABRicate with the NCBI and ResFinder databases.

Results

AST data from 167 *S. suis* isolates were evaluated, and a random subset of 37 isolates were further analyzed by whole genome sequencing. Isolates were recovered from lung or thoracic cavity (n=100), brain (n=26), heart/pericardium (n=17), or other sample types (n=24). Pneumonia/ respiratory diseases accounted for 56.9% of all infections associated with *S. suis.* Of the 167 isolates, 35.9% (60/167) were susceptible to all antibiotics. Resistance to one antibiotic was observed in 50.9% (85/167) of all isolates, and another 10.7% (18/167) were resistant to two antibiotics. Four strains were resistant to 3 or more antibiotic classes and thus were classified as multi-drug resistant. Of the 37 isolates sequenced, all were susceptible to florfenicol, ceftiofur and ampicillin. 11 isolates were resistant to tetracycline, and all had either *tetO* or *tetM* resistance genes. 3/37 isolates were resistant to penicillin, but none had corresponding genes for β-lactam resistance.

Conclusions

The findings from this pilot project demonstrate the utility of a national AMR monitoring and surveillance program for monitoring AMR at a national level for *S. suis* and other animal pathogens by providing AMR data to stakeholders that can help them in decision making efforts regarding these trends.

Financial Support
U.S. Department of Agriculture

Notes:

176 - Comparison of different biomass methodologies to adjust sales data on veterinary antimicrobials in the US

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Session: ANTIMICROBIAL RESISTANCE - MODELING AND SURVEILLANCE

Objective

Antimicrobial sales data for use in major food producing animals (cattle, swine, chickens and turkeys) in the United States (US) reported annually by Food and Drug Administration (FDA) is valuable but may be misleading because the sales estimates do not account for the differences in body mass among animal species. The aim of this study was to adjust the US antimicrobial drug sales data for the animal biomass using 4 different biomass adjustment methodologies proposed by: FDA, European Surveillance of Veterinary Antimicrobial Consumption (ESVAC), Public Health Agency of Canada (PHAC) and World Organisation for Animal Health (OIE).

Methods

For each biomass adjustment methodology, the 2018 US antimicrobial drug sales data expressed in milligrams was standardized by the corresponding biomass denominator. Although different for each methodology, a biomass denominator is typically defined as the population of a given animal category multiplied by the average weight of that animal category. US-specific national data was used to estimate biomass denominators for the FDA, ESVAC, PHAC and OIE methods.

Results

According to FDA's report, a total of 11.3 million kg of antimicrobials was sold in 2018 with the least amount sold for use in turkeys (9%) compared to cattle, swine and chickens. However, preliminary adjustment by the FDA methodology indicated antimicrobial use per kg of animal was the highest for turkeys (0.30 mg antimicrobial/kg of animal), followed by swine (0.16 mg/kg). Total adjusted estimate of antimicrobial sales for use in major food animals in the US was the highest when ESVAC methodology (0.32 mg/kg) was used, followed by PHAC (0.19 mg/kg), FDA (0.13 mg/kg) and OIE (0.11 mg/kg).

Conclusions

Our findings underscore the importance of implementing a consistent US-specific biomass methodology, as the estimates of the biomass adjusted antimicrobial sales changed depending on the methodology used. As adjusted sales data provided a better insight into how antimicrobials are used per kg of each animal category, interventions to reduce antimicrobials may be prioritized among food animals.

Financial Support
Pew Charitable Trusts

Notes:

177 - The economic costs of antibiotic use constraints in U.S. integrated beef supply chains: A systems approach

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Session: ANTIMICROBIAL RESISTANCE - MODELING AND SURVEILLANCE

Objective

The U.S. beef industry is one of the prominent antibiotic user, with an annual average usage of 2500 tons of medically important antibiotics in 2018. Our objective was to estimate the economic costs of antibiotic use constraints in a conceptual U.S. beef supply chain, to aid the beef industry in mitigating the ever-increasing risk of antibiotic resistance, by reducing their antibiotic use.

Methods

We developed a conceptual network model of U.S. integrated beef supply chain, illustrating the annual supply of 30 million animals through cow-calf, stockers, backgrounders, and feedlot operations. The entire supply chain was differentiated into 37 different nodes of production. Each node could only raise specific type of animals, differentiated based on the health status and hence could only follow a specific antibiotic use strategy. The cost as well as weight gain efficiency budget was developed for each node, based on average U.S. beef production budgets. The linear programming solutions to this network model provided the least cost path of beef supply, under various antibiotic use constraints.

Results

The budget calculated cost as well as weight gain efficiency of the 37 nodes, and the initial supply of 30 million calves weighing 0.68 million tons, and the final demand of 16.5 million tons of slaughter ready fed cattle were used as inputs/constraints to 3 different linear programming models, with different antibiotic use constraints. Our first model, the basic optimization model which had no antibiotic use constraints, estimated that the minimum economic cost was $11.5 billion to meet the final demand. Our second model which was constrained to use all the calves irrespective of their health status, increased the system cost to $13.7 billion. Our third model which avoided the feedlots using antibiotics from the network, incurred a total cost of $14.3 billion for antibiotic free beef production.

Conclusions

We concluded that the additional cost of $582 million for implementing antibiotic free beef production is relatively low (0.87%) when compared to the slaughter cash receipts of $67 billion.

Financial Support
Cornell University

Notes:

178 - Using Bayesian Networks to Identify MIC Distribution Profiles for *E. coli* Isolates from Dairy Heifers in California

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Session: ANTIMICROBIAL RESISTANCE - MODELING AND SURVEILLANCE

Objective

Antimicrobial resistance (AMR) continues to be an issue spanning multiple sectors. Many AMR studies use traditional analytic methods considering one bacteria and one antibiotic match at a time. These methods fail to describe associations between minimum inhibitory concentration (MIC) patterns for all investigated isolates, referred to as profiles. Bayesian network analysis (BNA) can identify profiles, adding to the growing body of research. This study describes reduced antibiotic susceptibility profiles in *Escherichia coli (E. coli)* isolates obtained from dairy heifers in California, using BNA.

Methods

Culture and sensitivity data from an existing data set was used. *E. coli* was selectively isolated for susceptibility against 15 antibiotics from 281 rectal swab samples obtained from weaned dairy heifers on five farms. Joint patterns of reduced susceptibility, defined as an increasing trend in MIC values, were identified using BNA. The PC learning algorithm and the Jonckheere-Terpstra test identified the best fitting model, and the joint probabilities were quantified. Analyses were conducted in R using the “bnlearn” package.

Results

The final graph contained 18 links between 18 variables. Linkages imply systematic dependency in trend or mutual dependence. Antibiotics of the same class were connected, as were those of different class including, Florfenicol and Tetracycline, Spectinomycin and Trimethoprim-Sulfamethoxazole, and Tetracycline and Sulfamethoxazole. The farm from which the sample was collected may influence associations involving Trimethoprim-Sulfamethoxazole. However, bootstrap analyses did not maintain this connection.

Conclusions

Although our analysis cannot infer causality, susceptibility profiles for antibiotics in the same class appeared related. Relationships between mechanistically different antibiotics were also identified and should be further investigated. Findings may have clinical implications as these relationships suggest reduced susceptibility for multiple drugs may be due to factors across both mechanistically related and unrelated drugs.

Notes:

179 - Partial budget analysis of culture and algorithm guided selective dry cow therapy

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Session: ANTIMICROBIAL RESISTANCE - MODELING AND SURVEILLANCE

Objective

The objective of this study was to estimate the herd-level net cash impact for switching from blanket dry cow therapy (BDCT) to culture- or algorithm-guided selective dry cow therapy (SDCT).

Methods

We conducted partial budget analysis using Monte Carlo simulation with @Risk software. Model simulations were run using 50,000 iterations, with each iteration representing 1 herd under a set of herd-level economic conditions. Expenditures associated with dry-off procedures and health outcomes (clinical and subclinical mastitis) during 1-30 DIM were used to model herd-level net cash impacts expressed in units of USD per cow-dry-off. Values and distributions were derived from previously published models, current industry databases, or from professional experience. Fixed values were used for economic components that were not expected to vary in the U.S. dairy population, and distributions were used for variables expected to vary among herds. Normal, pert, and beta distributions were used. As a sensitivity analysis we investigated the economic impact in situations where SDCT increased mastitis risk during the next lactation by 1%, 2%, or 5%.

Results

For culture-guided SDCT, on average, producers could expect to save $2.14 per cow-dry-off as compared to BDCT. For algorithm-guided SDCT, the mean net impact was $7.85. 76% and 100% of iterations had a net impact ≥$0 for culture- and algorithm-guided SDCT, respectively, indicating that the strategies were profitable in most of the herd conditions evaluated. The largest contributors to variance (~75% and ~25%) in both models were the percent of quarters treated at dry-off and the cost of antibiotics. Findings from the sensitivity analysis indicated that SDCT continued to have net benefits over BDCT when risk of clinical and subclinical mastitis was increased by small amounts (Risk difference = +0.01 and +0.02), particularly when use of these strategies resulted in substantially lower levels of antibiotic use.

Conclusions

This partial budget analysis indicated that in the majority of situations, 2 SDCT strategies produce a positive net cash impact over BDCT when udder health is equivalent.

Financial Support
USDA National Institute for Food and Agriculture

Notes:

180 - Establishing an AMR surveillance system in the USA to analyze the *E. coli* resistome across the One Health spectrum

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Session: ANTIMICROBIAL RESISTANCE - MODELING AND SURVEILLANCE

Objective

The continued emergence of antimicrobial resistance (AMR) is a global health crisis that threatens both human and animal health. Research in this area is difficult due to the complex epidemiology of the circumstances surrounding spread of resistance as these organisms exist in humans, animals, food, and our environment. In fact, it has been recognized by WHO, FAO, and the OIE, that the way to prevent further AMR is to create integrated monitoring systems. Thus, the development of a One Health AMR data surveillance platform is proposed in this project.

Methods

To create this nationwide surveillance system, AMR data sources for human, animal, and environmental samples were identified and integrated into a One Health AMR dataset. These data would be used to create a monitoring algorithm to track the transfer and emergence of resistant genes across host species. Historical AMR susceptibility test data, pathogen resistome, geographical data etc. are used to identify the spread of AMR across species as well as in and through environmental boundaries.

Results

Publicly available pathogen detection dataset from the National Center for Biotechnology Information (NCBI) has been selected for their One Health classifiers and inclusion of susceptibility test results with AMR genotypes. Data collection, munging and wrangling were accomplished using python programming language and MariaDb database management system. For the first stage, *Escherichia coli* resistome data and antimicrobial susceptibility test results along with geographical and temporal values were analyzed in 50920 samples obtained from humans, animals, and environment. Two of these genes were identified to match with the core resistome of *Escherichia Coli* in the created dataset. Further analysis of the dataset revealed a list of 45 genes among 367 genes present in the dataset to have a significantly higher frequency among the tested samples. These genes were further analyzed on the basis of epi-types, host organisms and susceptibility test results over time and geography.

Conclusions

The created One Health AMR dataset can be used to create a data model for a surveillance and analysis system. Geographical and temporal data should be made compulsory for all data submissions similar to the currently available datasets to be utilized for surveillance purposes in real-time. A standardization of data representation and a database model is essential to ease the surveillance and analysis of AMR data for real time results.

Notes:

181 - Modeling human exposure and risk to fluoroquinolone-resistant *Campylobacter* from retail chicken in Canada

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Session: ANTIMICROBIAL RESISTANCE - MODELING AND SURVEILLANCE

Objective

The World Health Organization identified antimicrobial resistance (AMR) as a significant global health issue and one of the top threats to human health. Infection with resistant *Campylobacter*, a primarily food- and water-borne bacterium, can result in increased risk of mortality. A critical task in combating AMR is quantifying transmission pathways and evaluating interventions to reduce transmission. The objective of this study is to conduct a risk assessment to quantify the Canadian public's exposure to, and the likelihood of infection from, fluoroquinolone-resistant (FLQR) *Campylobacter* in retail broiler chicken.

Methods

A quantitative microbial risk assessment framework with two analytic components will be used. The first will quantify the presence of FLQR *Campylobacter* at key nodes along the broiler chicken production chain. Epidemiologic and laboratory data will be used to build stochastic models to assess the probability of consuming meat with FLQR *Campylobacter*. The second analytic stage will pair these exposure distributions with a modified dose-response model to predict the likelihood of acquiring a FLQR infection. Sensitivity analysis will assess the influence of model parameters on risk of FLQR *Campylobacter* infection.

Results

This study will estimate the probability of FLQR-*Campylobacter* throughout the supply chain and provide a comprehensive description of the probability of exposure via consumption of broiler meat produced in Canada. A novel dose-response model that accounts for antimicrobial susceptibility will consider the likelihood of human exposure to FLQR *Campylobacter*. Influential data gaps in surveillance and research will be identified, and our understanding of the characteristics and transmission dynamics of FLQR *Campylobacter* via retail chicken exposure will be improved.

Conclusions

The findings will identify risk management options to reduce the dissemination of FLQR *Campylobacter* through broiler meat along the supply chain in Canada. Additionally, this work contributes to managing the risk of infection with resistant foodborne pathogens.

Financial Support
Government of Alberta; Government of Canada

Notes:

182 - Critical role of 3'-downstream region of *pmrB* in regulating colistin resistance in *Escherichia coli* BL21(DE3)

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Session: ANTIMICROBIAL RESISTANCE - MODELING AND SURVEILLANCE

Objective

Colistin is a drug of last resort to treat multidrug resistant Gram-negative infections in humans. Increasing colistin resistance has posed a serious threat to human health, warranting in-depth mechanistic research. *Escherichia coli* BL21(DE3), the commonly used model organism, was observed to resist colistin (MIC = 16 μg/mL). In this study, we examined molecular basis of colistin resistance in BL21 using functional cloning approach.

Methods

Both targeted genes (e.g. *pmrAB*) and sheared genomic DNA from BL21 were cloned into pZE21, an expression vector, to examine capability to confer colistin resistance in sensitive Top 10 host strain. The inserts from colistin resistant clones (MIC ≥ 2 μg/mL) were sequenced. Stepwise subcloning of one insert was performed to identify critical element required for colistin resistance. RT-PCR was performed to examine mRNA stability and expression level. Lipid A species were extracted and subjected to LC/ESI-MS analysis to examine lipid A modifications.

Results

Five recombinant strains (3.8 – 10.7 kb insert) displayed colistin resistance using functional cloning. Subcloning indicated $pmrB_{BL21}$ and its 3' untranslated region are required for colistin resistance. Secondary structure analysis revealed a complex stem-loop in $pmrB_{BL21}$ downstream region. Further delicate subcloning demonstrated at least 103 bp downstream region of $pmrB_{BL21}$ is still required for PmrB functionality and colistin resistance. The downstream region dramatically affected $pmrB_{BL21}$ mRNA level (up to 1,000 fold) but did not influence its half-life. The construct containing $pmrB_{BL21}$ with 86-bp downstream region did not show any lipid A modifications; however, the constructs containing $pmrB_{BL21}$ with longer downstream region (+103 or +126 bp) have L-4-aminoarabinose (Ara4N) and phosphoethanolamine (pEtN) modifications in lipid A.

Conclusions

The 3'-downstream region of *pmrB* is essential for PmrAB-mediated lipid modifications and colistin resistance in *E. coli* BL21(DE3). This study revealed a novel regulatory mechanism of colistin resistance in *E. coli*.

Financial Support
USDA National Institute of Food and Agriculture

Notes:

183 - Bayesian latent class mixture model with censoring for correlation in antimicrobial resistance across populations

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Session: ANTIMICROBIAL RESISTANCE - MODELING AND SURVEILLANCE

Objective

The emergence of antimicrobial resistance across populations is a global threat to public health. Surveillance programs often monitor human and animal populations to evaluate trends of emergence in these populations. Many national level antibiotic resistance surveillance programs quantify the proportion of resistant bacteria as a means of monitoring emergence and control measures. The reason for monitoring these different populations are many, including interest in similar changes in resistance which might provide insight into emergence and control options. In this research, we developed a method to quantify the correlation in antimicrobial resistance across populations, for the conventionally unnoticed mean shift of the susceptible bacteria.

Methods

With the proposed Bayesian latent class mixture model with censoring and multivariate normal hierarchy, we address several challenges associated with analyzing the minimum inhibitory concentration data.

Results

Application of this approach to the surveillance data from National Antimicrobial Resistance Monitoring System led to a detection of positive correlation in the central tendency of azithromycin resistance of the susceptible populations from *Salmonella* serotype typhimurium across food animal and human populations.

Conclusions

Our proposed approach has been shown to be accurate and superior to the naïve frequentist estimation by simulation studies. Further implementation of this Bayesian model could serve as a useful tool to indicate the co-existence of antimicrobial resistance, and potentially a need of clinical intervention.

Financial Support
USDA National Institute of Food and Agriculture

Notes:

184 - Antimicrobial use and stewardship practices on California dairies post restriction of over-the-counter sales

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Session: ANTIMICROBIAL STEWARDSHIP - CATTLE

Objective

In Jan 2018, California implemented legislation requiring veterinary prescriptions for all dosage forms of medically important antimicrobial drugs (MIADs) in food animals. The objective of this survey was to collect data regarding herd demographic, antimicrobial drug (AMD) use, stewardship practices, and producer expectations of impacts of current AMD regulations on CA dairies.

Methods

A 44-question survey was twice mailed to the 1,282 licensed Grade A dairies. A total of 141 (11%) survey responses from 19 of 31 dairy-producing counties in CA were received. The median (±SE) herd size of surveyed dairies was 1,575±181, 803±163, and 262±71 cows/herd for dairies in Greater Southern (GSCA), Northern San Joaquin Valley (NSJV) and Northern CA (NCA), respectively.

Results

Results showed 93.55% had a veterinary client-patient relationship, while the others had a veterinarian observe cows on a regular basis. The majority (83.20 %) of respondents confirmed the use of OTC and/or prescription AMD on their dairies prior to Jan 2018. About 52.14% of studied dairies had not made any changes since Jan 2018 regarding AMD that were previously available OTC. Approximately 47.8 % made changes which included: treating fewer animals with AMD (20.83 %); discontinued one or more AMD (11.66 %); use the same AMD but decreased dosage and duration (14.46%) or treat more animals with AMD (0.83%). A quarter (28.57%) of study dairies confirmed usage or increased use of alternatives to AMD since Jan 2018 such as vitamins, minerals, herbal remedies, and vaccines. Respondents clustered into 3 groups based on their responses, cluster 1 and 2 were mainly composed of dairies in NSJV and GSCA. While cluster 3 was mostly composed of dairies from NCA. More than 93% of dairies in clusters 1 and 2 were conventional, with 87.4% and 69.5% using blanket dry-cow treatment (BDCT), respectively. In contrast, 77.8% of dairies in cluster 3 were organic and did not use BDCT.

Conclusions

This survey identified regional and herd demographic differences in AMD stewardship practices on CA dairies that can guide future educational outreach efforts.

Notes:

185 - International survey of veterinarians' perceptions about antibiotic use and resistance on dairy cattle farms

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Session: ANTIMICROBIAL STEWARDSHIP - CATTLE

Objective

The objective of this study was to conduct a cross-sectional, international survey-based study to characterize perceptions and concerns of dairy veterinarians regarding antibiotic (AB) use and resistance in dairy cattle.

Methods

A questionnaire-based survey was designed. Paper and online versions of the survey were administrated to veterinarians in the context of the 2018 International Bovine Mastitis Conference, Italy. Survey responses were analyzed with a combination of quantitative and qualitative analyses. Logistic regression was used to identify predictors of veterinarians' level of concern about the development of AB resistance on their clients' farms and to compare perceptions of veterinarians working in the United States of America (USA) and European Union (EU). Responses about the reasons for overprescribing ABs by veterinarians were analyzed using thematic analysis.

Results

A total of 71 participants from 21 countries participated in the survey. Participating veterinarians perceived that nearly half of their clients' injudiciously use ABs, and nearly half of their colleagues are inappropriately prescribing ABs. Veterinarians with fewer years in clinical practice were more concerned about AB resistant infections on the dairy farms they serve. No differences with respect to concern about antibiotic use in dairy farming were observed between veterinarians working in the USA and EU. Thematic analysis identified four themes (knowledge, attitudes, barriers, and rules and regulations) explaining reasons for overprescribing of ABs by veterinarians.

Conclusions

This study fills a gap in the understanding of perceptions of an international sample of dairy veterinarians regarding AB use and resistance, particularly in those working in the USA and EU and proposes that age-focused initiatives might help improve knowledge about AB resistance emergence. Findings reported here will contribute to future research and aid in the development of strategies to improve AB use in dairy farming.

Financial Support
USDA National Institute of Food and Agriculture

Notes:

186 - Drivers of antimicrobial use in pastoral communities in Kenya

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Session: ANTIMICROBIAL STEWARDSHIP - CATTLE

Objective

Agricultural use of antimicrobials in the management of food-animal diseases contributes to the global emergence of antimicrobial resistance (AMR) and is of great importance to human and animal health as well as food safety. Research has focused predominantly on large-scale production systems in high-income countries, with minimal attention to rural small-scale production systems in low-and-middle-income countries despite the fact that in such areas antimicrobials are widely available and minimally regulated. This study aimed to determine decision-making drivers for use of antimicrobials in livestock among pastoral communities in Kenya.

Methods

Data were collected from 55 homesteads in the Maasai Mara ecosystem in Kenya using a cross-sectional community animal health survey between 2018-19. The data included household and herd demographics, herd management, animal health history, and disease control and management practices. We used multi-model logistic regression inference (a supervised machine learning approach) to ascertain trends in antimicrobial use (AMU) in animals within these homesteads. Analyses were stratified by different contexts of use (in cattle, in goats/sheep, and in response to foot-and-mouth disease [FMD] or contagious ecthyma [ORF] infections).

Results

AMU in cattle was associated with AMU in sheep and goats (P=0.05), implying some interdependency in decision making on AMU in the different species. Access to veterinary services was associated with less antimicrobial usage for vaccine-preventable diseases like ORF (OR=0.06, P=0.05), while occurrence of diarrhea was associated with higher usage (OR=67.3, P=0.08) for FMD in cattle.

Conclusions

Overall, decisions to use antimicrobials were associated with several factors depending on the species and animal diseases in this pastoralist community. Moreover, access to animal health advice and veterinary services were important correlates of antimicrobial usage. This hypothesis-generating study suggests that community and ethnologically relevant methods may be necessary to understand antimicrobial usage in pastoral communities.

Financial Support
University of Minnesota

Notes:

187 - The impact of a bundled intervention on the accuracy of veal calf producer diagnoses for antimicrobial treatments

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Session: ANTIMICROBIAL STEWARDSHIP - CATTLE

Objective

The objective of this farm-level, longitudinal experiment was to evaluate the impact of a bundled, antimicrobial stewardship intervention on the accuracy of veal calf producer diagnoses of cases requiring antimicrobial treatment.

Methods

Eight farms were assigned to receive the intervention (IF; n=4) or to the control group (CF; n=4). The intervention consisted of 1) didactic training on antimicrobial stewardship, clinical health assessment, and diagnosis and treatment of calfhood diseases, 2) decision-tree treatment protocols, and 3) veterinary training at 1 and 5 weeks after calves arrived to the farm. On IF at 1 and 5 weeks, producers and the veterinarian independently assessed the health of 75 calves and recorded antimicrobial treatment recommendations. On CF, the producer-reported treatment records were used for comparison with veterinarian observations. The relationship between the producer- and veterinarian-identified cases requiring antimicrobial treatment was expressed in terms of sensitivity and specificity, and Poisson regression models were used to test differences between IF and CF.

Results

The sensitivities of producers on IF and CF relative to the veterinarian were 50.0 (73/146) and 14.3% (9/63), and the specificities were 87.6 (664/758) and 99.1% (550/555), respectively. Stated differently, the percentage of undetected antimicrobial treatments on IF and CF relative to the veterinarian were 50 (73/146) and 85.7% (54/63), respectively, and the percentage of unnecessary antimicrobial treatments were 56.3 (94/167) and 35.7% (5/14). Producers of IF were more likely to identify cases requiring antimicrobial treatment than CF (RR: 3.50, 95% CI: 1.65–7.41; P=0.001), yet CF were more likely to diagnose calves as healthy (RR: 1.13; 95% CI: 1.01–1.27; P=0.03).

Conclusions

Results suggest the intervention improved producers' ability to identify cases of disease requiring antimicrobial treatment but may have reduced producer's diagnostic specificity. Future analyses will detail changes in producer knowledge and the quantity of antimicrobials used at the individual- and group-levels.

Financial Support
U.S. Centers for Disease Control and Prevention

Notes:

188 - A survey of individual and group-level antimicrobial usage on veal farms in the United States

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Session: ANTIMICROBIAL STEWARDSHIP - CATTLE

Objective

The objective of this cross-sectional study was to use a clinical vignette-based survey approach to evaluate the potential for reduced therapeutic antimicrobial use at the calf producer-level.

Methods

Study inclusion criteria consisted of raising 120 calves (e.g. 1 cohort) per year and having a valid veterinary-client patient relationship. The survey was mailed to producers within two veal production companies across the Midwestern and Northeastern U.S. The survey was piloted with two producers to improve question clarity and included questions on veterinary-written protocols and antimicrobial use at the individual calf- and group-level.

Results

The survey response rate was 35.6% (21/59). Nearly all (95%) producers reported having a veterinary-written treatment protocol for navel infection, diarrhea, and pneumonia. Roughly two-thirds of producers reported treating uncomplicated cases of diarrhea, as well as mild pneumonia, with antimicrobials when veterinary-written protocols suggest antimicrobials were not indicated. A one-sided, binomial test was used to compare treatment estimates for uncomplicated cases of diarrhea in veal calves to identical cases in dairy heifer calves based on results of a prior survey by our laboratory that reported 37.1% (165/445) of dairy producers treated uncomplicated cases with antimicrobials; producer-reported treatments were significantly higher for veal (66.7%, 95% CI: 43.0 – 85.4; $P = 0.002$) than dairy heifer calves. Producers reported, on average, using group antimicrobial therapy 9.7 ± 6.3 d for diarrhea and 14.4 ± 10.2 d for respiratory disease throughout the 24-wk growing period. However, the reported initiation of group therapy was highly variable with the proportion of diseased calves ranging from 6% to 50%. Two-thirds of producers reported between 6 to 10% mortality during the growing period.

Conclusions

These findings suggest calf producer-focused educational materials are needed to guide antimicrobial treatment decisions to improve producer adherence to veterinary-written treatment protocols, as well as antimicrobial stewardship in the veal industry.

Financial Support
U.S. Centers for Disease Control and Prevention

Notes:

189 - Inter-observer agreement between veterinarians and farm caretakers: a hierarchical survey of antimicrobial use

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Session: ANTIMICROBIAL STEWARDSHIP - CATTLE

Objective

Antimicrobial use in food-producing animals may contribute to the development of drug-resistant bacterial infections in humans. Veterinarians prescribe and dispense antimicrobials, but farm caretakers are responsible for judging disease severity and initiating on-farm treatments. The objective of this study was to estimate the level of agreement on initiating on-farm antimicrobial treatment between farm caretakers and the respective farm veterinarian at different levels of disease severity.

Methods

This was a cross-sectional study that used hierarchical surveys and clinical case vignettes to collect information from farm caretakers and veterinarians on their dairy farm clients. The survey included vignettes of mild, moderate, and severe cases of metritis, lameness, and mastitis. In 2019, we received responses from 35 veterinarians and 66 farm-matched caretakers in Ohio. Cohen's Kappa coefficients (k) were applied to the matching farm caretakers and farm veterinarian responses to evaluate the level of agreement for treating hypothetical but routine cases with antimicrobials.

Results

The results suggested that little agreement was reached on cases that require local or systemic antimicrobial therapy for severe (k= -0.010) and moderate (k= -0.053) cases of metritis. Additionally, there was only slight agreement on antimicrobial use between veterinarians and farm caretakers on local (k= 0.016, 0.017) and systemic (k= 0.019, 0.052) moderate digital dermatitis and mild interdigital pododermatitis respectively. Also, a slight agreement between veterinarians and farm caretakers was reached on intramammary antimicrobial on mild (k= 0.055) and severe (k= 0.026) cases of mastitis.

Conclusions

This study demonstrates that the level of agreement on initiating on-farm antimicrobial treatment at different levels of disease severity between veterinarians and farm caretakers is weak. Therefore, attention should be paid to implement on-farm educational campaigns to improve the criteria for the uinitiation of on-farm antimicrobial treatment therapies.

Financial Support
U.S. Department of Agriculture

Notes:

190 - Antimicrobial drug use and health outcomes in pre-weaned California dairy calves

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Session: ANTIMICROBIAL STEWARDSHIP - CATTLE

Objective

Antimicrobial drug (AMD) usage in food animals is a major concern for the rise of antimicrobial resistance. In dairy calves, diarrhea and pneumonia are the main indications for AMD use. The goal of this study was to estimate AMD usage in California dairy calves following FDA's changes in 2017 for requiring veterinary oversight for medically important AMDs administered in feed and water and before the implementation of California regulations in 2018 that restricted over-the-counter sales of AMDs without veterinary prescriptions. Together, these regulations increased veterinary oversight of antimicrobials in livestock.

Methods

The prospective cohort study followed 310 calves from 3 dairies and 4 calf ranches located in the California Central Valley. Calves were enrolled at birth (1 to 3 days old) and followed until weaning (60 days). Disease occurrence and treatments were recorded on cards attached to calf hutches.

Results

From the cohort, 44.18% of the calves were treated for pneumonia while 41.18% were treated for diarrhea. Antimicrobial usage was evaluated based on active substance and herd type using treatment incidence. Enrofloxacin, ceftiofur, and florfenicol were the most commonly used parenteral AMDs. Logistic regression models were used to investigate relationships between treatment and management practices. Our results showed higher odds of parenteral AMD therapy on farms that supplemented milk with antibiotics, while farms that fed medicated milk replacers or supplemented calf diets with vitamins had lower odds of treatment. Among the possible reasons for the observed differences in treatment odds between calves fed milk supplemented with antibiotics and medicated milk replacer could have been effects associated with increased bacterial loads in whole milk. The lower odds of treatment in calves receiving vitamins in diets was possibly due to enhanced nutritional and immune status which may have reduced disease occurrence.

Conclusions

The outcome of this study will help inform antimicrobial stewardship programs for dairy calves and potentially benefit both animal and human health.

Notes:

191 - Quantitative estimates of antimicrobial use in veal calves

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Session: ANTIMICROBIAL USE

Objective

Antimicrobials are necessary for the treatment of bacterial infections in food animals, but may result in the emergence of antimicrobial-resistant bacteria that are a critical threat to animal and public health. Antimicrobial use in neonatal veal calves is frequent due to the inherent susceptibility of the young calves to bacterial diseases. The objective of the study was to quantify and describe the use of antimicrobials on veal farms within a single production system.

Methods

The antimicrobial treatment incidence (TI) was estimated by collecting used antimicrobial containers, producer-recorded treatment records, and nutrition records from calf arrival though slaughter at approximately 23 weeks for 8 cohorts of calves located across 7 premises. The total mg of active substance per total kg of animal body weight and defined daily doses (DDD) per 100 days were calculated for each cohort based on the labeled dosage for each antimicrobial and the standardized weight.

Results

Treatment incidence for group therapy ranged from 6.5 to 16 doses per 100 calf-days across cohorts. For parenteral (individual) treatment, calves received a range of 6.4 to 19.8 doses of antimicrobials per 100 calf-days. There was a 1.8 times more group oral antimicrobial usage than individual parenteral usage, and there was a 1.9 times more antimicrobial usage during the first three weeks compared to the rest of the entire grow (Weeks 4-20+). The first three weeks ofafter arrival a calf received a mean of 0.71 doses of antimicrobials per day.

Conclusions

This study provides the first estimates for quantified antimicrobial use for veal calves in the United States. Group oral medications represented the largest amount of usage compared to individual parenteral treatments. Group oral antimicrobial usage was highest during weeks 1-3 after arrival and this is most likely due to the calves' high susceptibility of bacterial disease during this time. These data will be useful to monitor usage or set benchmarks for continued improvement in antimicrobial stewardship in veal production.

Financial Support
U.S. Centers for Disease Control and Prevention; Ohio State University

Notes:

192 - How does public perception of antibiotic use on dairy farms contribute to preference for organic?

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Session: ANTIMICROBIAL USE

Objective

Understanding the knowledge, attitudes, and behaviors of the public regarding organic farming and antibiotic use in animal agriculture is important, as these drive purchasing decisions, which in turn can affect antibiotic use practices in food animals. The aim of this study was to investigate the United States (U.S.) public's perceptions of organic dairy farming practices and antibiotic use on dairy farms and to assess whether these perceptions may affect their purchasing decisions for organic and conventional dairy products.

Methods

We used data from the phone-based 2018 Cornell National Social Survey, developed in collaboration with Survey Research Institute at Cornell University. The survey collected information about participants' (n = 1,000 U.S. adults): (i) knowledge of the legality of antibiotic use on dairy farms (conventional and organic) and (ii) frequency of purchasing organic instead of conventional dairy products, as well as several explanatory variables and demographic characteristics.

Results

Results indicate poor knowledge of the general public about antibiotics, as 35% (351/998) of respondents considered the use of antibiotics in any kind of dairy farming to be illegal, while 20% (200/998) thought that antibiotic use for growth promotion is still permitted in the U.S. Participants who were familiar with the current regulations of antibiotic use on dairy farms to treat or prevent cow illness would be more likely to accept the use of antibiotics in cows certified as organic (Odds Ratio = 1.52, 95% Confidence Interval = [1.11, 2.08]). However, the participants' perceptions about antibiotic use and antibiotic resistance in dairy farming had no effect on their purchasing decisions for organic or conventional milk.

Conclusions

These findings underscore the existing misconceptions of the public about farming practices and antibiotic use on dairy farms. Also, the results indicated that increasing consumer knowledge about antibiotic use regulations may be expected to shape public opinions, which in turn may promote change in socially acceptable practices in organic farming.

Notes:

193 - Economic impact of removing arrival metaphylaxis in the U.S. stocker and feedlot industry

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Session: ANTIMICROBIAL USE

Objective

Antibiotic resistance continues to be at the forefront of issues facing animal agriculture. As policies and regulations become more restrictive regarding antimicrobial use, producers, veterinarians, and industry representatives should cooperatively prepare to use less antimicrobials. The objective of this study was to use stock and flow value-chain models to understand how cattle markets would respond to various antimicrobial use policies based on profitability.

Methods

Vensim Personal Learning Edition, by Ventana Systems Inc., was used to model the system with causal loop diagrams and stock and flow value-chain models. A metaphylactic intervention was created within each sector of the beef chain to compare how the market would change if metaphylaxis was banned in certain sectors or across the cattle feeding industry.

Results

If metaphylaxis was in use, then there was a much higher count of high-risk calves. These high-risk calves were more likely to move directly to the feedlot. Removing arrival metaphylaxis and adding a 5% incentive for calves not treated with antimicrobial increased the number of low-risk calves relative to high risk. The feedlots then preferred the low-risk calves over their high-risk counterparts. If metaphylaxis was only permitted at the backgrounder stage, more high-risk calves moved towards backgrounding operations although low-risk calves were more numerous overall.

Conclusions

Low-risk calves marketed directly to feedlot were most numerous when arrival metaphylaxis was removed from the cattle feeding industry. Restrictive antimicrobial use policies might lead to important shifts in beef production sectors. Smaller cow-calf herds, which produce the most high-risk cattle, might be most affected.

Financial Support
Foundation for Food and Agricultural Research

Notes:

194 - Comparison of animal daily doses and days of therapy for antimicrobials in species of veterinary importance

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Session: ANTIMICROBIAL USE

Objective

Characterizing antimicrobial use in animal populations is critical for purposes of antimicrobial stewardship. Duration of therapy is emerging as a critical and intuitive metric. In theory, the number of animal daily doses (ADDs) should approximate the number of days of therapy (DOTs), but no studies have examined whether this is the case. The objective of this study was to compare antimicrobial ADDs with antimicrobial DOTs in three populations: canine patients, large animal hospital patients, and dairy herds.

Methods

In the hospital patient populations, dose-based metrics were calculated using administrative hospital records while duration-based metrics were ascertained from manual chart review of individual animals. In the dairy herds, both metrics were obtained via farmer self-report. The difference between the number of animal daily doses and days of therapy and Lin correlation coefficients were calculated. Bland-Altman plots were calculated to visually demonstrate the distribution of these differences.

Results

The correlation between the number of ADDs and DOTs was poor for hospital patients and there were large differences between the two metrics for all populations, with ADDs most often overestimating the number of DOTs. While the median (IQR) differences between the number of DOTs and ADDs were relatively small (-9.4 (-25.7 to -0.92), 0.34 (-5.0 to 4.0), and 0.0 (-18.0 to 9.0) among canine patients, large animal hospital patients, and dairy herds, respectively), the limits of agreement were likely too large to be acceptable for most investigative purposes. Increased discrepancies between the two metrics were significantly associated with certain animal species and drug classes, decreased animal weight, and increased length of hospital stay.

Conclusions

While the number of ADDs can approximate the number of DOTs under certain circumstances, the large limits of agreement between these two measurements suggest that the ADD is generally not a reliable proxy for the duration of therapy.

Notes:

195 - On-farm monitoring of antimicrobial use and resistance in U.S. broiler production

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Session: ANTIMICROBIAL USE

Objective

The objective of this project was to design a sustainable on-farm antimicrobial use (AMU) and antimicrobial resistance (AMR) monitoring program representative of the U.S. broiler chicken industry.

Methods

The program was implemented as a cross-sectional sampling of farms. The WATT Poultry USA list was used as the list frame of eligible companies. Each company that voluntarily participated selected the complexes to enroll; between one and five complexes were selected, with the number roughly proportional to company size. During each 3-month interval, each complex selected 4-8 farms for sampling, with one house on each farm being sampled. Litter samples were cultured for *Salmonella*, *Campylobacter* and *E. coli*. *Salmonella* isolates were serotyped, *Campylobacter* isolates were speciated, and antimicrobial susceptibility testing was performed with microbroth dilution. AMU data were recorded for every sampled flock.

Results

Even with COVID-19 constraints, 150 farms were sampled in the first 2 quarters of 2020. Farm level prevalence of *Salmonella* and *Campylobacter* was 55/150 (36.7%) and 67/150 (44.7%), respectively, with no association between *Salmonella* and *Campylobacter* farm status (OR: 1.18, 95%CI: 0.6-2.3). *S.* Kentucky and *S.* Infantis were the most common serotypes identified. Most *S.* Kentucky isolates were resistant to streptomycin (STR) and tetracycline (TET) whereas many Infantis isolates were resistant to STR, TET, sulfisoxazole, and ciprofloxacin (CIP). Most *Campylobacter* isolates were *C. jejuni,* and most were pan-susceptible or resistant to TET only; approximately one-third had resistance to CIP.

Conclusions

This program was designed for sustainable monitoring of on-farm AMU and AMR in the U.S. broiler chicken industry. The approach is intended to harmonize with NARMS activities and uses a sampling strategy similar to Canada's CIPARS program. Based on industry feedback, the program does not require an excessive time commitment and provides value to company participants. To capture long-term associations between AMU and AMR, these datasets need to be collected in parallel at the farm level.

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

Notes:

196 - Associations among management practices and antimicrobial use for calf diarrhea on Canadian dairy farms

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Session: ANTIMICROBIAL USE

Objective

Diarrhea is the most common disease in pre-weaned calves and previous studies demonstrated large between-farm variations in antimicrobial use (AMU) for treatment of calf diarrhea. The objective of this cross-sectional study was to explore the association between herd's attributes and management practices and AMU for diarrhea treatment in calves on Canadian dairy farms.

Methods

A questionnaire was administered to a convenience sample of 152 dairy farmers across 6 provinces [Alberta (AB): 30; British Columbia (BC): 28; Nova Scotia (NS): 24; Ontario (ON): 31; Prince Edward Island (PEI): 9; Quebec (QC): 30] at the first visit of a multi-year project. Responses to the questionnaire were recorded in an online data capture system (REDCap, Nashville, TN). We evaluated responses to whether farmers had a written treatment protocol for calf diarrhea, type of housing facility for calves, number of lactating cows (herd size), region the farm was located, and if they used antimicrobials to treat calf diarrhea (outcome). Association between variables and AMU for calf diarrhea were screened using univariable logistic regression and if the variables had $P < 0.20$ in univariable analysis, they were included in multivariable logistic regression with herd size forced into the model as a confounder.

Results

The average herd size was 138 lactating cows, and herds were divided into large (> 80 lactating cows) and small herds (≤ 80 lactating cows) based on the national average. Two farms were excluded due to an incomplete set of data on the dependent variable. Among 150 farms, 37% had a diarrhea treatment protocol, 47% housed pre-weaned calves in groups, and 73% used antimicrobials for diarrhea therapy. In the multivariable model, farms in NS had lower odds of AMU for calf diarrhea than AB (0.3 times odds) or BC (0.2 times odds) but not different from other provinces.

Conclusions

Difference between provinces could have been attributed to other farm management practices or calf care processes, and further research is necessary to understand important factors that may contribute to reducing AMU.

Financial Support
Dairy Farmers of Ontario

Notes:

197 - Isolation and characterization of outer membrane vesicles from the fish pathogen *Moritella viscosa*

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Session: AQUACULTURE

Objective

Outer membrane vesicles (OMVs) are nano-sized proteoliposomes shed from the cell envelope of all Gram-negative species. OMVs play an essential role in pathogenesis, delivering virulence factors to the host cells, including toxins, adhesins, and immunomodulatory molecules. *Moritella viscosa*, a Gram-negative pathogen causes winter ulcer disease in several marine fish species including an eco-friendly cleaner fish, Lumpfish (*Cyclopterus lumpus*). In this study, *M. viscosa* was tested for virulence in lumpfish, and its OMVs were isolated and characterized.

Methods

Lumpfish were intraperitoneally infected with *M. viscosa* (2.6×10^6, 2.6×10^7, and 2.6×10^8 CFU fish^{-1}) and PBS control. Tissues were sampled at different time points, and mortality was recorded until 30 days post-infection (dpi). OMVs of *M. viscosa* grown in iron-rich and iron-limited conditions were isolated and characterized by transmission electron microscopy and protein analysis.

Results

Lumpfish infected with 2.6×10^7 CFU fish^{-1} of *M. viscosa* showed rapid mortality, where more than 50% of the fish died and exhibited clinical signs of disease after 5 days post-infection. *M. viscosa* OMVs in both iron-rich and limited conditions are spheres of 39.8–370 nm diameter that contains small RNA and DNA. The main OMV proteins have molecular sizes of 45, 30, and 20 kDa. OMVs isolated from iron-limited condition harbor an additional protein of approximately 60 kDa which is absent in OMVs isolate from bacteria grown under iron-rich condition. The protein profile of the 60 kDa protein band has enzymes such as Metal-dependent carboxypeptidase, Glucose-6-phosphate isomerase, and Glucose-6-phosphate isomerase. Also, transport systems occupied with peptide ABC transporter, extracellular solute-binding protein, Oligopeptide transport system, and permease protein B.

Conclusions

According to the results, lumpfish are susceptible to *M. viscosa* infection in experimental challenges with a lethal dose (LD_{50}) of $2.6\ x10^7$ CFU fish^{-1}. Information from the characterization of *M. viscosa* OMVs will aid in their utility as vaccine candidates in future studies.

Financial Support
Canada-First Ocean Frontier Institute; 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:

198 - Response of Lumpfish (*Cyclopterus lumpus*) to *Renibacterium salmoninarum* chronic infection

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Session: AQUACULTURE

Objective

Renibacterium salmoninarum is a Gram-positive, intracellular fish pathogen that causes Bacterial Kidney Disease (BKD) in several fish species. Although BKD outbreaks have yet to be reported in lumpfish (*Cyclopterus lumpus*), this fish has a potential BKD risk due to *R. salmoninarum*'s horizontal transmission. Here, we evaluated the susceptibility and immune response of lumpfish to *R. salmoninarum* infection.

Methods

Groups of 60 fish were intraperitoneally infected with *R. salmoninarum* ATCC 33209 (1×10^7, 1×10^8, or 1×10^9 cells/dose) or PBS. Tissues were sampled at different time points and mortality was recorded until 98 days post-infection (dpi). Immune-relevant gene expression levels were measured in fish head kidney from high dose and PBS groups at 28 and 98 dpi using qPCR.

Results

Highest dose-infected fish showed a lower relative percent survival (65%) than the medium (93%) and low (95%) dose infected groups. Tissue *R. salmoninarum* loads in all surviving fish at different time points indicated a chronic infection pattern. Of the 33 transcripts that were evaluated, 12 were upregulated and 4 were down-regulated at both 28 and 98 dpi, whereas 17 were dissimilarly regulated. Significantly higher upregulation was observed for cytokines (*il1β*, *il8a*, *il8b*), and for pattern recognition (*tlr5a*), iron regulation (*hamp*), and acute phase reactant (*saa5*) related transcripts at 28 dpi. In contrast, strong down-regulation of *tnfα* and cell-mediated adaptive immunity-related transcripts (*cd4a*, *cd4b*, *ly6g6f*, *cd8a*, *cd74*) at 28 dpi revealed the immune suppressive ability of *R. salmoninarum* in the early stage of infection. Significant upregulation of *ifnγ* and *cd74* at 98 dpi suggests a triggered cell-mediated immune response against *R. salmoninarum* at the chronic stage of infection.

Conclusions

Lumpfish was susceptible to *R. salmoninarum* ATCC 33209 infection, which caused mortality to 35% of the fish, and chronic infection to the surviving fish in high-dose infected group. Lumpfish immune response to live *R. salmoninarum* was reported. Tolerance observed in the surviving fish suggests low BKD risk to lumpfish.

Financial Support
Ocean Frontier Institute; 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:

199 - An update from the Collaborative Immune Reagent Network for Aquacultured Species (CIRNAS)

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Session: AQUACULTURE

Objective

CIRNAS is an immune reagent network with a goal of serving the aquaculture community by advancing the availability verified immunological resources and providing a knowledge base for fish health. Antibody panels and immune assays are being developed to assess the contribution of different leukocyte subsets and effector molecules to measure immure responses during disease and vaccination.

Methods

Aquacultured fish represent a critical food and protein resource for humans. Importantly, disease outbreaks in culture facilities limits the sustainability and production potential for this industry. A major factor restricting advancement of basic and applied research for fish health is the lack of immunological tools to track and assess immune responses in fish during disease and vaccination.

Results

Cellular targets (receptors, chemokines) have been cloned into eukaryotic expression vectors for the production of recombinant proteins. We have been using a high-throughput approach to complement our standard protocols to produce reagents for the community. Target species for reagent development include rainbow trout, salmon, tilapia and catfish. We are currently assessing reactivity of newly produced pAbs for the project.

Conclusions

Availability of the reagents and assays produced by CIRNAS will permit fish health specialists to comprehensively address immune response potential and duration in fish. We are working in collaboration with researchers at US academic and government laboratories as well as fish health experts in Europe to minimize duplicated efforts so that more tools and reagents can be developed for the community.

Financial Support
USDA National Institute for Food and Agriculture

Notes:

200 - Sublethal microcystin LR exposure of channel catfish induces liver pathology and altered phagocyte function

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Session: AQUACULTURE

Objective

Cyanobacteria are the dominant algae of channel catfish aquaculture ponds during the summer. They can produce toxins that affect fish, other aquatic organisms and land animals that ingest the algae. Among the most studied of these toxins are the Microcystins. These are cyclic oligopeptides that cause liver damage by inhibiting protein phosphatases. As channel catfish production has intensified, cyanobacterial phytoplankton blooms have increased, and incidences of infectious diseases have increased. Given the importance of the liver in the immune system, our overall hypothesis is that sublethal microcystin exposure predispose channel catfish to infectious diseases (and reducing microcystin exposure could in turn reduce losses to infectious diseases).

Methods

To study this, we used microcystin Leucine-Arginine (LR) (MC-LR), the most common and most toxic of the microcystins, in challenge trials. Fish were given a single intracoelomic dose of 500ng/g MC-LR and 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.

Results

The MC-LR treated fish were not visibly affected but completely stopped eating, when sampled all treated fish through day 4 had no ingesta and had full gall bladders, most control fish demonstrated ingest a in the gut and lighter colored typical gall bladders. Serum AST and ALT levels were significantly elevated from 6 hours through 96 hours post-exposure indicating hepatotoxicity. Alkaline phosphatase and bilirubin levels were not substantially affected. Histology confirmed substantial hepatic injury among the treated fish. MC-LR was shown to decrease the number of cells that endocytosed dextran 40, and dextran 80 and the number that phagocytosed the bacterial pathogen *Edwardsiella ictaluri*.

Conclusions

When catfish are exposed to doses of MC-LR at levels below those that induce visible disease signs, it can compromise the function of the hepatocytes and phagocytic leukocytes, two critical components of the innate immune system.

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

Notes:

201 - *Streptococcus iniae* biofilms enhance environmental persistence and resistance to antimicrobials and disinfectants

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Session: AQUACULTURE

Objective

The globally distributed bacterium *Streptococcus iniae* is responsible for outbreaks of high mortality in a wide range of economically important freshwater and marine fish species. Despite the significance of *S. iniae*, our understanding of its transmission and pathogenesis dynamics remain incomplete. Biofilms are important for the persistence and pathogenesis of many bacteria, but there is a paucity of information on their role in the extra-host persistence of *S. iniae.* This study aimed to compare biofilm formation by isolates representing different *S. iniae* genotypes and to investigate the effect of biofilm formation on environmental persistence and resistance to common disinfectants and antimicrobials.

Methods

Twelve clinical isolates of *S. iniae* representing 4 distinct genetic groups and diverse host types were assessed for their ability to form biofilms. Planktonic bacteria or mature biofilms were exposed to in vitro aquatic microcosms of different temperatures to quantify the number of culturable bacteria in each system over time. The minimum biofilm eradication concentration (MBEC) Assay® system was used to determine biofilm resistance to 18 antimicrobials and 4 types of disinfectants commonly used in aquaculture.

Results

All isolates tested formed appreciable biofilms within 72 hours. Bacteria remained culturable notably longer in the biofilm form compared to the planktonic, with a significant impact from temperature and salinity ($p < 0.0001$). The MBEC was higher than the planktonic minimal inhibitory concentration (MIC) for at least one isolate in 17 out of the 18 antibiotics tested. The MBEC was also higher than the minimum biocidal concentration (MBC) for 12 out of 18 tested, including oxytetracycline and florfenicol. While both forms were susceptible to disinfection by bleach, hydrogen peroxide and Virkon® Aquatic, treatment with providone-iodine did not eliminate biofilms of multiple isolates.

Conclusions

The ability of *S. iniae* to form resilient biofilms has potentially important implications for understanding and controlling the infectious process of this widespread pathogen.

Financial Support
University of California at Davis

Notes:

202 - Role of CspB and CspD proteins in *aeromonas salmonicida* subsp. *salmonicida* genomics, physiology, and virulence

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Session: AQUACULTURE

Objective

Aeromonas salmonicida subsp. *salmonicida* (hereafter *A. salmonicida*), is one of the oldest known fish pathogens, causes furunculosis in marine and freshwater fish. In *A. salmonicida* chromosome, there is cold shock protein (CSPs) genes which express during different stress environments and help *A. salmonicida* to adapt with the harsh environment as well as to maintain genomics, physiology, and virulence. CspA, CspB, and CspD are found in the genome of *A. salmonicida*. In this study, we evaluated the roles of, CspB, and CspD on the *A. salmonicida* endogenous chromosomal mutagenesis, physiology, and virulence in lumpfish (*Cyclopterus lumpus*).

Methods

By using suicide vectors, in-frame deletion of *cspB* and *cspD* genes were done. Bacteriological techniques were used to characterize the mutants. Illumina sequencing of the RNA samples was done from the Genome Quebec, Montreal, Canada. Transcription analysis was performed by using CLC Genomic Workbench 11.0.1 (Qiagen).

Results

Δ*cspB,* Δ*cspD* and Δ*cspBD* mutants were constructed. *A. salmonicida* Δ*cspD* showed slower growth at 28ºC, low frequency of *vapA* modification after heat-shock, reduction in LPS, biofilm production and low virulence in lumpfish. *A. salmonicida* Δ*cspB* andΔ*cspBD* mutants showed a faster growth at 28ºC and reduced or no virulence in lumpfish. To get a complete in-depth insight into the function of Δ*cspB*, and Δ*cspD*, mutants' transcriptome analysis was performed. Differential gene expression was noticed for 167 genes for Δ*cspB* vs wildtype and 38 genes for Δ*cspD* vs wildtype. Among these 167 genes, 8 genes were upregulated, and 159 genes were downregulated in Δ*cspB* vs control. On the other hand, 18 genes were upregulated, and 20 genes were downregulated in Δ*cspD* vs control.

Conclusions

we noticed that CspD plays a major role in the *A. salmonicida* cell division at high temperatures, influencing growth, impacting endogenous mutagenesis. CspB and CspD influence virulence in lumpfish. The strongest induction was noticed for the IS21 family transposase gene and ATP-binding protein gene was highly down-regulated for both Δ*cspB* and Δ*cspD* mutants.

Financial Support
Ocean Frontier Institute; 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:

203 - Assessment of *F. psychrophilum* virulence in Atlantic salmon (*Salmo salar*) and brook trout (*Salvelinus fontinalis*)

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Session: AQUACULTURE

Objective

Salmonid diseases caused by infections of *Flavobacterium psychrophilum*, the causative agent of bacterial coldwater disease, remain difficult to manage as novel pathogenic strains continue to emerge in aquaculture settings globally. To date, much of the research regarding treatment options and vaccine development has focused on rainbow trout (*Oncorhynchus mykiss*). As inland aquaculture expands to more salmonid species, fish health professionals must understand how different bacterial strains affect various species.

Methods

Atlantic salmon and brook trout were challenged with five *F. psychrophilum* strains via intramuscular injection and observed for mortalities over 21 days. Strains were isolated from disease diagnostic cases in salmonids and included a well-studied standard (CSF259-93) known to be virulent in rainbow trout.

Results

In three separate virulence assessments (Trials A, B, and C), strains US063 (isolated from lake trout; *Salvelinus namaycush*) and US149 (isolated from Atlantic salmon) caused significantly higher cumulative percent mortality (CPM) relative to other strains in Atlantic salmon ($P<0.001$ for all trials). Strain US149 caused significantly greater mortality than US063 in Trials A (CPM 97% vs 65%, P-value = 0.010) and B (CPM 96% ± 2.3% vs. 81.33% ± 4.8%, P-value = 0.018). Trial C used a lower dose (1.86×10^8 CFU mL^{-1}) for US149, resulting in a lower mortality (78.67% ± 9.33%) relative to Trials A and B. In brook trout, the strain 03-179 (isolated from steelhead trout; *O. mykiss*) was significantly more virulent than any other strain (CPM 100%, $P < 0.001$), followed by US063 and US149 respectively. The standard strain CSF259-93 did not cause significant mortality relative to a mock challenge treatment in any of the trials.

Conclusions

Results provide information about the applicability of strain selection in *F. psychrophilum* virulence testing in Atlantic salmon and brook trout, demonstrating the high virulence of US063 and US149 for these salmonid species. Results will aid in the development of vaccines and other health management tools against *F. psychrophilum*.

Financial Support
EU Framework Programme for Research and Innovation

Notes:

204 - Transcriptional response of lumpfish (*Cyclopterus lumpus*) to *Aeromonas salmonicida* infection reveals novel pathways

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Session: AQUACULTURE

Objective

Lumpfish (*Cyclopterus lumpus*), a native fish of the North Atlantic Ocean, is utilized as cleaner fish to biocontrol sea-lice (*Lepeophtheirus salmonis*) infestations in Atlantic salmon (*Salmo salar*) farms. *Aeromonas salmonicida* subspecies *salmonicida* is a Gram-negative facultative intracellular pathogen that causes lethal furunculosis in several fish species, including lumpfish. The molecular immune response of lumpfish to *A. salmonicida* has not been explored. In this study, we established an *A. salmonicida* infection model in lumpfish and examined the transcriptome profile of central lymphoid tissues after infection.

Methods

Groups of lumpfish (50 g; *n*=60 per group) were intraperitoneally (ip) injected with different doses of *A. salmonicida* to calculate the median lethal dose (LD_{50}). Samples of blood, head kidney, spleen, brain, and liver were collected at different time points (0, 3, and 10 dpi) to determine the infection kinetics. The transcriptomic analysis was performed using the Trinity pipeline.

Results

We determined that *A. salmonicida* lethal dose 50 (LD_{50}) is 10^2 bacterial cells per dose. The infection kinetic analysis indicated that the head-kidney is the primary target organ for *A. salmonicida* infection. Triplicate biological samples were collected from infected spleen, liver, and head kidney at 3 and 10 days post-infection (dpi) and compare to non-infected organs. Transcriptome sequencing was performed in the Nova-Seq Illumina platform and generating 1,034 million pair-end reads. The *de novo* assembly resulted in 403,204 transcripts with an average read length of 497 bp, representing 270,150 Trinity genes. In total, 11,795 significantly differentially expressed genes (DEGs) were identified.

Conclusions

Gene and pathways enrichment analyses showed that *A. salmonicida* induces the apoptotic process, precludes lysosome acidification, caused the metabolic arrest, and immune suppress the adaptive immune pathways. These results are in concordance with the fish behaviour prior to disease onset and mortality, and also coincident with *A. salmonicida* virulence factors.

Financial Support
Ocean Frontier Institute

Notes:

205 - Characterization of maternal immunity following vaccination of broodstock against IHNV or *F. psychrophilum* in trout

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Session: AQUACULTURE

Objective

Infectious hematopoietic necrosis (IHN) is a significant viral disease affecting salmonid species, while *Flavobacterium psychrophilum* (Fp), which causes bacterial coldwater disease (BCWD) remains one of the most significant bacterial pathogens of salmonids. We explored maternal immunity in the context of IHN and BCWD management in steelhead trout.

Methods

Females broodstock steelhead trout were separated to 6 groups. Group 1 was unvaccinated fish, while group IHNV-1M-2yrs or IHNV-1M-3yrs were vaccinated with an IHNV DNA vaccine at 1 month (M) prior to spawning. Fish in group IHNV-2M were vaccinated with the same IHNV vaccine at 2M prior to spawning. Fish in group Fp-1M were immunized with an live attenuated *F. psychrophilum* (Fp B.17-ILM) vaccine. The fish in group IHNV+Fp-1M were immunized with both the IHNV and Fp vaccine. Progeny from IHNV vaccinated and unvaccinated groups were challenged with low or high dose of IHNV once sac fry reached 13 days post hatch (dph) and also at 32dph. Progeny from group1 and Fp-1M were challenged with *F. psychrophilum*. The group 1 and IHNV+Fp-1M were challenged with both pathogens.

Results

Broodstock developed specific or neutralizing antibodies respectively to *F. psychrophilum* and IHNV. A significant reduction in mortality due to CWD was found when 13 dph sac fry, originating from broodstock immunized with Fp B-17-ILM. This suggests that maternal immunity via broodstock immunization is stimulated and may provide benefit to progeny. The specific mechanism is still unclear since antibody titers were low or undetectable in eggs or sac fry. Observations of 13 dph sac fry from vaccinated broodstock showed a clear benefit when challenged with a low dose of IHNV in comparison to progeny from unvaccinated group. However, the protective effect appeared to be overwhelmed by a greater viral dose and transient since fry challenged at 32 dph exhibited mortality similar to controls.

Conclusions

This study is suggested that limited active transfer IgM occurs from mother to eggs in steelhead trout and benefits observed may be linked to non-specific immune factors.

Notes:

207 - Comparative genomics and virulence of *Pseudomonas fluorescens* isolated from wild cunners (*Tautogolabrus adspersus*)

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Session: AQUACULTURE

Objective

The use of cleaner fish to biocontrol sea lice infestation in the Atlantic salmon aquaculture is considered a sustainable and effective strategy. The utilization and aquaculture of cleaner fish are progressing rapidly, and information on infectious diseases affecting this fish species is essential for their sustainable utilization. Cunner is utilized during the summer period due to its high predatory activity. Native infectious diseases of cunner have not been described to date. Here, we described the phenotypic and genomic characteristics of *Pseudomonas spp.* J380 isolated from infected wild cunners in North Atlantic Canada.

Methods

Cunners collected during spring exhibited a skin lesion and internal colonization of a single bacteria colony. The genome of this bacterium was sequenced using Illumina and PacBio platforms.

Results

The isolate was characterized as a Gram-negative motile rod, which grows in TSA at 4-28 °C. Biochemical and genomic characterization indicated that the isolated bacterium belongs to the *Pseudomonas fluorescens* group. The genome length was 6,261,650 bp, and the GC content was 59.7%. Iron deprived culture conditions were utilized to study differential expression of virulence factors and environmental adaptation mechanisms. Koch's postulates were verified in wild healthy cunners intraperitoneal (i.p.) infected with doses of 10^3, 10^5 or 10^7 CFU mL-1. After 30 days post-infection, the mean survival percentage was 85.2% with no significant differences between doses, indicating chronic infection. To evaluate the host range and virulence, lumpfish and Atlantic salmon were also i.p. injected with a dose of 10^6 CFU mL^{-1}. After 4 weeks post-infection, the survival of lumpfish and salmon was respectively 50% and 95%.

Conclusions

These results indicated that *P. fluorescens* J380 isolated from cunners in Newfoundland is specific to wrasse species and causes chronic infection in cunners.

Financial Support
Genome Canada; Natural Sciences and Engineering Research Council of Canada; Canada-First Ocean Frontier Institute

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

Notes:

208 - Development of an anti-IgM monoclonal antibody for burbot (*Lota lota*)

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Session: AQUACULTURE

Objective

Burbot (*Lota lota*) is the only freshwater member of the cod-like fish (Gadiformes) and are an ideal candidate for cool/cold-water aquaculture. Burbot are susceptible to disease outbreaks, and may become asymptomatic carriers, however they are refractory or even show limited mortality to most salmonid pathogens. However, larval and juvenile burbot have been shown to be susceptible to *Aeromonas sp.*, with mortality from ranging from 10 to 20% following laboratory challenges. Outbreaks of a species of *Aeromonas* most closely related to *A. veronii* have been observed to cause mortality up to 92% in juveniles. Development and optimization of a serological method using monoclonal antibodies to survey juvenile burbot for *Aeromonas sp.*, and aid in the generation of a vaccine, is paramount to the success of burbot aquaculture.

Methods

IgM proteins were isolated from burbot serum via an agarose affinity column containing immobilized mannan binding protein. The purified burbot IgM was injected into three mice for the generation of antibodies demonstrating specific affinity to burbot IgM, determined via ELISA (enzyme linked immunosorbent assay). Mice possessing antibodies for burbot IgM were culled and the spleens harvested to generate hybridoma cell lines. Using an ELISA the hybridoma cell lines were screened against the heavy and light chains of burbot IgM to identify a candidate for an anti-burbot IgM monoclonal antibody.

Results

Four hybridoma cell lines yielded specificity to burbot IgM heavy and or light chain. Three cell lines had affinity for the heavy chain, and two showed affinity to the light chain.

Conclusions

The cell line showing the highest absorbance for burbot heavy chain will be used to develop and optimize an ELISA to measure immune response of burbot to Aeromonas infection. This optimized ELISA will then serve as a primary tool to aid in the development of a vaccine to prevent *Aeromonas sp*. infections in burbot aquaculture.

Notes:

209 - Implications of specialization of a salmonid rhabdovirus for disease management

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Session: AQUACULTURE

Objective

Managing diseases in animals requires studying host-pathogen interactions across different levels of biological organization and under ecological settings that reflect natural communities and environmental variability. Juvenile fish mortality caused by infectious hematopoietic necrosis virus (IHNV) can be very high and is an important management challenge in salmonid conservation programs that rear juvenile fish for release into natural environments. To determine whether specialist and generalist modes of pathogen infection affect disease prevalence and thus management decisions, we test for evidence of IHNV specialization within different salmonid hosts, within hatcheries, and across hatcheries in the Pacific Northwest of the U.S

Methods

To achieve this, we analyze data collected from laboratory experiments, hatchery outbreaks, and landscape-level diagnostic testing.

Results

We show that IHNV genogroups have varying degrees of host specialization and generalism that result in significant variation in transmission to different host populations at the landscape level.

Conclusions

Disease transmission in hatcheries is likely enhanced by the higher replication of specialist IHNV genogroups. However, because of specialized host-pathogen associations, disease transmission may be limited by the host composition of hatcheries across the landscape. In addition to managing water sources, rearing strategies that minimize the prevalence of specialized lineages could have positive effects on IHNV control.

Financial Support
U.S. Department of Agriculture

Notes:

210 - Trained macrophages and non-target protection against *Edwardsiella ictaluri* and *E. piscicida* in channel catfish

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Session: AQUACULTURE

Objective

Trained Immunity (TI) is the immunomodulation of innate immune cells that provides non-target protection following stimulation. TI has two main signatures: metabolic changes that modify immune cell function, and protection against multiple pathogens. Our study was performed to determine if TI can occur in catfish leukocytes.

Methods

Channel catfish were intra-peritoneally (IP) injected with PBS, or 50 micrograms of mannan/gm of fish or 50 micrograms of beta glucan/gm of fish. Fourteen days later, anterior kidney (ak) leukocytes were isolated. Flow cytometry analyzed phagocytosis or binding of mcherry:*Edwardsiella ictaluri* and mcherry:*E. piscicida* by cells labeled with monoclonal antibodies L/CD207, mpeg-1, 51a, nccrp-1, 9E1, or C24a for dendritic cells, macrophages, neutrophils, Non-specific cytotoxic cells, B-cells or T-cells, respectively. Reactive oxygen species bursts (ROS), nitrite oxide production (NOS) and lactate dehydrogenase (LDH) assays were performed. Expression analyses of MHC I, MHC II, TLR2, TLR4, IL-6, tnf alpha, and GAPDH were performed by quantitative pcr.

Results

Neutrophils and B cells from catfish exposed to mannan phagocytosed significantly more mcherry:*E. ictaluri* than those cells from fish exposed to PBS. Dendritic cells, neutrophils and B cells from fish exposed to beta glucan phagocytosed significantly more mcherry:*E. ictaluri* than those cells from fish exposed to PBS. Leukocytes from fish exposed to mannan and beta glucan demonstrated significantly greater ROS, NOS and LDH than leukocytes from control fish.

Conclusions

Exposure to mannan enhanced bacterial phagocytosis by catfish neutrophils and B cells. Exposure to beta glucan enhanced bacterial phagocytosis by dendritic cells, neutrophils, and B cells. Exposure to beta glucan also enhanced bacterial binding by catfish NCCs.

Notes:

211 - Haplotype and genomic variation in atypical A*eromonas hydrophila* (aAh) of channel catfish aquaculture in the US

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Session: AQUACULTURE

Objective

Since the late 2000s, an atypical form of the gram-negative bacterial pathogen, *Aeromonas hydrophila,* has caused dramatic losses to the channel catfish *Ictalurus punctatus* aquaculture industry in the southeastern US. Previous studies have shown that two distinct haplotypes exist (ML09-119 and S14-452); however, information on haplotype distributions within the industry is lacking. This study aimed to evaluate the relative prevalence of the two haplotypes in three distinct catfish aquaculture regions of the southeastern United States as well as key genomic differences in the haplotypes.

Methods

A. hydrophila bacterial cultures were collected from case submissions at three diagnostic laboratories representing distinct regions of catfish aquaculture in Mississippi and Alabama. Cryostock isolates were revived on plates and DNA was subsequently extracted from individual colonies. Identification of each isolate was achieved using duplex end-point PCR. A subset of confirmed haplotype isolates was subjected to genome sequencing using nanopore technology and the homology of genes was compared.

Results

A total of 232 cultures were confirmed as aAh. Both, ML09-119 (68%) and S14-452 (32%), haplotypes were represented in the aAh isolates. Isolates from the Mississippi Delta showed a rapid haplotype shift from primarily ML09-119 to S14-452 between 2014 and 2017. A similar trend was seen in cases from East Mississippi between 2016 and 2018. The two aAh haplotypes showed a largely clonal relationship across most genes analyzed. However, important differences arose in the type VI secretion system (T6SS). All T6SS genes were present in S14-452, while nearly all were absent from ML09-119-like isolates.

Conclusions

The rapid haplotype shift seen in the Mississippi Delta raises important questions about the ecology and selective pressures placed on the pathogen. Selective pressures may the S14-452 haplotype over that of the original ML09-119. Thus, any future work on vaccination and treatment options should evaluate efficacy on both haplotypes to better estimate feasibility in the catfish aquaculture industry.

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

Notes:

212 - DTU-DADS-Aqua: a simulation framework to assist infectious disease management planning in marine aquaculture

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Session: AQUACULTURE

Objective

Infectious diseases often have highly adverse consequences on marine aquaculture. Simulation modelling approaches can be used to predict the dynamics and magnitude of spread of such diseases in aquatic environments, thus informing best management practices. Despite recent modelling endeavors in this context there has been no substantial progress in delivering a versatile simulation framework adapted to an aquaculture setting. We aimed to develop a modeling platform to study waterborne transmission of viral and bacterial pathogens in farmed aquatic populations.

Methods

We developed a stochastic, spatiotemporal hybrid simulation model (DTU-DADS-Aqua) that incorporates an agent-based model (ABM) for infection spread between net-pens within and between marine production sites and a compartmental model for infection spread within net-pens. Seaway distance among marine sites was used to estimate site hydro-connectivity. Different model processes replicate surveillance, detection and depopulation strategies. Using historical data from the 2003 - 2004 Canada and U.S.A infectious salmon anaemia (ISA) outbreak and information collected from the available literature to estimate model parameter values, we demonstrated the application of the model in a farmed Atlantic salmon (*Salmo salar*) population.

Results

Model outcomes from different simulated outbreak scenarios varying in control strategies adopted for ISA suggest that improved disease detection, combined with frequent surveillance visits to production sites and increased depopulation rate of infected net-pens may reduce the number of infected net-pens and outbreak duration, however the number of ISA-infected sites was minimally affected.

Conclusions

DTU-DADS-Aqua is a flexible modelling framework, which allows to explore alternative transmission and control dynamics of waterborne infectious diseases. This tool is able to provide vital information to assist the aquaculture industry and regulatory agencies in establishing decision-making policies, such as siting of marine production sites and designing protocols for disease mitigation efforts.

Financial Support
Ocean Frontier Institute

Notes:

213 - *Vibrio coralliilyticus* RE22 kills oyster larvae and prey bacteria using two Type Six Secretion Systems

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Session: AQUACULTURE

Objective

The oyster pathogen, *Vibrio coralliilyticus*, is a major cause of disease in oyster hatcheries. However, the mechanisms of pathogenesis are not fully established. Here, we show that strain RE22Sm uses two distinct Type VI Secretion Systems (T6SSs) as virulence factors against larval eastern oysters, *Crassostrea virginica*, and bacteria prey cells.

Methods

Mutations in the T6SS genes *hcp1* or *hcp2* (hemolysin co-regulated pilus) and *vgrG1* or *vgrG2* (valine-glycine repeat protein G) were created by allelic exchange mutagenesis using homologous recombination. Wild-type (wt) and mutant strains were tested for killing of T6SS null *E. coli* Sm10 cells by mixing RE22 and prey cells at an MOI of ~4, co-incubating for 4h, and quantifying cell changes by spot plating. Larval oyster challenges were accomplished by exposing ~100 oyster larvae to bacteria at a CFU/mL of 1×10^5 for 24h.

Results

Mutation of *hcp1* resulted in a complete loss of virulence toward larval oysters and attenuated virulence toward *E. coli* Sm10 (1.2 log decline of prey cells versus 3.34 log decline from RE22Sm wt). Mutation of *hcp2* resulted in reduced virulence against larval oysters (80% survival versus 53% survival with RE22Sm infection control), and significantly reduced killing of prey *E. coli* cells (0.47 log decline of prey cells versus 3.34 log decline from RE22Sm wt). Mutation of *vgrG1* also resulted in the complete loss of virulence toward oyster larvae but did not affect virulence toward *E. coli* cells. Mutation of *vgrG2* significantly reduced virulence against larval oysters (~90% relative survival versus 53% with RE22Sm infection control) but maintained full virulence toward *E. coli*.

Conclusions

V. coralliilyticus RE22Sm has two T6SSs that enable killing other bacteria and oyster larvae. T6SS1 plays a more significant role in virulence against oyster larvae. Mutations in either T6SS reduce killing of *E. coli* cells, though mutations in T6SS2 show a greater effect on bacteria killing. We propose that full pathogenic activity of *V. coralliilyticus* RE22Sm requires both T6SS1 and T6SS2 to kill competing microbes and attack oyster larvae.

Financial Support
USDA National Institute of Food and Agriculture

Notes:

214 - Contributions of organized nasopharynx-associated lymphoid tissue to rainbow trout immune response to vaccination

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Session: AQUACULTURE

Objective

Previous work identified the presece of nasal immunity in teleost fish. Studies described the nasopharynx-associated lymphoid tissue (NALT) of rainbow trout as a diffuse network of immune cells. We recently identified a lymphoid aggregate (O-NALT) in the nasal cavity of rainbow trout, outside the olfactory epithelium which has been overlooked in the context of fish immunity. The goal of this study is to determine how trout O-NALT contributes to the innate and adaptive immune response of rainbow trout during vaccination.

Methods

We have used a nasal vaccination model in rainbow trout using a live attenuated infectious hematopoietic necrosis virus vaccine. Rainbow trout were mock vaccinated or vaccinated with IHNV vaccine and the diffuse NALT and O-NALT compartments were sampled 15 min, 1 day, 7 days, 30 days and 90 days post-vaccination (dpv). Fish were injected with Edu 24 hours before sampling to detect proliferating cells. Samples were collected for immunofluorescence microscopy to determine changes in immune cells. Additionally, samples were collected for laser capture microdissection (LCM).
In a developmental series experiment, we sampled rainbow trout from hatch to 5 months of age to perform routine histological examination.

Results

Thus far we have identified that trout O-NALT first appears 150 days post-hatch. Vaccination studies show that the numbers of IgM or IgT B cells do not change in trout O-NALT in response to vaccination. We observed significant increases of numebrs of CD8 T cells in trout O-NALT following nasal vaccination with IHNV at 1 and 30 dpv. We detected a significant increase in IgM B cells 7 dpv. Proliferation studies indicate that the increase in CD8 T cells in O-NALT is not due to local proliferation. RNA samples isolated by LCM capture from diffuse and O-NALT will reveal the key immune molecules and markers that make these two tissue microenvironments unique.

Conclusions

The results obtained from our first year of work indicate that trout O-NALT plays a unique role during the course of vaccination in both innate and adaptive immune responses.

Financial Support
U.S. Department of Agriculture

Notes:

215 - *Vibrio anguillarum* stress response through ncRNAs to different temperature and limitation of nutrients.

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Session: AQUACULTURE

Objective

V. anguillarum is the causative agent of vibriosis that causes hemorrhagic septicemia a recurrent disease in cold or warm water fishes of economic relevance worldwide. These recurrent outbreaks are translated into important economic losses. Since the discovery of the critical regulatory role of ncRNA, scientists of diverse fields have put special effort in predict and characterize ncRNAs in different organisms of interest to analyze how these RNA molecules regulate biological processes, especially in pathogenesis. In this study, we identify and analyze *V. anguillarum* ncRNAs expressed under nutrient lack stress, in different grown temperature, and their relation with pathogenesis.

Methods

We extracted RNA from V. anguillarum J360 an outbreak isolate, grown under iron-limited conditions and rich conditions at 15 -28 ºC. High-quality RNA was sent to Genome Quebec and was sequenced by Illumina NovaSeq. Identification of ncRNAs repertory in V. anguillarum J360 was performed by the sRNAdetect RNA-seq based method and StructureRNAfinder that use secondary structure and covariance models. The transcriptomic analysis was performed using CLC Genomic Workbench 10.2 and Rockhopper 2.03. pipeline.

Results

We identified 87 ncRNAs in intergenic regions among chromosomes one and two of *V. anguillarum,* those were used as input to differential expression analyses in 15-28 ºC and iron-limited conditions compared to controls by biological triplicate. We identified 77 and 24 ncRNAs differentially expressed by chromosome under the iron-limited conditions at different temperatures.

Conclusions

We found differentially expressed the ncRNA RyhB related to iron storage also RsaF and NrrF related to iron regulation by the Ferric uptake regulator (Fur), we found highly overexpressed the quorum sensing related sRNA Qrr. *V. anguillarum* J360 is able to mount a response to temperature and nutrient lack through ncRNAs, like Qrrr, NrrF, RsaF, and RyhB that can trigger pathogenic factors to uptake iron from the host.

Financial Support
Ocean Frontier Institute; 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:

216 - Efficacy of glucans as immunostimulants to white sturgeon, *Acipenser transmontanus*

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Session: AQUACULTURE

Objective

Veronaea botryosa infections cause significant loses in cultured sturgeon. We aimed to investigate protection against *V. botryosa* challenge conferred to white sturgeon fed b-Glucans and to characterize the immunostimulatory effects induced by b-Glucans in feed.

Methods

Twelve replicate tanks (20 fingerlings/tank) were fed diets containing 0, 0.1 or 0.3% b-Glucans daily. After three weeks, fish from six tanks per diet were challenged with 10^5 *V. botryosa* spores by intramuscular injection. Control fish received PBS. Six weeks post-challenge, survival probability was calculated for each treatment and 10 randomly collected survivors were euthanized for necropsy. Splenic tissue was evaluated histologically for compatible lesions, fungal quantification, and expression of immune relevant genes using quantitative reverse-transcription PCR.

Results

Six weeks post-challenge a significant difference in survival probability between fish groups fed 0 and 0.1% b-Glucans ($p = 0.0142$). Similarly, a significant difference in survival was observed between groups fed 0% and 0.3% b-Glucans ($p = 0.0067$). Higher survival of glucan fed fish was also associated with lower fungal DNA levels in survivors. Similar transcript levels to controls were detected for all immune relevant genes after 21 days. However, 6 weeks post-challenge, compared to controls, significantly higher levels of Il-17 were detected in challenged fish fed 0.1 and 0.3% ($p=<0.05$) b-Glucans. Transcript abundance of haptoglobin, serum amyloid, serotransferrin, and cathelicidin was also significantly higher in challenged fish but did not differ by diet, and challenged fish fed 0.3% b-Glucans ($p=0.0006$) had significantly higher transcripts of IRF8. Similar transcript levels of MHC-II, TNFa, and TGFb were detected in exposed and control fish 6 weeks post-challenge.

Conclusions

Results indicate dietary supplementation with 0.1 or 0.3% b-Glucans for 21 days is a safe and effective prophylactic option to decrease susceptibility of white sturgeon to *V. botryosa* infection. Protection is at least partially associated with greater expression of IL-17.

Financial Support
USDA Animal Health Formula Funds

Notes:

217 - Challenge model of piscine streptococcosis in white sturgeon (*Acipenser transmontanus* l.) fingerlings

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Session: AQUACULTURE

Objective

Streptococcus iniae is a zoonotic pathogen and one of the major aetiologic agents of streptococcosis. To date, host-pathogen interactions between *S. iniae* and white sturgeon have not been well characterized. The aim of this study was to develop a challenge model to better understand the pathogenesis of this important disease in white sturgeon.

Methods

White sturgeon fingerlings were challenged with a single dose of 1.3×10^8 colony forming units of *S. iniae* per fish utilizing intracoelomic/intraperitoneal (IC) or intramuscular (IM) routes. Clinical signs and mortality were monitored for 30 d, and bacterial persistence as well as associated lesion, investigated in moribund, fresh-dead and surviving fish following microbiological and histopathological examination.

Results

Acute mortalities were present in IM challenged fish only, with first mortality occurring four days post-challenge and the mortality rate reaching 18.3% after nine days. Challenged fish presented erratic swimming, ulcerative skin lesions and hemorrhages in the liver and swim bladder. *Streptococcus iniae* was recovered from kidney and brain of moribund and dead fish. Histopathologic analysis of fish that died acutely revealed massive proliferation of bacteria in the muscle at the injection site and within vascular organs such as the heart and spleen with variable amounts of tissue necrosis including a necrotizing myositis. Fish that died closer to nine days post-challenge demonstrated more pronounced multifocal to locally extensive granulomatous inflammation of skeletal muscle at the injection site, liver, kidney, and spleen. No mortality, clinical signs or gross changes were observed in control or IC challenged fish. Post-mortem evaluation of ten survivors in each treatment was performed to determine carrier status in brain and posterior kidney. The prevalence of *S. iniae* in survivors was 10% and 0% in IM and IC challenged groups, respectively.

Conclusions

Results from this study suggest that IM injection challenge methods are not only suitable to induce streptococcosis in white sturgeon, but they may be the preferred method to study the pathogenesis of the naturally occurring disease in this species.

Notes:

220 - *Staphylococcus aureus* enhances biofilm formation, aerotolerance, and survival of *Campylobacter* at low temperature

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Session: BACTERIOLOGY

Objective

Campylobacteriosis remains a leading diarrheal illness in developed countries. High prevalence of *Campylobacter* (mainly *C. jejuni* and *C. coli*) as well as *Staphylococcus aureus* has been reported in retail meat and liver products. Polymicrobial presence of *Campylobacter* with other bacteria might enhance the survival of *Campylobacter* under adverse conditions encountered during retail meat processing and storage. *Campylobacter* has shown enhanced biofilm formation in the presence of other common microbial contaminants found in retail meat products. The objective of this study was to investigate the role of *Staphylococcus aureus* - if any - in enhancing the survivability and/or aerotolerance of *Campylobacter* species.

Methods

Experiments were carried out to investigate the survival of retail meat isolates *C. jejuni* [n=3] and *C. coli* [n=3] during a polymicrobial existence with retail meat isolates *S. aureus* [n=2] strains at low temperature [4ºC], and also under aerobic conditions. Biofilm formation was also tested. The effect of both *S. aureus* cells and filter sterilized *S. aureus* grown media [at 4ºC, 25ºC and 37ºC] on the survival and aerotolerance of *Campylobacter* strains was investigated.

Results

Results showed that both *S. aureus* cells and cell free grown media were able to prolong the survival of all tested *Campylobacter* strains at low temperature and under aerobic conditions. Biofilm formation of *Campylobacter* strains seemed to be significantly enhanced in the presence of *S. aureus* cells but results were not conclusive when the cell free media was used.

Conclusions

In conclusion, the presence of *S. aureus* cells enhances biofilm formation, aerotolerance, and survivability of *Campylobacter* strains at low temperature. Further investigations are warranted to identify possible mechanisms of interaction between the two species, which in turn might facilitate the design of effective intervention strategies to reduce both foodborne pathogens in retail meat and liver products.

Notes:

221 - Development and application of CRISPR-interference (CRISPRi) in pathogenic *Leptospira*

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Session: BACTERIOLOGY

Objective

Leptospirosis is a neglected widespread zoonosis caused by pathogenic bacteria of the genus *Leptospira*. Symptoms can range from mild flu-like disease to severe multi-organ failure. Pathogenic mechanisms of infection are poorly understood and virulence factors remain underexplored, due to the lack of effective, easy and affordable tools for genetic manipulation of this pathogen. To overcome this, we applied CRISPR-interference by creating plasmids to concomitantly express the dCas9 protein (dead Cas9) and a single-guide RNA. This variant of Cas9 lacks nuclease activity, and therefore, RNA-guided dCas9 provokes a blockage in transcription of target genes, causing gene silencing rather than gene disruption. Conjugation protocols were optimized for plasmid delivery using HAN media.

Methods

Protospacer within the *lipL32* gene from *L. interrogans* serovar Copenhageni strain FIOCRUZ L1-130 were evaluated by the webserver CHOPCHOP. sgRNA cassettes were obtained by PCR and ligated into pMaOri.dCas9 plasmid, previously developed for silencing in saprophytic leptospires. Distinct donor *E. coli* and recipient *L. interrogans* proportion and conjugation times were assayed for filter mating. Recovered transconjugants were evaluated regarding LipL32 expression by quantitative RT-PCR, immunoblotting and the Fluorescent Antibody Test (FAT).

Results

The plasmid pMaOri.dCas9sg32 was successfully obtained and used to generate recombinant donor *E. coli* β2163 for conjugation to *L. interrogans*. The most efficient transconjugant recovery was using at 1:1 proportion of donor and recipient for 24h. Transconjugant colonies could be observed within 12 days on HAN agar plates. Plasmid insertion was confirmed by PCR and gene silencing was corroborated by qPCR. Immunoblotting and FAT confirmed no detection of LipL32 protein in recombinant leptospires.

Conclusions

This is the first demonstration of complete gene silencing in pathogenic *Leptospira* by CRISPRi. The application of this technology to gene and sRNA targets of pathogenic leptospires provides a novel tool to elucidate pathogenic mechanisms of leptospirosis.

Financial Support
U.S. Department of Agriculture

Notes:

222 - ESBL-producing bacteria detected in commercial poultry farms

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Session: BACTERIOLOGY

Objective

Extended spectrum beta-lactamase (ESBL)-producing *Enterobacteriaceae* like *Escherichia coli* are serious threats to public health. Epidemiology of these bacteria and their potential to transfer their resistance to other bacteria is crucial. The objective of this study was to investigate the presence of ESBL-producing *E. coli* in hens at three maturity stages in conventionally caged (CC) and cage-free (CF) commercial farms and analyze transferability of resistance, as well as their virulence potential.

Methods

Feces from hens in three maturity stages (early, peak, and late lay) were initially screened for ESBL-producing bacteria using CHROMagar selective media. Presumptive *E. coli* colonies were verified using PCR amplification of the *β*-glucuronidse (*uidA*) gene and for the presence of avian pathogenic *E. coli* (APEC) virulence genes (*iutA*, *iss*, *iroN*, *cvaC*). Plasmids were extracted via phenol-chloroform, visualized via gel electrophoresis, and replicon typed via PCR multiplex. *In vitro* conjugation assays were performed to determine the potential spread of ESBL resistance between bacterial species and the plasmid type associated with this transfer.

Results

ESBL *E. coli* were found in both CC and CF environments in the early lay maturity stage only. Virulence screening detected the presence of *iss* and *iutA* in both groups. Conjugation assays indicated the transfer of a large, K/B plasmid conferring cefotaxime resistance to an APEC strain, χ7122, containing 3 plasmids and its plasmid-cured strain χ7368. χ7368 transconjugants were able to further transfer the plasmid to the *E. coli* K-12 strain χ6092.

Conclusions

From this investigation, ESBL *E. coli* can be found in both CC and CF environments. The presence of ESBL-production on transferable broad-range plasmids and its ability to transfer resistance to virulent and avirulent strains with and without plasmids is alarming. The detection of virulence genes in ESBL *E. coli* presents serious threats for chicken and human health because of their potential in causing deadly urinary tract and sepsis infections.

Financial Support
Iowa State University

Notes:

223 - Analysis of initial stage of *M. avium* subsp. *paratuberculosis* infection with *in vitro* granuloma model

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Session: BACTERIOLOGY

Objective

Mycoabcterium avium subsp. *paratuberculosis* (MAP) is a causative agent of paratuberculosis (PTB) or Johne's disease (JD), which is a chronic and debilitating disease in ruminants. MAP is also considered to be a potential cause of human Crohn's disease (CD) because the MAP was associated in the CD patients. Currently, the mechanism of survival of MAP at initial stage of infection is not fully elucidated. In the current study, therefore, an *in vitro* granuloma model based on 3-D culture method was set to analyze host-MAP interaction.

Methods

Bovine peripheral blood mononuclear cells (PBMCs) were cultured with collagen extracellular matrix (ECM) to make 3-D environment and MAP was infected for 10 days (MOI=1:1). During infection, cellular aggregation was observed by phase-contrast microscopy. Samples at 7 days p.i were fixed and embedded with paraffin for H&E staining. Additionally, scRNA-seq was performed using Human THP-1 macrophages. After 3h infection (MOI=10:1), cells were harvested and then scRNA-seq was performed using 10X Genomics platform.

Results

MAP induced aggregation of PBMCs was began to be observed after 3 days p.i. The granuloma-like lesion (formation of multi-nucleated cells and infiltration of lymphocytes) was observed by H&E staining at 7 days p.i. Single cell level transcriptomic profiles of THP-1 cells were clustered infected cell and control cells into different groups. Differentially expressed genes of infected cells showed upregulation of genes related to initial inflammatory responses.

Conclusions

In vitro granuloma model apperes to be able to reproduce initial stage of infection. Therefore the analysis of phenotypic characteristics using this model could reveal the pathogenesis of MAP. Further scRNA-seq will be performed with PBMC cells with this model. 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 Ministry of Education (No. NRF-2019R1I1A1A01063387), BK21 PLUS and the Research Institute for Veterinary Science, Republic of Korea.

Notes:

224 - Relative isolation rates of bacteriophages lytic to *Fusobacterium necrophorum* from rumen and sewage samples

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Session: BACTERIOLOGY

Objective

Liver abscesses are a major problem in the beef cattle industry, currently controlled by tyloisin, a macrolide. Bacteriophages that lyse *Fusobacterium necrophorum,* the primary etiologic agent, are a potential antimicrobial alternative for prevention of liver abscesses. Our aim was to isolate lytic phages active against *F. necrophorum* from bovine ruminal fluid and city sewage samples and perform comparative analysis on relative frequencies of isolations between the two source samples.

Methods

Pooled ruminal fluids at slaughter and pooled untreated city sewage samples were each collected on five separate dates in 2019-2020. Across different sampling dates, each source sample, in portions, was screened for presence of bacteriophages on ~50 *F. necrophorum* strains previously isolated from feedlot-cattle liver abscesses. Presumptive phage plaques were harvested, and the viruses purified by serial passaging on the susceptible bacterial strains. The bacteriophage isolation frequencies were compared between the source samples, sampling dates, and *F. necrophorum* strains.

Results

A total of 93 bacteriophages lytic to *F. necrophorum* were isolated from five samples of pooled bovine rumen fluids and five samples of untreated city sewage. Thirty-three (11.8%) phages were isolated from the rumen fluid samples and 60 (21.7%) from city sewage samples. From each sampling date of pooled rumen fluid samples, phages were isolated with relative frequencies of 29.0, 18.2, 3.5, 0.0, or 6.3%. The relative frequencies of phages from each sampling date of city sewage samples were 24.5, 22.8, 12.3, 29.1, or 20.0%. Twenty-nine of the bacterial strains did not yield any phages, while the remaining yielded 1 to 6 phages. A few bacterial strains yielded phages more frequently than other bacterial strains.

Conclusions

Bacteriophages that are lytic to *F. necrophorum* were isolated from pooled bovine ruminal fluids samples and untreated city sewage samples. Comparative analysis suggested that phages lytic to *F. necrophorum* have a higher probability of isolation from untreated city sewage than pooled bovine ruminal fluids.

Financial Support
USDA-NIFA Food Animal Residue Avoidance Databank

Notes:

225 - Animal Models for *Mycobacterium avium* subsp. *paratuberculosis* Infection

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Session: BACTERIOLOGY

Objective

Mycobacterium avium subsp. *paratuberculosis* (MAP) is the causative agent of Johne's disease that is an important chronic and wasting diseases in ruminants such as cattle, sheep and goats. It is essential to understand the interaction of the pathogen with host to control the infection. However, the use of ruminants as animal models for Johne's disease is expensive and complicated due to the long incubation period. Therefore, developing an efficient animal model that mimics the natural infection has been required to identify a potential control strategy. In this study, we evaluated the effect of MAP in their ability to colonize murine tissues following oral and intraperitoneal (IP) infection. Oral is representative of the natural route of infection and IP is effective route to induce infection in mice which is not natural host for MAP.

Methods

The 6-week-old C57BL/6 female mice were infected with the MAP ATCC 19698 (10^9 CFU/mouse) by oral and IP routes. Mice were sacrificed at 6-, 12-, and 18- weeks post infection (wpi). At time of sacrifice, samples from the liver, spleen, mesenteric lymph node, peyer's patches and intestines were collected for bacterial, histopathological, and immunological examination.

Results

At the 6wpi, all mice were colonized with MAP, whereas the oral-immunized group showed significantly lower level than the IP-infected groups. In the splenocyte, TNF-α and IL-10 were sigificantly increased in all group. RNA-seq analysis from the MLNs and spleen showed that genes related to immune responses and fatty acid metabolism was differentially regulated.

Conclusions

In this study, we developed a reproducible model of MAP in mice by different routes of infection. Reproducible model of MAP in mice may be useful for future investigations for understanding of pathogenesis and prophylactic studies. This work was supported by the Strategic Initiative for Microbiomes in Agriculture and Food, MAFRA (No. IPET918020-4), the BK21 PLUS and RIVS, SNU, Korea.

Notes:

226 - Inoculation of lactobacilli mixture reduces lesions and modulates gut microbiota in chicken necrotic enteritis

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Session: BACTERIOLOGY

Objective

Necrotic enteritis (NE) is an important chicken intestinal disease caused by NetB positive *Clostridium perfringens* (CP) which imposes economic losses to the poultry industry worldwide. The objective of this study was to evaluate the effect of a multi-species lactobacilli mixture on experimental necrotic enteritis in chickens.

Methods

One-day-old male broiler chickens were divided into 6 groups. Groups 1- 4 received orally two different concentrations (10^7 and 10^8 CFU/ml) of the lactobacilli mixture, *Lactobacillus (L) acidophilus*, *L. reuteri, L. salivarius, L. crispatus* and *L. johnnsonii* once a week at 1, 7, 14 and 20 days of age. Groups 5 and 6 served as controls. At 21 days of age, the chickens in groups 3, 4 and 6 were challenged orally with 10^8 CFU/ml of a pathogenic *C. perfringens* isolate, twice daily for 3 days. At 24 days of age, all birds were euthanized and intestinal lesions were scored. Cecal contents of chickens in the groups 1, 2, 5 were collected for microbiome analysis by real-time PCR at days 21 (before challenge) and from all groups at day 24 (after challenge). Segments of duodenum and jejunum were also collected for histomorphometric analysis.

Results

Using both concentrations of lactobacilli (10^7 and 10^8 CFU/ml) decreased lesion scores by 28% and 52%, respectively. Evaluation of microbiome phyla showed that lactobacilli reduced *Actinbacteria* and *Enterobactriacae*, while increased *Bacteroides, Firmicutes* and *Proteobacter* in the cecal content of chickens. Histomorphometic analysis showed higher villous/crypt ratio in lactobacilli treated chickens.

Conclusions

It was concluded that using a mixture of lactobacilli could result in beneficial effects on the chicken gut health marked by a reduction in lesions associated with NE.

Notes:

227 - Channel catfish virus targeted management approaches in catfish aquaculture

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Session: BIOSECURITY & INFECTION CONTROL

Objective

Catfish industry is the largest aquaculture industry in the U.S. contributing 74% of total finfish production. Channel catfish virus (CCV) is the most relevant virus causing significant mortalities in catfish fry and fingerlings. The current unavailability of therapeutic measures and commercial vaccines against CCV is a great concern. This project envisions to provide detailed information on CCV transmission and pathogenesis to develop CCV-targeted management strategies, which will reduce virus-associated production and economic losses. Effective disinfection protocols, fish immunization, identification of less susceptible catfish populations, and evaluation of environmental stress factors triggering CCV disease outbreaks would direct the industry to employ the best preventative strategies to limit disease incidences.

Methods

A preliminary study was carried out to determine the susceptibility of channel and hybrid catfish to CCV. The CCV concentration used in the immersion challenge was $10^{3.5}$ $TCID_{50}$ /mL. Percent fish mortalities were determined. Effect of stocking density on the activation of CCV was carried out. CCV attenuation was carried out by frequent passage of CCV on catfish cell lines.

Results

The differential pathogenicity of CCV to catfish fingerlings were determined. The percent mortality was found to be higher in channel catfish fingerlings. Significantly higher mortality was observed in channel catfish exposed to CCV in high density tanks. The attenuation of CCV is in progress.

Conclusions

Increased understanding of CCV pathogenesis and effective management strategies will reduce CCV associated losses in the hatchery and production units. Identification of catfish populations that are less susceptible to CCV field strains, effective disinfection protocols, fish immunization, identification of less susceptible catfish populations, and evaluation of environmental stress factors triggering CCV disease outbreaks would direct the industry to employ the best preventative strategies to limit disease incidences.

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

Notes:

228 - Effect of eugenol nanoemulsion on the structure, composition, and microbial load in *Listeria monocytogenes* biofilm

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Session: BIOSECURITY & INFECTION CONTROL

Objective

Listeriosis is an important infectious disease of ruminants caused by *Listeria monocytogenes* (LM) that leads to encephalitis and abortion in affected animals. LM is known to survive in farm environment for long durations through formation of sanitizer tolerant biofilm. Therefore, it is critical to develop strategies to control LM biofilms and reduce LM environmental persistence. This study investigated the effect of eugenol (EG; isolated from cloves) or its nanoemulsion (EGNE) on LM biofilm architecture, composition, and underlying microbial population.

Methods

Biofilm of LM (Scott-A, AT19115 strain) was developed at 25°C for 4 days on borosilicate surface either in the presence or absence of sub-lethal concentrations of EG/EGNE. Biofilm architecture, proportion of live-dead cells, and eDNA in the matrix was visualized using confocal microscopy. Effect of EGNE/EG treatments on exopolymeric substance (EPS), that provides protection to the underlying microbes, was quantified using ruthenium red staining followed by spectrometry at 450 nm. Effect of EGNE and EG treatments on LM population in the biofilm developed at 25°C (4 days)/10°C (24 days) was studied using glass beads-based microbial extraction protocol. All experiments had duplicate samples, repeated twice and analyzed with one-way ANOVA at $p<0.05$

Results

In both LM strains, biofilm developed in presence of sub-lethal concentrations (< 700 ppm) of EG and EGNE lead to reduced eDNA and reduced EPS production as compared to control ($p<0.05$). In addition, LM population in the biofilm was reduced by ~1.5 log CFU/ml as compared to control ($p<0.05$). Exposure to bactericidal concentrations of EG and EGNE (2750 ppm-Scott A; 2300 ppm-AT19115) reduced LM population in the biofilm (developed at 25°C or 10°C) to below detection limit, as early as 1 min of treatment time ($P<0.05$).

Conclusions

Eugenol nanoemulsion could be used as a potential disinfectant in the farm environment.

Financial Support
USDA National Institute for Food and Agriculture

Notes:

229 - A systematic review on emergency mass-depopulation methods for swine

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Session: BIOSECURITY & INFECTION CONTROL

Objective

Mass depopulation involves euthanasia of very large numbers of pigs, and may include not only pigs infected with a disease, but also healthy pigs in a facility or surrounding areas. The most common reasons for depopulation include immediate disease control, where euthanasia of swine on farm is necessary to minimize the risk of highly virulent pathogen spread, foreign diseases; response to natural or human-made disasters; and to protect public health in the case of potential zoonoses. Other emerging applications include euthanasia to reduce economic and welfare costs associated with slaughter delays result of decreased demand for meat or limitations in slaughterhouse processing capacity as observed during the COVID-19 pandemic. The objective of this study were to summarize available literature information on swine depopulation methods and to highlight critical gaps in knowledge.

Methods

Relevant peer reviewed articles from pilot, small-scale and large-scale studies were identified through a systematic search in electronic databases of PubMed, Cab Abstract/Web of Science and MEDLINE using key words and themes. Eligible studies were screened according to the schematic framework of Preferred Reporting Items for Systematic reviews guidelines.

Results

A total of 68 publications were identified and reviewed for criteria including chemical/physical agent used, time to loss of consciousness, insensibility, and death, ease of carcass disposal, potential personnel safety and animal welfare risks, skill necessary for application, as well as equipment and facilities requirements. Collectively, gaseous carbon dioxide was the most studied depopulation method, and from those reports, only two articles were identified as large-scale field trials. Despite limitations inherent to each method previously reported, none of the published studies demonstrated an ideally safe and reliable way to induce rapid unconsciousness in large groups of pigs.

Conclusions

For successful emergency preparedness, further development of rapid mass depopulation methods for use in large groups of pigs is necessary to provide industry partners with suitable and low cost emergency preparedness procedures adhering to personnel safety and animal welfare standards.

Financial Support
National Pork Board

Notes:

230 - Perceived risks and benefits for participation in poultry disease monitoring programs in the US: a cluster analysis

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Session: BIOSECURITY & INFECTION CONTROL

Objective

The development and implementation of disease monitoring systems is useful for rapid and efficient communication during outbreaks of infectious diseases affecting the poultry industry. The objective of this study was to describe perceived benefits and risks for participation in disease mapping and monitoring projects specific to the poultry industry; and to identify groups of poultry professionals with similar perceptions and attitudes towards monitoring/mapping projects.

Methods

An anonymous online survey was developed and distributed to poultry professionals through industry and associations. Information on participant's demographics, perceptions on risk and benefits from participation on voluntary poultry disease mapping and monitoring programs, and biosecurity and disease concern information was collected. Multiple correspondence analysis and hierarchical clustering were performed to identify groups of professionals with similar characteristics.

Results

A total of 65 participants filled out the survey representing 21 states in the US. The cluster analysis yielded two distinct groups of respondents, each comprising approximately 50% of respondents. Cluster 1 subjects could be characterized as optimistic, perceiving major benefits of sharing farm-level poultry disease information. However, they also had major concerns, mostly related to potential accidental data release and providing competitive advantages to rival companies. Cluster 2 subjects were characterized as perceiving a lesser degree of benefits from sharing farm-level poultry disease information. This second cluster was mostly composed of production and service technicians. Participant's roles and perceptions of risk and benefits contributed significantly to cluster assignment; while their represented commodity and geographical location in the US did not.

Conclusions

For the successful development of poultry disease mapping and monitoring programs in the future, various groups of poultry professions will likely need to be approached in different manners to achieve maximum participation in the voluntary program.

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

Notes:

231 - Connecting livestock disease and market dynamics to human biosecurity decisions

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Session: BIOSECURITY & INFECTION CONTROL

Objective

The acceleration of animal disease spread worldwide due to increased animal, feed, and human movement has driven a growing body of epidemiological research as well as a deeper interest in human behavioral studies aimed at understanding risk attitudes. Biosecurity measures can reduce the risk of infection but human risk tolerance can hinder biosecurity investments and/or compliance.

Methods

We help untangle the complexity of the hog production system with disease threats, human decision making and economic dependencies using an agent-based model. We focus our work on swine production subsequent to the 2014 outbreak of Porcine Epidemic Diarrhea Virus (PEDv) in the U.S. This outbreak presented dramatic disease spread in the hog production systems and unexpected outcomes in the supply-demand market.

Results

Monte Carlo simulations of scenarios designed with different distributions of risk attitudes amongst the simulated swine producers provide distributions and trends of disease incidence, market prices and producer budgets during a modeled PEDv outbreak. A shift toward risk aversion in the producer population - meaning higher and engaged biosecurity - can reduce disease incidence by 20% (+/- 10%). In a scenario of high risk tolerance, the model shows that the delayed bisoecurity response leaves the hog production system vulnerable to unpredictability and high chance of pandemics. In this scenario, up to 40% of the swine producers can experience income losses.

Conclusions

In an effort to support effective disease prevention, our model results can inform policy making and stakeholder communication to move towards more resilient and healthy production systems. The modeled dynamics of risk attitude have the potential to improve strategies for nudging risk aversion and the economic patterns emerging from the model can heighten awareness of disease consequences and direct adaptation to disease shocks.

Financial Support
USDA National Institute of Food and Agriculture

Notes:

232 - Prevalence factors associated with fluoroquinolone resistant *Campylobacter jejuni* in broiler flocks in Canada

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Session: BIOSECURITY & INFECTION CONTROL

Objective

Campylobacter infections in humans are usually self-limiting, however in the case of severe infection antibiotic intervention may be necessary. The antimicrobial of choice for treating campylobacteriosis are fluoroquinolones (FQ), however resistance to these drugs can develop rapidly. Approval of use of FQ in animals has coincided with increasing resistance to FQ in human infections, therefore isolation of FQ resistant (FQr) *campylobacter* in broiler flocks is concerning. Data collected by the Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) from 2013-2018 was utilised to investigate prevalence factors associated with the isolation of FQr *C. jejuni* from broiler faecal samples.

Methods

Mixed effects logistic regression models accounting for clustering of flocks within hatcheries, with (tet) and without (no-tet) a fixed effect for the presence of flock level tetracycline resistance were used to assess prevalence factors among 536 *C. jejuni* isolates from 158 flocks.

Results

Both models indicated that the type of bird, use of virginiamycin, the use of traps to control rodent populations, and the number of birds in the barn were significant prevalence factors for increased FQr *C. jejuni* in a flock. In the tet model the odds of FQr *C. jejuni* increased by 16 (95% CI: 3.74, 68), and the magnitude of the effect of each of the identified prevalence factors was larger. Both models indicated that methods of disinfection of water lines between production cycles is important, with the use of chlorine protective in the tet model , and the use of hydrogen peroxide a risk factor in the no-tet model. The use of hot water to wash the barn between production cycles was also a significant protective factor in the no-tet model.

Conclusions

Results indicate that biosecurity and sanitation procedures play a role in the dissemination of FQr *C. jejuni* in broiler flocks. A better understanding of the management of cleaning and disinfection practices and their effect on the isolation of FQr *C. jejuni* may allow for the reduction of this enteric pathogen in broiler flocks in Canada.

Financial Support
Alberta Agriculture and Forestry

Notes:

233 - Harnessing emergent digital tools for quantifying agricultural disease risk preferences

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Session: BIOSECURITY & INFECTION CONTROL

Objective

Understanding how human behavior impacts the spread of disease is extremely relevant yet challenging to quantify. Our research harnesses digital technology to recreate complex decision mechanisms related to agricultural disease risk management. We investigate how risk measured in an experimental gaming environment compares to traditional survey methods.

Methods

We designed two experimental games that emulate decision making within the hog production industry. The biosecurity compliance game simulates operational farm labor during an infectious disease outbreak. Participants can choose to comply with a line of separation, which carries an associated opportunity cost affecting their earnings. The protocol adoption simulation allows participants to manage a farm's biosecurity investment during an outbreak. Participants are confronted with varying knowledge of neighboring disease spread and biosecurity. Recruits then completed a digital version of Holt and Laury's risk assessment survey.

Results

We found very weak positive correlation (Spearman:rho = 0.18;$p < .01$;N = 239) between risk measured in our compliance simulation and the survey. Surprisingly, no significant correlation (Spearman:rho = 0.08;$p < .01$;N = 1000) was found between risk in our protocol simulation and survey. We found participants classified as risk averse in the survey adopted more biosecurity in the protocol game than the risk tolerant (MWU:$p < .01$). Overall survey risk assessment was not an adequate predictor of biosecurity investment strategies. This may indicate that the immersion presented in our experimental games captures nuanced behavioral signals that prove elusive using generalized risk assessment surveys.

Conclusions

Experimental gaming simulations have great potential for measuring risk associated with complex decision mechanisms. Tailored interfaces using game design software provides an adaptable tool for targeted behavioral analysis. Understanding risk using experimental games can aid in epidemiological modeling as well as a tool for testing efficacy of risk communication strategies.

Financial Support
USDA National Institute of Food and Agriculture

Notes:

235 - Molecular surveillance of foot-and-mouth disease virus at slaughterhouses in Vietnam

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Session: BIOSECURITY & INFECTION CONTROL

Objective

The genetic diversity of foot-and-mouth disease virus (FMDV) poses a significant challenge to successful control. In endemic settings such as Vietnam, it is important to identify the emergence and distribution of different strains promptly to implement control measures.The objective of this study was to evaluate sampling of asymptomatic livestock at slaughterhouses as a strategy for molecular surveillance of FMDV. We investigated the extent to which viruses recovered from slaughterhouses reflect the diversity in the source population,and whether they can serve as sentinels for the early detection of emerging outbreak strains.

Methods

Oropharyngeal fluid (OPF) samples were collected from 944 asymptomatic cattle and buffalo in two slaughterhouses at bi-monthly intervals in Long An and Tay Ninh provinces from 2017 to 2019. Sequences of the VP1 region were obtained from 72 animals, which were assumed to represent sub-clinical infection.To characterize viral diversity in the source population, sequences obtained from longitudinal sampling of OPF from farms (2015-2017) from eight provinces were also included.Outbreak sequences from clinical cases were available (2009-2019) from GenBank. A time-scaled phylogenetic tree was created for serotypes O using BEAST.

Results

Within a given serotype time-scaled trees showed that a series of viral clades emerged, spread, and subsequently declined over time. For several serotype O clades that included outbreak, slaughterhouse and farm sequences, subclinical slaughterhouse sequences pre-dated the outbreak sequences by 2-6 months.For other clades, sequences isolated from animals without clinical signs from farms clustered with older outbreak sequences, suggesting the sub-clinical circulation of the viral strain may occur up to 18 months after the observed outbreak.

Conclusions

Routine sampling at slaughterhouses may provide a cost-effective means for molecular surveillance to identify circulating and emerging FMDV strains. Within this study virus sequences obtained in this manner provided a subset of the diversity present in other regional viral populations.

Financial Support
USDA National Institute for Food and Agriculture

Notes:

236 - Isolation of potential therapeutic, lytic bacteriophage for *Mycoplasma ovipneumoniae (M. ovi).*

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Session: BIOSECURITY & INFECTION CONTROL

Objective

M. ovi causes respiratory disease in domestic and bighorn sheep. Morbidity in infected domestic sheep is modest, however, introduction into naïve bighorn sheep is quite lethal. In the absence of effective countermeasures, current management practices restrict grazing locations for domestic sheep, which causes hardships for the industry. Our goal is to identify novel approaches to eliminate *M. ovi* infection in domestic sheep.

Methods

This Seed Project is focused on isolating bacteriophages that lyse *M. ovi* from infected sheep that could be used alone or in combination with other treatments to clear infection in future studies. In the first year of this project we established cultures of *M. ovi* (Y98, ATCC) and developed screening approaches to identify lytic phage. We also collected nasal washes, lung lavage fluids, and lung tissues from infected animals as potential sources of phage.

Results

We began combining *M. ovi* cultures with extracts of lung tissue from *M. ovi* infected sheep. This combination halted bacterial growth, suggesting a strong antibacterial component in *M. ovi* infected lung extracts. We planned to assess DNA sequences in these culture supernatants for potential novel phage patterns. However, problems arose that hindered progress. First, we experienced unexpected issues with our original cultures of *M. ovi* from ATCC (loss of growth). As such, we expanded our efforts to include cultures of *M. ovi* isolated directly from infected animals. Another unexpected problem that hindered progress was the impact of COVID-19 on our research efforts in the Spring of 2020.

Conclusions

Though progress was not as expected in our first 9 months, we still anticipate that completion of this Seed grant will provide the tools and preliminary data in support of a larger grant. This effort will focus 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
USDA National Institute of Food and Agriculture

Notes:

237 - Control of *Salmonella* Dublin in a dairy herd

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Session: BIOSECURITY & INFECTION CONTROL

Objective

The objective of this study was to describe control methods implemented on a dairy farm that were successful in eradicating an S. Dublin outbreak, as measured by changes in herd prevalence.

Methods

Five groups of Holstein cattle divided by origin and arrival date were sampled and determined positive or negative for S. Dublin based on ELISA results from the Animal Health Diagnostic Center at Cornell University. Bulk tank milk samples were also collected and evaluated in this way. Prevalence estimates were calculated and compared to control measures implemented on the dairy during the outbreak.

Results

Two-hundred and eighty three cows were sampled for a total of 700 observations. The group of cows originating from Iowa in 2015 had the greatest overall seroprevalence (76.53%), but seroprevalence for all groups decreased throughout the study, coinciding with testing and management changes. All bulk tank milk samples for the herd were negative for S. Dublin.

Conclusions

It is possible to eradicate S. Dublin and avoid great economic loss on dairy farms if proper biosecurity measures take place.

Financial Support
University of Tennessee

Notes:

238 - Descriptive and multiple factor analysis of the North Macedonian pig sector: implications for disease transmission

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Session: BIOSECURITY & INFECTION CONTROL

Objective

Provide the first description of the pig farming system of North Macedonia, highlighting traditional backyard pig farming, biosecurity and husbandry practices, disease awareness and pig trade patterns. Assess the implications for disease spread, as outbreaks of African swine fever (ASF) in Bulgaria, Greece and Serbia put the N. Macedonian pig sector at imminent risk of incursion.

Methods

A closed questionnaire survey was implemented through a network of private veterinarians collaborating with FAO and N. Macedonian veterinary services. Across farm types, we gathered data on pig numbers, husbandry practices and veterinary care, with emphasis on feed, sick and dead pig protocols, and disposal methods. We characterized the pork value chain from buying sources to selling locations, including the type and fate of products sold. Each of these practices were classified by risk level and combined using weighted linear combination to generate a biosecurity risk score. Multiple factor analysis (MFA) was used to identify and group factors associated with highest ASF risk and to generate “farm profiles” based on farm type, biosecurity, and other farm characteristics.

Results

We interviewed 457 pig farmers: commercial(7%), family(32%) and backyard(61%). Biosecurity practices differ across farm type and region based on sanitation, containment, and exposures to outside people, domestic pigs and wild boar. Commercial farms have the most consistent and highest-level biosecurity practices, with isolation of new pigs and worker restrictions being the most variable factors. In general, farms with the highest risk scores are backyard farms in the eastern regions.

Conclusions

These results provide the first step to characterize biosecurity gaps, define risk profiles, and to identify targets for mitigation measures. We provide baseline information to design realistic, risk-based and sustainable prevention, surveillance, and control strategies for ASF and other swine diseases in North Macedonia, using an approach that could easily be adapted to other countries with similar epidemiological settings.

Financial Support
USDA National Institute of Food and Agriculture

Notes:

239 - Alternative techniques to protect environmental surfaces

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Session: BIOSECURITY & INFECTION CONTROL

Objective

Environmental cleaning and disinfection are critical in controlling infections. Traditional methods involve a 2 to 3 step process consisting of cleaning with a detergent, followed by a liquid disinfectant. Disinfectants have difficult to attain contact times, leading to sub-optimal disinfection. Furthermore, poorly designed drainage systems in some animal care facilities, makes cleaning and rinsing difficult. After a surface had been cleaned and disinfected, quick pathogen re-contamination occurs. Despite the fact that environmental surface disinfectants are widely accepted, concerns about safety exist. Procedures implemented for cleaning and disinfection are frequently inadequate, and the overuse of disinfectants could induce tolerance or cross-resistance with antibiotics. Lastly biofilms are not always effectively removed by routine cleaning.
Due to these drawbacks, innovative methods for improved environmental cleaning and disinfection should be investigated.

Methods

Literature review

Results

Combining ultra-microfiber cloth (UMC) and steam is an efficient cleaning and disinfection method. UMC, made of polyester and polyamide, which due to absorption and static attraction are able to effectively remove surface microorganisms and prevent cross-transfer of pathogens during wiping.
Steam technology uses temperatures of 140°C under pressure (97% dry steam), that loosens surface dirt and microorganisms, allowing the microfiber cloths to remove it. Steam technology has been effectively applied to kill pathogens on environmental surfaces with a short contact time. This method of cleaning and disinfection has the advantage of reducing water usage by 90%, is environmentally friendly, eliminates the need for chemicals, shortens the time needed for cleaning and disinfection and improves surface cleanliness. Despite the initial capital investments, the whole process is more effective and improves the return on investment. Safety and risk hazards are reduced, and adoption and compliance improve.

Conclusions

The combination of UMC and steam can be used to supplement or replace chemical disinfection in animal care facilities. Even though this technology was proven successful in controlling outbreaks in human hospitals, careful testing and evaluation would be recommended before its implementation in animal health.

Notes:

240 - Novel Therapeutic Leads; Demonstration of efficacy, safety, and applicability of anti-APEC molecules in chickens

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Session: BIOSECURITY & INFECTION CONTROL

Objective

Avian pathogenic E.coli (APEC) is a leading cause of morbidity and mortality in poultry and results in multi-million dollars annual losses to the poultry industry. Currently, APEC infections are controlled by antibiotic medication and vaccination. However, APEC isolates are becoming more resistant to antibiotics and the currently available vaccine doesn't confer sufficient protection against heterologous APEC serotypes. Therefore, there is a critical need for developing novel anti-APEC therapeutics to improve the control of this key endemic avian disease. The objective of this study is to measure the efficacy, safety, and applicability of identified anti-APEC molecules in chickens and further elucidate their mechanisms of action (MOAs).

Methods

Anti-APEC molecules were identified through screening of small molecule library followed by testing their efficacy and toxicity in cultured epithelial, macrophage and red blood cells, wax moth larva, and in broiler chickens. In this proposed study, we will; 1) optimize the delivery of lead molecules in drinking water of chickens, 2) determine their efficacy under conditions mimicking the field settings and compare with currently used antibiotics, and 3) identify the antibacterial target(s) and elucidate their MOAs using proteomics and metabolomics approaches.

Results

We have identified four promising anti-APEC lead molecules acting either by inhibiting the APEC growth (GI-7 and GI-10; growth inhibitors) or quorum-sensing/virulence (QSI-5 and QSI-10; quorum-sensing inhibitors). These lead molecules in small pilot studies reduced the mortality, severity of APEC lesions, and APEC load in internal organs of chickens. In addition, these leads were effective against diverse APEC serotypes, antibiotic-resistant APEC strains, and biofilm protected APEC.

Conclusions

The demonstration of the efficacy, safety, and applicability of these lead molecules in chickens is vital to advance them as novel anti-APEC therapeutics. Our study will help to promote sustainable poultry production worldwide together with improved food security and food safety.

Financial Support
USDA National Institute for Food and Agriculture

Notes:

241 - Bacterial chondronecrosis with osteomyelitis in broilers: pathogen genomics, and management strategies

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Session: BIOSECURITY & INFECTION CONTROL

Objective

Lameness is a major animal welfare and food safety issue in meat type birds. Bacterial chondronecrosis with osteomyelitis (BCO) is a form of lameness, often associated with outbreaks and significant economic loss. Numerous bacterial species have been associated with BCO lameness. We were the first to report association of *Staphylococcus agnetis* with BCO, a species previously associated with mastitis in cattle.

Methods

We developed a model for spread of BCO lameness in a broiler facility under standard growth conditions using a source and target population where the source is challenged with *S. agnetis*. We have used this model to screen for commercial products that can reduce BCO incidence. Surveys of BCO outbreaks have provided additional species. Genomes for representative isolates have been sequenced and assembled.

Results

We identified commercial probiotics and feed supplements that can significantly reduce BCO lameness in our *S. agnetis* challenge model. Phylogenomics of BCO isolates suggest that BCO in individual farms may be largely clonal, but remarkably different between farms. *S. agnetis* in chickens appears to have arisen from a single clade within isolates from cattle. However, detailed genome analyses failed to reveal genes associated with the jump to chickens.

Conclusions

Identification of management strategies to mitigate BCO lameness is critical to improving animal well-being. Understanding the spectrum of bacterial species and the phylogenomic relationships will elucidate the breadth of measures necessary to control BCO.

Financial Support
Arkansas Biosciences Institute; Chris Hansen Inc

Notes:

242 - Interactions and innovations generate insights for influencing biosecurity adoption in agricultural animal systems

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Session: BIOSECURITY & INFECTION CONTROL

Objective

Biosecurity has strategic or conceptual, tactical or structural, and operational or procedural components. A multi-disciplinary team explored decision-making at these levels in the context of food animal production systems. Our primary objectives were (a) to better understand the roles of information and communication methods on risk perception and how factors such as economic and social concerns influence people to adopt or engage in biosecurity strategies, (b) to visualize the impact of human behaviors on disease spread in production systems, and (c) to create new tools to educate the next generation about biosecurity.

Methods

This work was supported by the USDA National Institute of Food and Agriculture, under award number 2015-69004-23273. Interactions with private and public sector stakeholders informed the development of our work. The team deployed innovative interviews, surveys, and digital field experiments to gather human behavioral data. We integrated epidemiologic information, human risk perception, and socio-economic influences into first-of-their-kind agent-based models to explore how changing human behaviors affects disease spread dynamics.

Results

The research outputs have identified several key findings. For instance, we found more risk averse behavior following graphic (threat gauge) risk messages, or seeing where disease is but not what biosecurity is in place on neighboring facilities. An interactive online curriculum about biosecurity and hands-on activities were developed for youth audiences, and a website focused on farm biosecurity was launched. Recordings of presentations at our project symposium held in May 2019 are available through a project website. We are developing evaluation tools to gauge the impact of the online educational modules.

Conclusions

This Coordinated Agricultural Project funded by USDA NIFA in the Food Security Challenge area has generated innovations in research design and outreach as well as insightful findings for stakeholders concerned with protecting animal health in agricultural production systems.

Financial Support
USDA National Institute of Food and Agriculture

Notes:

243 - Controlling Bovine Leukemia Virus in young stock and cows by identifying and removing super-shedders

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Session: BIOSECURITY & INFECTION CONTROL

Objective

Our research strives to improve the sustainability of the US dairy industry by developing and testing methods to reduce economic loss from bovine leukemia virus (BLV). We will evaluate the efficacy and cost effectiveness of a protocol to identify and remove the most infectious adult cattle as well as a testing protocol for the young stock so they are free from BLV when they enter the milking herd.

Methods

We will conduct a longitudinal study of ≥150 heifers on 5 farms over a 5-year period. Antibodies to BLV (ELISA) and qPCR measures of proviral load (PVL) will be measured three times before animals enter the milking herd. Once cows enter the milking herd, ELISA and PVL will be measured at 60 days in milk of their first, second, and third lactations. Following these animals from birth through adulthood provides the ability to track BLV incidence among the young stock, providing insights into how and when animals become infected as well as the longevity of infected animals into adulthood as compared to their herdmates.

Results

In our first pilot intervention trial, three small farms used blood lymphocyte counts and PVL to identify the most infectious cattle for segregation or culling. Each farm saw a significant ($P < 0.0001$) decrease in their whole-herd BLV incidence and prevalence by the end of this 2.5-year trial. A second trial removed ELISA-positive cows to eradicate BLV from the milking herd. In total, 3 farms dropped from the trial due to re-infection of BLV from incoming heifers. These two studies prompted the current project to develop an integrated program to simultaneously manage BLV in both the young stock and the milking herd. To estimate the return on investment, the cost of BLV management in the young stock and the milking herds will be compared with previously measured economic impacts of BLV infection.

Conclusions

This proposed research should provide our dairy producers with a proven path forward to control this costly disease.

Financial Support
U.S. Department of Agriculture

Notes:

244 - Significant inactivation of airborne coronavirus in a ducted ultraviolet-C (UV-C) System

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Session: BIOSECURITY & INFECTION CONTROL

Objective

Control technologies to inactivate airborne viruses effectively are needed during the ongoing SARS-CoV-2 pandemic, and to guard against future pandemics where airborne transmission is an important route of infection. In this study, we examined the efficiency of a relatively compact (1.14 m length x 30.5 cm diameter), low pressure Hg lamp (monochromatic UV-C light) flow tube reactor tested at high flow rates of 684 L min^{-1}, 1674 L min^{-1}, and 2349 L min^{-1} in a duct mounted system to evaluate the inactivation of aerosols of porcine respiratory coronavirus (PRCV), a surrogate of SARS-CoV-2.

Methods

PRCV virus was nebulized in a custom-built 3.86 m long wind tunnel housed in a biosafety level class II facility. Air samples using an Anderson Cascade impactor were collected upstream and downstream of the device, and tested by RT-PCR and titrated using cell culture methods.

Results

We demonstrate that sealed UV-C flow tube reactors (252 nm light) can be operated at flow rates of 684 – 2439 L min^{-1} and with fluences near 252 +/- 1 nm of 13.9 – 49.6 mJ cm^{-2} efficiently inactivating airborne coronaviruses. The single pass log_{10} reduction of active coronavirus was in excess of 2.2 (99.4% removal efficiency) at a flow rate of 2439 L min^{-1} and in excess of 3.7 (99.98% removal efficiency) at 684 L min^{-1}. Because virus titers resulting from sampling downstream of the unit were below the limit of detection, the true log reduction and removal efficiencies are likely even higher than measured. Comparison of virus titration based log reductions to those based upon reverse transcriptase quantitative PCR (RT-qPCR) and measurement of fluorescein concentrations (doped into the nebulized aerosol) reveals that the reduction in viable PRCV is primarily due to UV-C based inactivation, as opposed to physical collection of virus within the unit.

Conclusions

The results presented in here confirm that UV-C flow tube reactors can efficiently inactivate coronaviruses through incorporation into HVAC ducts or recirculating air purifiers.

Notes:

245 - Associations of pen management characteristics and cattle morbidity in the first 45 days after feedlot arrival

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Session: BOVINE RESPIRATORY DISEASE

Objective

Bovine Respiratory Disease (BRD) is a leading cause of economic loss, impaired animal welfare, and reduced sustainability in beef production. BRD results from interactions among environment, host, and pathogens. We aim to evaluate potential relationships between pen- and yard- level management factors and morbidity during the first 45 days on feed.

Methods

Pen characteristics (pen area per head, bunk space per head, and stocking density) were combined with retrospective feedlot data which included gender, arrival weight, number of head in arrival group, and arrival date. Generalized linear mixed models were used to evaluate potential associations between pen characteristics, cattle attributes and BRD morbidity in the first 45 days post-arrival.

Results

Overall, cattle had a mean 0.34 m and a median 0.42 m of bunk space per head and a mean 28.81 m^2 and a median of 23.65 m^2 of pen area per head. An association between BRD morbidity and pen characteristics was determined and was modified by cattle attributes. For example, cattle weighing greater than 362.87 kg at arrival had higher BRD morbidity in pen densities less than 23.22 m^2 per head compared to greater pen densities. Cohorts with pen area per head greater than 32.51 m^2 had lower BRD morbidity compared to those with less available pen space. No clinically significant associations were found between available bunk space and BRD morbidity.

Conclusions

Further research is needed to explore if pen- and yard- level characteristics are associated with BRD incidence in other feedlot settings. Additional associations discovered will enhance information on management strategies towards reducing BRD risk during the feedlot period.

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Financial Support
USDA National Institute for Food and Agriculture

Notes:

246 - Comparison of 3 sampling methods for recovery of *Mannheimia haemolytica* from feedlot cattle

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Session: BOVINE RESPIRATORY DISEASE

Objective

Bovine respiratory disease (BRD), the most economically impactful disease of feedlot cattle, is caused by interactions between stressors and microbial pathogens. The current standard for antemortem recovery of pathogens uses guarded nasopharyngeal swabs to sample the nasopharynx. However, this can be technically challenging, costly, and waste generating. The objective was to compare recovery rates of *Mannheimia haemolytica* (Mh) by culture and qPCR using guarded deep nasopharyngeal swabs (DNPS), and 16" proctology swabs (PS) and less invasive 6" nasal swabs (NS), which are both less expensive than DNPS.

Methods

Samples were collected from two groups of beef steers and bulls (n=60 per group, mean weight=262.2 ± 12.5 kg). The 3 swabs were used to obtain samples through the left nostril, in random order, placed in Modified Ames Transport media, then streaked onto blood agar plates. At 24-48 hours, a colony with morphology and biochemical reactions consistent with Mh was selected from each plate and submitted for MALDI-TOF-MS identification, genotype classification, and antimicrobial susceptibility testing. McNemar's Chi-square test was used to compare results of Mh isolation between swab types for each animal, and logistic regression was used to determine effect on isolation results and group on antimicrobial susceptibility results.

Results

Mh was recovered from 69% (124/180) of swabs from the 1st group of cattle (42 NS, 40 DNPS, 42 PS), and 44% (79/180) of swabs from the 2nd group (26 NS, 27 DNPS, 26 PS); all isolates were Genotype 2. Nearly all isolates (200/203) were resistant to 2 drug classes (MDR); 3 isolates recovered from one animal were pansusceptible. The frequency of Mh isolation and recovery of MDR isolates was not statistically different for the different swab types ($P>0.05$).

Conclusions

All 3 swabs provided comparable results for Mh recovery from beef feedlot cattle. Use of nasal swabs or long proctology swabs can provide comparable results with less cost and waste than deep guarded swabs, and could improve producer willingness to participate in investigations of the ecology of BRD pathogens

Financial Support
Texas A&M University

Notes:

247 - The role of the global regulator Hfq and small RNAs in the regulation of virulence factors in *Histophilus somni*

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Session: BOVINE RESPIRATORY DISEASE

Objective

Histophilus somni is an important opportunistic pathogen responsible for bovine respiratory disease and other systemic diseases in cattle. Many virulence factors have been identified in *H. somni*, and these factors are tightly regulated. However, there is essentially no current information on *H. somni* regulatory mechanisms. Small RNAs (sRNA) have been reported in *H. somni* and are known to control the expression of genes, often in association with the global chaperone regulator Hfq. Our aim is to establish the role of Hfq in regulating virulence genes, and to identify and over-express the sRNAs that complex with Hfq in *H. somni* to determine their role in regulating virulence factors, particularly biofilm formation.

Methods

A DNA fragment containing the 5' region of *hfq* and upstream DNA, a kanamycin resistance cassette (Kan^R), and the 3' region of *hfq* and downstream DNA were prepared by fusion PCR, ligated into pLSGA-C-SK, and cloned into *Haemophilus influenzae*. This cloned region was also electro-transformed into *H. somni* strain 2336. Anti-Hfq IgG was purified by affinity chromatography from the serum of rats immunized with recombinant Hfq.

Results

A putative *hfq* mutant confirmed to contain Kan^R was isolated that grew slower than the parent, and is being extensively tested for genotypic and phenotypic properties. Recombinant *H. somni* His-tagged Hfq was over-expressed in *E. coli*, and used to obtain high-titer anti-Hfq IgG. The purified IgG will be coupled to magnetic beads to isolate Hfq-bound sRNAs from *H. somni* followed by identification of these sRNAs. The *H. somni* sRNAs will be over-expressed in a virulent *H. somni* strain to determine their phenotypic and genotypic effects.

Conclusions

This study will enable better understanding of the functional aspects of the global regulator Hfq and sRNAs in controlling the expression of *H. somni* virulence factors. The development of additional mutants or recombinant strains with altered regulation of key genes will facilitate the development of new and improved strategies to control diseases due to *H. somni*.

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

Notes:

248 - *In vitro* characterization of poly-microbial biofilms by bovine respiratory disease pathogens

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Session: BOVINE RESPIRATORY DISEASE

Objective

Bovine respiratory disease (BRD) is one of the most economically important problems affecting the bovine industry. Chronic BRD infections caused by bacterial agents are likely present as a biofilm, which enhances bacterial resistance to antibiotics and host defenses. To enhance treatment and prevention of BRD greater understanding is needed of how biofilms develop *in vivo*, how the bacteria interact in a poly-microbial biofilm, and the discovery of compounds that dissolve the biofilm matrix.

Methods

Biofilms were quantified by crystal violet staining. Fluorescence *in situ* hybridization was used to examine the interaction of *Histophilus somni, Pasteurella multocida*, and *Mannheimia haemolytica* in a poly-microbial biofilm. A capsule-deficient mutant was isolated following ethyl methane sulfonic acid mutagenesis and confirmed by electron microscopy. Purified polysaccharides were characterized by gas chromatography-mass spectrometry. Biofilm formation was examined on bovine cell lines, and on tissue culture-treated and non-treated plates (polystyrene, polyvinyl chloride, flat bottom, round-bottom, V-bottom).

Results

M. haemolytica formed a loosely attached biofilm. A distinct exopolysaccharide in the biofilm matrix was not identified, but a capsule-deficient mutant of *M. haemolytica* did form more biofilm than the wild-tpe. *M. haemolytica* did interact with *H. somni* and/or *P. multocida* in a poly-microbial biofilm. Biofilms were formed on bovine turbinate and pulmonary artery endothelial cells by all three species, but biofilm formation was much more substantial on turbinate cells. An initial screen of common compounds for biofilm removal indicated that basic and acidic amino acids were most effective, and of these arginine, as low as 10 mM, was most effective at removing the biofilm formed by all three species.

Conclusions

This project will aid us in understanding how BRD poly-microbial biofilms form and develop with the eventual use of 3-D tissue cultures, and identify compounds that can disrupt an established biofilm to enhance the efficacy of antibiotics and host defenses.

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

Notes:

249 - Progesterone stimulates bovine herpesvirus 1 gene expression and reactivation from latency.

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Session: BOVINE RESPIRATORY DISEASE

Objective

Bovine herpesvirus 1 (BoHV-1) infection of the ovary and/or fetus increase the incidence of reproductive failure in cattle. The goals of this study were to test whether progesterone and the progesterone receptor (PR) stimulate viral gene expression, productive infection, and trigger reactivation from latency in calves.

Methods

Transient transfection assays were used to examine regulation of key viral promoters with progesterone and PR. Chromatin immunoprecipitation (ChIP) studies were also used to test whether PR specifically bind viral promoters during productive infection or in transfected cells.
Calf studies were performed to test whether progesterone stimulated BoHV-1 reactivation from latency.

Results

PR and KLF4, a stress-induced cellular pioneer transcription factor, stimulated bICP0 E promoter activity and expression of productive infection in a cooperative manner. Chromatin immunoprecipitation studies demonstrated PR and KLF4 occupy bICP0 E promoter sequences in transfected Neuro-2A cells and at late times after productive infection of bovine kidney cells.
Since PR and the glucocorticoid receptor (GR) bind similar DNA sequences and the synthetic corticosteroid dexamethasone induces reactivation, we tested whether progesterone stimulated reactivation of heifers latently infected with BoHV-1. Strikingly, progesterone triggered viral reactivation from latency in all 4 heifers that were examined.

Conclusions

1. Progesterone and KLF4 or KLF5 cooperatively stimulate BoHV-1 productive infection and bICP0 E promoter activity.
2. Progesterone treatment triggered BoHV-1 reactivation from latency in four latently infected heifers.
3. The ability of progesterone and stress-induced transcription factors to stimulate BoHV-1 productive infection and key viral promoters directly correlate with the ability of progesterone to trigger reactivation from latency.

Financial Support
USDA National Institute for Food and Agriculture; Oklahoma State University; U.S. National Institutes of Health

Notes:

250 - Characterization of the respiratory microbiome and virome associated with bovine respiratory disease complex

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Session: BOVINE RESPIRATORY DISEASE

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)). To date, nasal swabs have been collected from herds in the US and Ireland for year one, and sampling has been initiated for year two in the US herd. During year one sampling of the US herd, a BRD outbreak occurred prior to weaning in a subpopulation of the herd.

Results

Evaluation of these samples revealed all calves were nasally shedding bovine coronavirus and a large percentage had a coinfection with *Mycoplamsa sp.*

Conclusions

Further evaluation of year one and year two samples will occur in 2020 and 2021.

Financial Support
USDA National Institute of Food and Agriculture

Notes:

251 - Applications of thoracic ultrasonography to evaluate progression and severity of bovine respiratory disease

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Session: BOVINE RESPIRATORY DISEASE

Objective

Bovine respiratory disease (BRD) is a complex syndrome that can cause severe or even fatal pneumonia in neonatal calves. While there are preventative measures against BRD, early diagnosis is crucial to success. Early detection of subclinical disease in calves is challenging, often causing delayed treatment and progression to severe clinical illness or reduced animal performance. BRD is commonly initiated by a primary viral infection associated with subclinical pneumonia, followed by a secondary bacterial infection, which can result in severe clinical disease. Recent research suggests that thoracic ultrasonography (TUS) can be used to improve early detection of subclinical pneumonia; however, it is unknown how TUS results correlate with other common parameters of disease progression (i.e., pathogen shedding, pathogen lung burden, clinical disease) when comparing viral versus bacterial pneumonia. Therefore, the study's objective was to compare the progression of lung disease, as measured by TUS, between viral and bacterial models of infection in young calves, and determine correlations between pathogen shedding and the manifestations of clinical disease and gross pathology results at necropsy.

Methods

Three-week-old calves were infected with either Bovine Respiratory Syncytial Virus (BRSV), a model of viral pneumonia, or Mannheimia haemolytica, a model of bacterial pneumonia. TUS videos were collected at designated time points following viral or bacterial challenge, and the disease progression was scored based upon the total area and location of pneumonic lesions. TUS results were correlated with daily clinical illness scores, viral or bacterial shedding determined by qPCR or quantitative culture, and gross lung pathology scoring.

Results

Results point toward a positive correlation between TUS, clinical observations, and pathogen shedding and burden, suggesting that TUS is a valuable tool for assessing disease presence and progression.

Conclusions

Thus, TUS may provide a rapid and reliable method for improving the diagnosis of subclinical pneumonia on-farm and increasing treatment success.

Financial Support
U.S. National Institutes of Health; USDA National Institute for Food and Agriculture

Notes:

252 - Replication of bovine herpesvirus 1.1 is influenced by co-infection with *Mannheimia haemolytica*

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Session: BOVINE RESPIRATORY DISEASE

Objective

The Bovine Respiratory Disease (BRD) is a multifactorial condition that affects cattle worldwide with high mortality and morbidity rates. The condition is caused by a combination of viral and bacterial and viral pathogens such as Bovine Herpesvirus-1 or Bovine Respiratory Syncytial Virus, among others, and comensal respiratory bacteria such as *Mannheimia haemolytica* and *Pasteurella multocida*. Triggered by a a variety of stressors, these pathogens infect and immunosuppress animals to ultimately develop severe pneumonia. Much of previous research has focused on individual/combination of pathogens and their effect on host gene expression. Instead, our work has focused on studying these pathogens in co-infection to establish how they affect each other's metabolism.

Methods

Bovine herpesvirus type 1.1 (BoHV-1) and *Mannheimia haemolytica* were used to co-infect bovine tissue cultures at varying times and multiplicity of infection. Microbial replication, culture conditions and host cell viability were assessed.

Results

When bovine cells were co-infected with BoHV-1 and increasing doses of *M. haemolytica*, infectious virus production decreased in a dose-dependent manner. However, when higher viral doses were used for the co-infection, viral replication was not affected. Similar results were observed in respiratory-derived cultured cells. The acidification caused by bacterial growth did not severely affect host cell viability, yet it had an effect on viral infectivity. Both timing of infection and microbial density impacted this observation. Other pathogen combinations are also being assessed in ongoing experiments.

Conclusions

Preliminary conclusions suggest BoHV-1 replication program can be adversely affected by co-infection of *M. haemolytica.*

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

Notes:

253 - Blood biomarker discovery in high-risk stocker cattle at-arrival: differentiating respiratory health and disease

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Session: BOVINE RESPIRATORY DISEASE

Objective

Complex immunological interactions and genomic mechanisms underlining bovine respiratory disease (BRD) risk have been investigated using RNA sequencing (RNA-seq). These studies are typically performed with single populations and findings remain controversial. Our objective is to classify and validate BRD-associated genes and mechanisms from blood samples across multiple independent populations of post-weaned beef cattle.

Methods

At-arrival blood was collected from 48 calves in two independent populations; each population (n=24) included twelve cattle not developing BRD and twelve cattle diagnosed with BRD within 14 days of arrival. Gene counts from sequenced reads (Novaseq 6000; ~50M paired-end reads/sample) were assembled in a HISAT2/Stringtie/prepDE pipeline. Samples were categorized into severity categories (healthy, treat_1, treat_2+) based on frequency of antimicrobial treatment and mortality. Differentially expressed genes (DEGs; FDR<0.05) were identified with edgeR glmLRT testing. Data visualization and dimensional reduction analysis was performed in R. WebGestalt and STRING were utilized for downstream functional analysis.

Results

132 DEGs were identified across all groups. Principle component correlations with disease severity, population (year), and weight gain were identified. Genes involved with lipid metabolism, inflammatory downregulation, and T-cell survival were DE in healthy cattle. Genes involved with neutrophil activation, antimicrobial defense, and cellular cornification were DE in treat_1 cattle, and those involved with nitric oxide production, ligand scavenging, and alternative complement activation were DE in treat_2+ cattle.

Conclusions

Several DEGs identified corroborate findings from other bovine transcriptome analyses; these could serve as candidate biomarkers for BRD prediction. This study provides information relevant for developing novel diagnostic and therapeutic modalities to decrease BRD impact.

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

Notes:

255 - Analysis of TLR expression in the macrophages derived from different hosts of the stimulation with *Brucella canis*

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Session: BRUCELLOSIS

Objective

Due to importance of the disease in both public health and clinical fields, a lot of researches have been conducted to understand the immunopathological mechanism of *Brucella canis* infection. However, the previous mechanism related with the infection is still remained to be solved. In addition, there are reports of differences in immune response why *B. canis* infection has been known compared with other *Brucella* species. In this study, an analysis was conducted on the TLR expression patterns in the different hosts.

Methods

Therefore, the time-dependent expression patterns of Toll-like receptor (TLR)s were analyzed with immune cells originated three different hosts; THP-1: human cell line, Raw 264.7: mouse cell line, DH82: canine cell line. DPBS was used as control and TLR expression patterns were analyzed over time. The expression of TLR was analyzed through real-time time PCR samples of 2-hours, 12-hours, and 24-hours after infection of *B. canis* in each cell line.

Results

The expression patterns of TLR showed higher different depending on the host. Even though time-dependent expression were also observed. In THP-1 cells, TLR 1, 4 and 8 were expressed by the stimulation with B. canis in cells while expression of TLR 9 and 10 were induced at 24-hours of the stimulation. Only TLR 1 and 3 were expressed in Raw 264.7 cells, at 2-hours after the stimulation. TLR 3, 6 and 9 were expressed all the times in DH82 cells. In addition, TLR7 was significantly increased at 24-hours after stimulation with *B. canis*.

Conclusions

In the infection of *Brcella* spp., TLR is playing an important role in the immune response of the host. These results indicated species and time difference in the expression of TLRs after stimulation with *B. canis*. Also, important roles of TLR7 in the natured host were suggested in *B. canis* infection.
This work was carried out with the support of CCRC (Project No. PJ013985) RDA.

Notes:

257 - Incidence of *Brucella* spp. in various livestock species raised under the pastoral production system in Kenya

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Session: BRUCELLOSIS

Objective

Brucellosis is an important zoonosis with a worldwide distribution. The disease is caused by multiple species of *Brucella* that can infect a wide range of mammalian hosts. In sub-Saharan Africa, many studies have been implemented to determine the prevalence of the disease in livestock, but not much is known about its incidence. We implemented a longitudinal study to determine the incidence of *Brucella* spp. infection in cattle, camels, sheep, and goats that were being raised in a pastoral area in Isiolo County, northern Kenya.

Methods

An initial cross-sectional survey was implemented to identify unexposed animals for follow up; that survey used 141 camels, 216 cattle, 208 sheep and 161 goats. A subsequent longitudinal study recruited 31 cattle, 22 sheep, 32 goats and 30 camels for follow up. All the samples collected were screened for *Brucella* spp. using the Rose Bengal Plate test (RBPT), a modified RBPT, and an indirect multispecies Enzyme-Linked Immunosorbent Assay (iELISA) kit. Samples that tested positive by any of these serological tests were further tested using real-time PCR-based assays to detect genus *Brucella* DNA and identify *Brucella* species. These analyses targeted the *alkB* and *BMEI1162* genes for *B. abortus*, and *B. melitensis,* respectively. The longitudinal study took 12 months and data were analysed using Cox proportional hazards model that accounted for the clustering of observations within herds. Changes in anti-*Brucella* IgG optical values between successive sampling periods were determined to confirm primary exposures.

Results

The mean incidence rate of *Brucella* spp. was 0.024 (95% confidence interval [CI]: 0.014 – 0.037) cases per animal-months at risk. *Brucella* spp. incidence in camels, cattle, goats and sheep were 0.053 (0.022 – 0.104), 0.028 (0.010 – 0.061), 0.013 (0.003 – 0.036) and 0.006 (0.0002 – 0.034) cases per animal-month at risk, respectively. A higher incidence rate of *Brucella* spp. was found among females (0.020, 0.009 – 0.036) than males (0.016, 0.004 – 0.091), while young animals (0.026, 95% CI; 0.003 – 0.097) had a slightly higher incidence rate compared to adults (0.019, 95% CI; 0.009 – 0.034). Real-time PCR analyses showed that *B. abortus* was more prevalent than *B. melitensis* in the area. The results of multivariable Cox regression analysis identified species (camels and cattle) as an important predictor of *Brucella* spp. exposure in animals. On the diagnostic tests, modified RBPT provided similar findings as the iELISA test.

Conclusions

Our findings indicated that camels and cattle have a higher incidence of *Brucella* spp. exposure compared to the other livestock species. This could be due to the higher prevalence of *B. abortus*, which readily infects these species, than *B. melitensis*. More studies are underway to identify ecological factors that influence the persistence of the key *Brucella* species in the area. The study further concluded that the modified RBPT test can give reliable results as those of a formal iELISA test, and it can, therefore, be used for routine surveillance in the region.

Financial Support
U.S. Defense Threat Reduction Agency

Notes:

258 - Immune responses and efficacy of two different vaccination strategies in elk against brucellosis

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Session: BRUCELLOSIS

Objective

Elk (*Cervus elaphus*) in Yellowstone National Park and surrounding areas demonstrate high levels of seroprevalence for brucellosis. During winter months, elk may enter areas where they can transmit brucellosis to domestic livestock. Developing a successful vaccination strategy for elk would decrease the risk of brucellosis spillover to livestock in affected areas. We have previously demonstrated that elk predominantly develop humoral responses after vaccination, which are not protective against intracellular bacteria. In the current study, we sought to test the ability of two vaccination platforms in providing protection against brucellosis in elk. .

Methods

We utilized a live modified, *Mannheimia hemolytica* recombinant vaccine expressing *Brucella* outer membrane proteins, administered mucosally, and a parenterally-delivered, polymer-based vaccine containing killed RB51. Immune responses were compared to those of non-vaccinated animals and animals vaccinated with 10^{10} CFU of RB51 parentally. To determine protection, vaccinated animals were bred and then challenged in mid-gestation with 10^7 CFU of *Brucella abortus* strain 2308. Bacterial loads were determined from tissue samples collected at necropsy after parturition or abortion.

Results

Vaccinated elk demonstrated humoral responses after vaccination that were greater ($P > 0.05$) than responses of elk in the control treatment. None of the control elk (n=5) aborted, whereas 1 abortion occurred in the parenteral RB51, polymer-based vaccine, and *Mannheimia* vaccine groups (n=5, 6, and 8, respectively). Infection rates in mammary, fetal, or maternal tissues did not differ between treatments.

Conclusions

Overall, our data indicate that both vaccination strategies can effectively deliver antigens, but continued modification will be required for development of a highly efficacious brucellosis vaccine for elk.

Financial Support
U.S. Department of Agriculture

Notes:

259 - Studies on intra-ocular vaccination of adult cattle with reduced-dose *Brucella abortus* strain-19 vaccine

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Session: BRUCELLOSIS

Objective

Brucella abortus vaccines play a central role in bovine brucellosis control with tremendous success worldwide for decades. The study was aimed to evaluate the efficacy of reduced dose ($5.0x10^9$ cfu) of S19 vaccine in adult cattle and its shedding in the milk of vaccinated cattle using molecular techniques

Methods

The OIE recommended tests (RBPT, SAT, and iELISA) for brucellosis screening in cattle were used. Seronegative cattle (n=90) of different age groups (n=30 each) were selected for the vaccine trials. Antibody titers were recorded at 7th, 21st, 30th, 60th, 90th and 120th days post-vaccination (DPV) to monitor the immune responses following vaccination and subsequent booster dose to an intraocular group at 150th, 180th, 210th, and 240th days post-booster (DPB). Molecular detection techniques employing bcsp-31 and IS711 targeted PCR and Taqman assay qPCR was used to confirm the shedding of *Brucella abortus* S19-vaccine in milk from all the groups

Results

The humoral immune responses observed by RBPT and ELISA proved that antibody titers persisted in the subcutaneous group compared to the intraocular group in all categories. The IFN-γ stimulation (CMI) due to reduced dose vaccination was noticed early as 30th in all groups and declined after 90th DPV, with higher IFN-γ stimulation among the subcutaneous group. *Bcsp31* gene revealed the presence of *Brucella* DNA in milk samples (n=120) from the vaccinated cows (n=30) a t 30PV, 60PV, 90PV, and 120PV DPV and Confirmed by *IS711* targeted PCR and qPCR (TaqMan assay)

Conclusions

A Significant number of 70% (7/10) *Brucella* DNA was detected in the subcutaneous group by the qPCR. *Bcsp31* sequences were deposited at NCBI GenBank (accession no. MK881173-6). The PCR and qPCR techniques could provide reliable diagnoses of brucellosis from milk. The intraocular remains the safer route for vaccinating adult cattle than the subcutaneous route.

Financial Support
Indian Council of Agricultural Research

Notes:

260 - A new drug to fight Brucellosis?

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Session: BRUCELLOSIS

Objective

Brucellosis is an infectious zoonosis caused by bacteria of the *Brucella* genus. These pathogens can infect a wide range of mammals (mainly cattle, goat and swine). In animals, the main symptoms of the disease are abortions and perinatal death followed by chronic infection, causing huge economic losses in livestock. Bacteria can be transmitted to humans upon consumption of unpasteurized milk products, direct contact with tissues/secretions of infected animals or aerosolization. The human disease presents as a flu-like syndrome with a characteristic undulating fever and can lead to severe complications if untreated. More than 500,000 new human cases are reported each year worldwide. No vaccination is available for humans to date, and the current treatment consists of a double antibiotherapy (rifampicin + doxycycline) for at least 6 weeks. However, this treatment has many side effects and relapses can occur. In order to try improving the management of human brucellosis, we adopted a drug repositioning strategy and tested the ability of already existing drugs to inhibit the intracellular replication of these bacteria, which is a key aspect of their pathogenicity.

Methods

We performed *in vitro* infection of cultured cells (human trophoblasts, murine macrophages) treated by different drugs. We then measured bacterial intracellular replication using gentamycin protection assays and different readouts.

Results

We performed *in vitro* infection of cultured cells (human trophoblasts, murine macrophages) treated by different drugs. We then measured bacterial intracellular replication using gentamycin protection assays and different readouts.

Conclusions

These results are very promising and could provide new tools to fight *Brucella* infections.

Notes:

261 - A multiplex immunochromatogarphic lateral flow assay for detection of ESBL and carbamenemase-producer bacteria

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Session: DIAGNOSTIC TESTING

Objective

Carbapenemase and ESBL-producer pathogenic bacteria pose a global threat to one-health because they are also resistant to other multiple antimicrobial classes. We evaluated the sensitivity and specificity of a rapid multiplex antibody based immunochromatogarphic lateral flow assay for detection of ESBLs and carbamenemases-producing *E.coli* in dogs using disk diffusion and selective CHROMAgar as a reference.

Methods

We isolated 21 ESBL-*E.coli* from feces of 92 dogs in Long Island and obtained 14 non-ESBL *E.coli* from Pennsylvania State University. For quality control, we used 2 carbapenemase, 2 ESBL, and 2 non-ESBL ATCC isolates. CTX-M lateral flow assay was compared with CHROMAgar ESBL and cefotaxime (CTX) and ceftazidime (CFZ) alone or with clavulanic acid (CA). CARBA-5 (KPC, OXA, VIM, IMP, and NDM) multiplex lateral flow assay was compared with mSuperCARBA and disk diffusion of meropenem (MEM) or MEM + other compounds. The sensitivity (Se), specificity (Sp), test agreement (Kappa), ROC curve and area under ROC curve (AUC) of the anti-CTX-M lateral flow assay was compared with selective CHROMAgar, disk diffusion and PCR.

Results

CTX-M (anti-ESBL$_{CTX-M}$ antibody) based lateral flow assay in reference to CHROMAgar ESBL had Se = 100%, Sp = 85%, and Kappa = 0.856 (p=0.000). CTX-M had Se = 100%, Sp = 75%, and Kappa = 0.759 (p=0.000) in reference to CTX disc. CTX-M had Se = 81.8%, Sp = 90%, and Kappa = 0.715 (p=0.000) in reference to CTX+CA disc. CTX-M in reference to CFZ disc had Se = 90.9%, Sp = 80%, and Kappa = 0.712 (p=0.000). The AUC of anti-CTX-M positive explained by CTX, CTX+CA, CFZ, and, CFZ+CA was 97.5% (95%CI 93.6 – 100%; p=0.000), 84.5% (95%CI 70.7 – 98.4%; p=0.000), 90.5% (95%CI 79.6 – 100%; p=0.000), and 91.6% (95%CI 81.7 – 100%; p=0.155), respectively. 85% (17/21) of them had CTX-M by PCR. None of them produced carbapenemase apart from ATCC isolates. Like MEM discs and mSuperCARBA agar, the CARBA-5 lateral flow assay detected KPC and NDM in the ATCC isolates correctly.

Conclusions

Anti-CTX-M lateral flow assay had very good Se and Sp versus CHROMAgar, disk diffusion and PCR as a reference.

Notes:

262 - Application of pathogen-specific biomarkers to enhance specificity of bovine TB diagnosis

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Session: DIAGNOSTIC TESTING

Objective

Bovine tuberculosis (bTB) is a zoonotic disease that is primarily transmitted from cattle to humans. This requires its control in animals to be of foremost importance to eliminate public health impact. Using validated *Mycobacterium bovis*-specific biomarkers, we aim to develop a rapid, inexpensive, yet highly specific detection platform, which is the need of the hour.

Methods

Previously, 32 host proteins and 16 *M. bovis* specific proteins were identified as biomarkers of bTB. Of these, 3 *M. bovis* proteins-Pks5, MB2515c, MB1895c, were selected for high-precision DNA ligand (aptamer) selection. Two short regions per biomarker were selected as targets for aptamer selection. These short peptides had high specificity to the *Mycobacterium tuberculosis* complex and predicted antigenicity by MHCpanBoLA server. A combinatorial aptamer library and one-step selection was used to identify Pks5-binding aptamers. Validation of selected aptamers was performed against larger Pks5 peptides.

Results

Selection resulted in 4 redundant pairs of anti-Pks5 aptamers. Of these, 2 were selected based on their predicted 3-dimensional structure, high GC content and low enthalpy that suggested stronger binding of the aptamers with target peptides. Selected aptamers were biotinylated and tested against the target peptides using dot blots followed by ELASA. The tests lacked sensitivity for the detection of aptamer-peptide binding. A sandwich ELISA using monoclonal anti-Pks5 antibodies from our earlier work suggested that the peptides' small size confounded effective plate-binding, likely impairing aptamer binding. Thus, longer Pks5 peptides were expressed in *E. coli* BL21(DE3) and purified. Dot blot assays revealed that both selected aptamers bound 100ng of expressed Pks5 at 1000nM concentration.

Conclusions

The aptamers will be further validated with field animal TB serum samples before their application for diagnostics, ultimately leading to development of a field diagnostic device to test for bovine tuberculosis.

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

Notes:

263 - ELISA protocol for novel caprine coronavirus and serologic survey of exposed goat herds in Northern California.

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Session: DIAGNOSTIC TESTING

Objective

Coronaviruses cause respiratory and gastrointestinal disease in a range of animals including humans, cattle, small ruminants, other mammals, turkey, and other avian species. A novel goat coronavirus was identified in 2017, associated with an outbreak of respiratory and gastrointestinal disease in show animals in Northern California. Sequencing revealed a novel coronavirus closely related to bovine coronavirus. The first objective of this study was to adapt a bovine coronavirus ELISA protocol for the novel goat coronavirus. The ELISA was then used to characterize seropositivity in exposed herds and individuals over time.

Methods

A bovine coronavirus ELISA kit (Svanovir BCV-Ab, SVANOVA Biotech), was adapted using a monoclonal anti-goat IgG antibody (mouse anti-goat IgG-HRP, Santa Cruz Bio tech). Cut point was established by using the mean plus 3x SD of negative controls.
Serum from three exposed goat herds (including herds with PCR positive animals in 2017) collected in 2016, 2017, 2018, 2019 and 2020 were analyzed with the developed ELISA protocol, to determine seropositivity in the herds and trends over time.

Results

The percentages of seropositive individuals in three exposed goat herds from Northern California in 2017, 2018, 2019, and 2020 were determined (9-100%), and there was an increase in the percent of seropositive animals within the herds over time. Serial samples from individual goats also showed and an increase in individual titers over time.

Conclusions

An ELISA to detect antibodies against a novel caprine coronavirus was successfully adapted from a commercial bovine coronavirus assay. Herd percentages of seropositive individuals indicate that this coronavirus is a significant pathogen within the Northern California show goat population. Furthermore, tracking changes in individuals and herds with time showed that individuals maintain antibodies against this novel coronavirus for years. Further research needs to be done to determine whether this is due to the virus circulating within the herd or re-exposure to subclinical individuals during show season.

Financial Support
USDA Animal Health Formula Funds

Notes:

264 - Development of a PCR screening test for *Mycobacteria tuberculosis* in Elephant Trunk Washings

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Session: DIAGNOSTIC TESTING

Objective

Develop a PCR method to detect *Mycobacteria tuberculosis* (M. tb) complex organisms in trunk washes to enable infected elephants to be detected at an earlier time point and lower cost.

Methods

Samples from captive elephants submitted for routine M. tb screening were tested in parallel by PCR and culture after obtaining consent from submitters. Over 2000 samples were tested from 87 elephants (African and Asian). Trunk wash samples were mixed and debris allowed to settle for 15-30 minutes. Supernatant was removed and a sample for PCR taken from the debris that settled (sediment). The supernatant was centrifuged to pellet any bacteria and another sample was taken for PCR from the pellet (inoculum).

Results

The PCR method detected 42/43 M. tb culture positive trunk washes. To determine if the assay could be reduced from two PCR reactions to one, PCR of the sediment and inoculum were considered separately. If PCR was performed only on the inoculum, 40/43 culture positive trunk washes were detected compared to 40/43 using PCR on the sediment. Each method detected 1 sample that was missed by the other, resulting in better detection when both PCR reactions are run.

Conclusions

Culture is the industry standard for diagnosis of M. tb in elephants, but trunk wash samples are heavily contaminated with bacteria, fungus, as well as nontuberculous mycobacteria (NTM). To remove contaminating bacteria and fungi, the samples undergo a 2 day decontamination process. Identification and differentiation of NTM from M. tb lengthens the time from sample submission to report of results. Further determination of elephant tuberculosis status is in process to determine the sensitivity and specificity of the PCR test on the US captive elephant herd with a goal to replace annual trunk wash cultures with the PCR and only culturing when M. tb DNA is detected in the trunk wash sample. Using PCR as a screening test will allow for a more rapid result for elephant caretakers thus allowing tuberculosis control methods to be implemented sooner.

Financial Support
U.S. Department of Agriculture

Notes:

265 - MinION-based RACE-Seq of infectious bronchitis viruses from tissues and FTA cards

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Session: DIAGNOSTIC TESTING

Objective

Infectious bronchitis virus (IBV, species: *Avian coronavirus*) is the cause of infectious bronchitis, which is one of the most important and economically devastating diseases of poultry and has a diverse range of clinical signs. Similarly, IBV has a diverse genetic background that manifests in the S1 subunit of the spike gene, leading to the emergence of new serotypes and genotypes. For this reason, the, S1 is commonly used for IBV genotyping and lineage typing (i.e., subgenotyping). Therefore, accurate IBV genotyping is an important step IBV identification, vaccine planning, and tracking of this global virus. While the diversity of the S1 is important for defining genotypes, it also means that mutations may interfere amplification. The objective of this study was to develop a RACE (rapid amplification of cDNA ends)-based method to detect and characterize IBV from diagnostic samples.

Methods

Total RNA was extracted from trachea, lungs, cecal tonsils, and a Flinders Technology Associates (FTA) card using routine diagnostic methods. IBV RNA was reverse transcribed in a strand switching reaction, followed by amplification in a RACE PCR and the MinION PCR-based barcoding PCR. MinION libraries were multiplexed and synthesized using standard protocols. Raw reads were basecalled, demultiplexed, and taxonomically classified using a desktop PC.

Results

IBV RACE-Seq was able to accurately identify varying IBV lineages in tissues, including cecal tonsils, and in the FTA card sample. The FTA card sample was a sample in which other sequencing methods, including MinION amplicon sequencing failed.

Conclusions

The results demonstrate the utility of using RACE-Seq with the MinION for the identification and characterization of IBV, including a case in which other sequencing methods were inadequate.

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

Notes:

266 - A Rapid Test to Monitor the Immune Status Change and Disease Risk of Dairy Cows during the Periparturient Period

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Session: DIAGNOSTIC TESTING

Objective

During the periparturient period, from approximately 30 days before calving to 30 days postpartum, the immune system of the dairy cows undergoes a multitude of changes to prepare for parturition, colostrum production and lactation. One such change is the transfer of a large amount of immunoglobulin G proteins, especially subclass IgG1 to colostrum, leading to a reduction of IgG1 in the blood. As IgG1 and IgG2 need to maintain a balance to protect animals from intracellular and extracellular pathogens, a disruption of this balance compromises the immune protection of the animals. Rapid tests that can detect these immunological changes may be potentially useful for predicting the risk of dairy cows developing infectious diseases and other adverse health conditions following parturition.

Methods

We report here a new rapid test to detect certain immune status changes in the blood serum of dairy cows. This test uses a nanoparticle probe to evaluate the relative quantity of IgG1 and IgG2 in a sample. The nanoparticle probes are aggregated together upon interaction with bovine IgG2, while bovine IgG1 inhibits such interactions. The nanoparticle aggregates are detected by monitoring the color change of the assay solution using a handheld device.

Results

We tested the serum samples from 230 dairy cows collected during periparturient period, from 14-7 days before calving to 7-14 days postpartum. Results show that the test clearly detected an immune status change associated with IgG1/IgG2 relative quantity change around the time of parturition (P value = 0.001). Data analysis using mixed liner model in SAS (Statistical Analysis System) revealed a significant difference (P value = 0.042) in their test responses between healthy cows and cows with mastitis and/or lameness.

Conclusions

The new rapid test we report here can be used to detect and monitor certain immune status change in dairy cows during the periparturient period. The test results may be potentially used to evaluate and predict the health risk of the dairy cows following parturition.

Notes:

267 - Development of capture antigens & sensor functionalization procedures for viral respiratory bovine health screening

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Session: DIAGNOSTIC TESTING

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 simultaneous on-farm screening and detection of bovine viral respiratory infections of key importance.

Methods

Recombinant BPI-3 hemagglutinin-neuraminidase, BVDV non-structural protein 3 and BRSV fusion protein viral capture antigens for application within immuno-based diagnostic analyses were designed based on selected consensus sequences of common circulating strains, and synthesized within baculovirus expression vector systems. To reduce potential cross-reactivity to non-viral antibodies, proteins were expressed in Sf9 cells and Hi-5 insect cells providing for variation in glycosylation profiles. Western Blot and ELISA analyses were applied to characterize the efficacy of antigen synthesis and to assess protein immune-specificity and antibody cross-reactivity. Surface Plasmon Resonance was used to develop capture antigen functionalization approaches.

Results

Immunoassays were developed using expressed recombinant antigens and compared to ELISA, demonstrating comparable test performance. Potential to use assays to measure IgM specific responses as early infection markers was also investigated through calve antibody level profiling. To facilitate translation to on-farm diagnostic tests, approaches were investigated utilizing the interaction of terminal His-tag regions incorporated within synthesized viral proteins to functionalize sensor surfaces with antigen and facilitate epitope recognition to maximize antigen-antibody interactions. Covalently-bound capture antigen layers formed through interaction of sensor surface associated cobalt ions with protein His-tag regions stabilized via oxidative processes were shown to offer potential to develop reusable sensor assay tests.

Conclusions

Recombinant bovine respiratory viral proteins have been produced for antibody screening assays and alternative immobilization procedures developed enhancing levels of capture antigen functionalization of sensor chip surfaces.

Financial Support
USDA National Institute of Food and Agriculture

Notes:

268 - Inducing *Streptococcus suis* disease in nursery piglets challenged by serotype 2

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Session: DISEASE PATHOGENESIS

Objective

The objective of this study was to induce clinical signs of *S.suis* infection in nursery piglets challenged by *S.suis* serotype 2.

Methods

Twenty-four, 3-week old pigs were transported to Animal Isolation Facility and assigned to 1 of 4 different groups including oral, intranasal, combination (oral and nasal) challenge and control groups. Pigs were challenged 6 times over a 6-week period (3 consecutive days 3 and 4 weeks post weaning) with serotype 2. Prior to challenge nostrils were treated with 1% acetic acid. Throughout the trial health parameters and rectal temperature were recorded, and nasal, tonsil and rectal swabs taken. Pigs showing clinical signs of *S.suis* disease were euthanized and samples from meninges, blood, spleen and ileum collected. At the end of the trial all remaining pigs were euthanized, and tonsil tissue and ileum samples collected. All samples were tested for presence of *S.suis* and isolates were serotyped by multiplex PCR. A linear regression analyzing method was used to analyze differences in rectal temperature.

Results

Five pigs (3 in combined, 1 in nasal, 1 in control group) developed clinical signs and were euthanized. Serotype 2 was recovered from blood, meninges and spleen of those pigs except the pig in the control group. It was also recovered from tonsil tissue of a euthanized pig in the combination group. In the oral treatment group serotype 2 was found in the tonsil tissue of 2 pigs with no clinical signs of infection. Serotype 16, 21, 29, 31 and untypable isolates were recovered from non-systemic sites in most pigs. Two days after the 1st challenge, rectal temperature of pigs in the combination group was higher compared to pigs in the oral group ($P<0.05$). Eight days after the 6th challenge, rectal temperature of pigs in the combination group was higher compared to pigs in the nasal group ($P<0.05$).

Conclusions

Clinical signs of *S.suis* disease could be induced in piglets with oral/nasal inoculation as the most successful method. Serotype 2 did not colonize the tonsils or nasal cavities of most pigs however non-systemic sites were colonized with other serotypes.

Notes:

269 - Host-virus interactions mediating Equine Arteritis Virus persistence in the stallion reproductive tract

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Session: DISEASE PATHOGENESIS

Objective

Alphaarterivirus equid (previously known as equine arteritis virus [EAV]) can establish long-term persistent infection (LTPI) in the reproductive tract of stallions. Recent studies showed that long-term persistence is associated with a specific allele of the *CXCL16* gene (*CXCL16S*). Our objective is to understand the molecular mechanisms that drive EAV persistence in the stallion reproductive tract.

Methods

The *in vivo* immunological milieu during long-term EAV persistence was comprehensively characterized via transcriptomic analysis, single/dual immunostaining, *in situ* hybridization (RNAscope® and miRNAscope®), and exosomal miRNA sequencing.

Results

We demonstrated that the ampulla is the primary tissue reservoir for EAV during LTPI, with viral specific tropism for $CD8^+$ T and $CD21^+$ B lymphocytes but not glandular epithelium. Furthermore, EAV persistence is associated with a significant mucosal antibody and local inflammatory response characterized by diverse mucosal anti-EAV-specific immunoglobulins (IgA, IgG1, IgG3/5, and IgG4/7) with variable neutralizing efficacy; and significant infiltration of T lymphocytes (mainly $CD8^+$ and lower numbers of $CD4^+$/$FOXP3^+$ T lymphocytes), Iba-1^+ macrophages and plasma cells (IgA^+, $IgG1^+$, and $IgG4/7^+$). Transcriptome analysis revealed a $CD8^+$ T lymphocyte transcriptional profile with upregulation of inhibitory receptors, homing chemokines (including CXCL16/CXCR6), and specific transcription factors (EOMES, PRDM1, NFATC2, TBX21) with the predominance of $EOMES^+$ and $NFATC2^+$ lymphocytes. Finally, the downregulation of seminal exosome-associated miRNA eca-mir-128 inversely correlated with an enhanced expression of CXCL16 (a putative target of eca-mir-128) in infiltrating lymphocytes and lining epithelium. Expression of eca-mir-128 in the ampulla was overall very low and limited to the luminal epithelium.

Conclusions

The findings presented herein suggest that complex host-pathogen interactions shape the outcome of EAV infection in the reproductive tract and that the CXCL16/CXCR6 axis is strongly implicated in EAV persistence.

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

Notes:

270 - Experimental Infection of Specific-Pathogen-Free Domestic Lambs with *Mycoplasma ovipneumoniae*

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Session: DISEASE PATHOGENESIS

Objective

Mycoplasma ovipneumoniae (*M. ovi*) is an opportunistic respiratory pathogen that is prevalent in domestic sheep herds. *M. ovi* causes mild to moderate symptoms in domestic lambs, asymptomatic infections in adult domestic sheep, but lethal illness in bighorn sheep. Why *M. ovi* causes such variable symptomology is not fully understood. To address this issue, we established a specific pathogen free (SPF) and immunologically naive herd of domestic lambs for experimental *M. ovi* infection.

Methods

Thirty lambs were separated from *M. ovi* infected ewes at birth and raised in an ABSL-2 facility on colostrum replacer and milk replacer. To confirm absence of *M. ovi* and *Pasteurellaceae* infection, nasal swabs were tested by PCR and bacterial culture, respectively. At 4 months of age, 4 lambs were inoculated with *M. ovi* positive nasal wash fluid; 4 lambs were inoculated with PBS. All lambs were monitored for symptoms for 2 months and were tested weekly for *M. ovi*-specific antibody levels and colonization with *M. ovi* and aerobic bacteria.

Results

Survival rate of SPF lambs raised without their mothers was 94%. Effective passive transfer of antibodies from the colostrum replacer was shown by serum antibody ELISA. Average daily gain for all lambs for the first 8 weeks was 0.78 lbs/day. All lambs tested negative for *M. ovi* on days 1 and 30 after birth. *M. ovi* infection was successfully established in the 4 inoculated lambs and peaked at 2 weeks before decreasing and plateauing. All infected lambs mounted an antibody immune response that peaked at 4 weeks. Despite the infection and antibody response, no lambs exhibited symptoms.

Conclusions

Supervised lambing followed by artificial rearing generated a herd free from and immunologically naïve to *M. ovi* and *Pasteurellaceae*, closely mimicking the situation in bighorn sheep. When challenged with *M. ovi*, colonization was established and the lambs mounted an immune response, although they did not develop clinical symptoms. Our results indicate that *M. ovi* alone is not sufficient for causing respiratory disease in otherwise healthy domestic lambs.

Financial Support
U.S. Department of Agriculture

Notes:

271 - The role of outer membrane proteins in attachment and pathogenicity of *Fusobacterium necrophorum*

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Session: DISEASE PATHOGENESIS

Objective

Fusobacterium necrophorum is a Gram-negative, obligate anaerobe that causes necrotic infections in animals and humans. This bacterium causes liver abscess, foot rot, and calf diphtheria in cattle resulting in a high financial impact on the feedlot industry. Currently, antibiotic administration is the main measure to control infections, however, resistance is inevitable. Attachment to the host cell is a known key step in the pathogenesis of most Gram-negative bacteria. The role of the outer membrane proteins (OMPs) in the attachment and pathogenicity of *F. necrophorum* has not been thoroughly studied.

Methods

High binding affinity adhesins were identified by binding assays and pull-down assays with bovine endothelial (EJG) cells; were sequenced and cloned in the expression plasmids. The binding assay identified four adhesins with high binding affinity, 17, 24, 40, and 74kDa protein. The current study focused on 17 and 24kDa OMPs and were identified as OmpH and OmpA family protein, respectively. The recombinant proteins were purified, and polyclonal antibodies were generated against these putative proteins. The effects of these polyclonal antibodies in inhibiting the bacterial binding to the cells were studied through adhesion inhibition assay, *in-vitro*.

Results

Our results showed that individual polyclonal antibody treatment exerted a decreasing, but not statistically significant, trend in bacterial adhesion inhibition. In contrast, the treatment in combination (17 & 24kDa) showed a significant decrease in bacterial adhesion to the host cells. Furthermore, by pull-down assay, we identified another OMP, a 67kDa protein, which can be further studied to identify its role in pathogenicity.

Conclusions

We identified OMPs with potential roles in bacterial attachment to the host cell. These proteins could serve as a target for directed therapy, or for the development of vaccines to curb bacterial attachment and hence avert the disease. Further investigation is required to confirm the role of OMPs *in vitro* and *in vivo* and to develop OMP-based vaccine against fusobacterial infection.

Financial Support
Purdue University

Notes:

272 - Genomic screens to identify causative polymorphisms accounting for Marek's disease genetic resistance in chicken

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Session: DISEASE PATHOGENESIS

Objective

Marek's disease (MD), a lymphoproliferative disease of chickens caused by the highly pathogenic Marek's disease virus (MDV), is the most serious chronic disease problem that costs the worldwide poultry industry ~$2 billion per year. Despite widespread use of vaccines, more virulent MDV strains have repeatedly arisen. Consequently, alternative control methods, especially improving MD genetic resistance, are needed and highly desired.

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 will identify candidate regulatory elements that contain the causative polymorphisms. In Experiment 1, we use splenic-derived lymphocytes from uninfected and MDV-infected experimental chickens to reveal promoters and/or enhancers with specific transcription factors (TF) motifs that regulate gene expression in response to viral infection. In Experiment 2, the same design will be conducted except MDV will lack Meq, the viral oncogene and a bZIP transcription factor. Results from this experiment should help identify genes that are regulated by Meq. In Objective 3, we validate our experimental predictions by screening key regions in progeny-tested commercial layer sires.

Results

All samples have been collected, and Hi-C and RNA seq datasets have been generated. Despite clear differential gene expression and clustering samples based on this information, initial visualization of the Hi-C results do not indicate clear differences in topologically associating domains (TADs) between sample groups. Other efforts to further interrogate the results have been on hold due to the pandemic; ARS has not allowed any work since mid-March on this project.

Conclusions

None yet as the experiments are still underway and not proceeding as planned. As a contingency, once we're approved to come back into the facility, we will run a number of more simpler samples, e.g., uninfected and MDV infected chicken embryo fibroblasts.

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

Notes:

273 - Endotoxin enhances lipolytic responses and impairs insulin sensitivity in bovine adipose tissue

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Session: DISEASE PATHOGENESIS

Objective

Intense and protracted adipose tissue (AT) lipolysis increases the risk for metabolic periparturient diseases in dairy cows. This vulnerability escalates when cows have endotoxemia, but the mechanisms are unknown. Lipolysis induces a remodeling process that is characterized by macrophage infiltration and inflammation that may be enhanced by exposure to endotoxin (ET). We hypothesized that extended exposure to ET increases AT lipolytic responses by activation of inflammatory lipolytic pathways and reduction of insulin sensitivity (IS)

Methods

Subcutaneous AT (SCAT) explants were collected from 57 multiparous Holstein dairy cows and incubated for 3 and 7 h in the presence of ET (lipopolysaccharide, LPS=20µg/ml; CON=0µg/ml). The effect of ET on stimulated lipolysis was determined using the adrenergic agonist isoproterenol (ISO=1uM; ET-ISO). The impact of ET on the anti-lipolytic responses induced by insulin (1µL/L, ET-IN) was determined during ISO stimulation (ISO-IN; ET-ISO-IN). Lipolysis was quantified by glycerol release. Protein expression of osteopontin (OPN), a potent chemoattractant for macrophages and marker of AT inflammation, was assessed by capillary western immunoassay

Results

Results indicate that ET is an effective lipolytic agent increasing glycerol release at 3h by 30.5±18% (P=0.06 n=9) and at 7h by 61.6±14% (*P*<0.001 n=9) compared to CON. ET-ISO potentiated the lipolytic response at 7h by 52±15% compared to ISO (P=0.005 n=9). As expected, IN reduced lipolysis induced by ISO and ET at 3 h by -75±13% and -59±14% respectively (P<0.05 n=52). However, Insulin did not have an antilipolytic effect in ET-ISO-IN at 3h (-1.2% P=0.5 n=28) and at 7h to ET-IN (7h) (-15.8% P=0.56 n=9). Inducing lipolysis for 7h with ISO and ET enhanced OPN synthesis by 56% compared to 3h (P=0.06 n=4)

Conclusions

Prolonged exposure to ET, reduces IS and increases lipolysis in SCAT. Higher expression of OPN suggests the initiation of AT macrophage infiltration during extended lipolysis. Therefore, dairy cows with endotoxemia, may be predisposed to metabolic diseases by having higher AT lipolysis, impaired IS, and AT macrophage infiltration

Notes:

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

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Session: DISEASE PATHOGENESIS

Objective

Transmission from host-to-host (interindividual spread) is an essential component in the herpesvirus' life cycle. Herpesviruses are typically associated with a single host species in nature. Using our chicken model for alphaherpesvirus transmission, we identified a conserved viral gene – namely glycoprotein C (gC) – to be essential for interindividual spread of Marek's disease alphaherpesvirus (MDV). The main objective of this project funded through USDA-NIFA-AFRI grant no. 2019-67015-29262, is to determine the importance of the gC protein in host-to-host transmission that will have dual benefit to both humans and agriculturally relevant chickens and turkeys. We hypothesize that gC is important, if not essential, for host-to-host transmission of alphaherpesviruses and will address our overall hypothesis in two Specific Aims.

Methods

In Specific Aim 1, we will determine the importance of gC during interindividual and interspecies transmission via our avian-herpesvirus transmission model using gC-null avian herpesviruses. We will also determine the role gC plays during interspecies spread and the role the originating host plays in transmission. Previous studies have suggested that gC of alphaherpesviruses evolved with their respective host based their ability to interact with host factors, such as the complement component system. To determine whether gC facilitates host specificity of alphaherpesvirus, we will perform horizontal transmission studies. Briefly, 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. In Specific Aim 2, we will determine the role gC homologs play during replication in human skin, the tissue in which human alphaherpesviruses are disseminated into the environment. We will examine the importance of gC during VZV replication in human skin using the skin organ culture (SOC) model using VZV gC-null virus. Additionally, we have established that MDV expresses secreted forms of gC and these secreted forms, along with the full-length form, are important for interindividual spread of MDV. HSV-1 and VZV also express secreted forms of gC. Therefore, we will also determine whether alternative forms of gC are expressed in human (VZV) and avian (MDV, GaHV3, and HVT) skin cells using RT-PCR and western blot assays.

Results

We hypothesize that gC of other alphaherpesviruses are also required for host-to-host transmission and direct host-specificity. To test our hypotheses, we deleted gC in GaHV3 strain 301B/1 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.
To evaluate whether other avian alphaherpesvirus gC proteins can compensate for MDV gC during natural infection, we removed MDV gC and replaced it with gC proteins from chicken ILTV and HVT. Our results showed that MDV replication in experimentally infected chickens was not dependent on MDV, ILTV, or HVT gC, consistent with previous reports. However, when natural infection was examined, only HVT gC was able to facilitate transmission of MDV, while MDV expressing ILTV gC could not naturally infect chickens.

Conclusions

Further studies are required, but our results suggest that alphaherpesviruses did not necessarily evolve dependent on the host, but dependent on cellular tropism and pathogenesis based on the pathogenesis of MDV and HVT compared to ILTV. We are currently testing whether HVT gC is required for HVT natural infection, as well as developing vaccines targeting transmission of MDV in chickens. We are also examining the functions these gC proteins play in natural infection and the identification of specific regions or motifs of gC proteins that are important for interindividual or interspecies spread that could explain our preliminary results.

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

Notes:

275 - Big-data Genomic Investigation to Improve Dairy Cattle Health

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Session: DISEASE PATHOGENESIS

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:

277 - Role of maturation of lipoproteins in the pathogenesis of the infection caused by *Streptococcus suis*

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Session: DISEASE PATHOGENESIS

Objective

Streptococcus suis is one of the most important swine pathogens. Knowledge on virulence factors and the pathogenesis of the infection remains incomplete. Lipoproteins are membrane-anchoring proteins with a variety of pathogenic functions in bacteria, such as adhesion and cell activation (inflammation). Maturation of lipoproteins requires the prolipoprotein diacylglyceryl transferase (Lgt) and the lipoprotein signal peptidase (Lsp). Studies on the role of lipoprotein maturation in the pathogenesis of the *S. suis* infection are scarce. The aim of this study was to understand the role of maturation of lipoproteins in the pathogenesis of the infection caused by *S. suis*.

Methods

S. suis serotype 2 isogenic *lgt* and *lps* mutants (as well as double mutants), derived from two virulent *S. suis* serotype 2 strains were constructed and characterized. Using these mutants, the role of such proteins in adhesion to swine respiratory epithelial cells was evaluated. In addition, activation of murine dendritic cells by these mutants was assessed by measuring cytokine levels by ELISA. Finally, the virulence of mutants was evaluated in a mouse infection model.

Results

Identical results were obtained with mutants from both *S. suis* wild-type strains. It was shown that the maturation of lipoproteins does not influence the adhesion of *S. suis* to swine epithelial cell. However, *lgt* mutation reduced the secretion of different pro-inflammatory mediators when live bacteria were used. Either (*lgt* or *lps*) mutations affected the activation of cells by killed bacteria or by bacterial-culture supernatant. Finally, and more importantly, all mutants showed reduced virulence in a mouse model of infection

Conclusions

The requirement of *lgt* and *lsp* for cell activation provides evidence that lipoprotein maturation plays an important role in inflammation caused by *S. suis*. Moreover, this process would significantly contribute to virulence. Maturation of *S. suis* lipoproteins may play an important role in the pathogenesis of infection and may lead to new therapeutic possibilities.

Financial Support
Fonds de recherche du Québec

Notes:

278 - Isoprostanes reduce reactive oxygen species production and apoptosis in a bovine model of oxidative stress

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Session: DISEASE PATHOGENESIS

Objective

Transition dairy cattle are prone to oxidative stress, which has been associated with several economically important diseases and results in damage to tissue macromolecules. Isoprostanes (IsoP) are molecules generated from interactions between free radicals and membrane phospholipids, thus serving as excellent indicators of free radical-mediated lipid damage during times of oxidative stress. Previous studies have shown that IsoP concentrations change during times that dairy cattle are susceptible to oxidative stress, such as the transition period and coliform mastitis. While IsoP are recognized as excellent biomarkers of oxidative stress, their physiological role remains largely unknown. As the vascular endothelium is a primary target of lipid peroxidation during oxidative stress, this experiment aimed to determine the effect of the most extensively studied IsoP, 15-F2t-IsoP, on bovine endothelial cells during oxidative stress conditions.

Methods

Bovine aortic endothelial cells (BAEC) were incubated in the presence of 10 nM 15-F2t-IsoP alone and in combination with known oxidizers 2,2′-azobis(2-methylpropionamidine) dihydrochloride (AAPH) and lipopolysaccharide (LPS). Statistics were performed by using one-way ANOVA with Tukey's adjustment and significance set at $P<0.05$.

Results

15-F2t-IsoP decreased ROS production in BAEC incubated with AAPH for 12 h compared with cells incubated with AAPH alone. Additionally, 15-F2t-IsoP decreased apoptosis in BAEC incubated with LPS for 12 h when compared with cells incubated with LPS alone.

Conclusions

The results of this study indicate that 15-F2t-IsoP may have a cytoprotective role during times of oxidative stress. Future studies should be directed toward investigating if IsoP alter other factors associated with vascular damage during oxidative stress, such as endothelial cell barrier integrity. This research advances animal health by indicating how a well-known biomarker of oxidative stress in dairy cattle may contribute to the pathophysiology of economically important diseases.

Financial Support
USDA National Institute of Food and Agriculture

Notes:

280 - Characterizing the regulatory interactions of small RNAs CjNC110 and CjNC140 of *Campylobacter jejuni* IA3902

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Session: DISEASE PATHOGENESIS

Objective

Campylobacter jejuni sheep abortion clone, represented by isolate IA3902, is responsible for the majority of sheep abortions caused by *Campylobacter* spp. within the U.S., and has been linked to human gastrointestinal disease outbreaks. Small non-coding RNAs (ncRNAs) have been demonstrated to be transcribed by *C. jejuni*, however, characterizing the functional roles of these ncRNAs has been slow to follow. We have recently demonstrated that ncRNA CjNC110 alters phenotypes necessary for virulence of IA3902, and that these phenotypes may be associated with a second ncRNA, CjNC140. Here we characterize the regulatory roles of CjNC140 and its relationship to CjNC110 in IA3902. Our hypothesis is that CjNC110 and CjNC140 interact within the same regulatory networks to alter expression of mRNAs, which are critical to IA3902 virulence.

Methods

To test this, IA3902 ΔCjNC140 and ΔCjNC140ΔCjNC110 were created and validated using Sanger sequencing and western blotting, and then phenotypically tested.

Results

Growth curves demonstrated growth of mutants were normal and northern blot testing confirmed CjNC140 was no longer transcribed when comparing ΔCjNC140 to wild-type. RNAseq comparing wild-type to mutants demonstrated significant differential expression of mRNAs, particularly those related to motility, iron acquisition, and capsule formation. Phenotypic assays demonstrated that when disrupting CjNC140 alone, motility significantly increased similar to previous studies of ΔCjNC110, but when disrupting both CjNC140 and CjNC110, motility significantly decreased. Extracellular AI-2 concentrations significantly increased in ΔCjNC140; however, in ΔCjNC140ΔCjNC110, AI-2 concentrations significantly decreased, phenotypically matching ΔCjNC110. Autoagglutination significantly increased for both ΔCjNC140 and ΔCjNC140ΔCjNC110, contrasting previous studies of ΔCjNC110.

Conclusions

These results support the idea that CjNC110 and CjNC140 interact within the same regulatory networks to influence virulence related phenotypes, indicating these ncRNAs may provide a critical frontier for further study of *C. jejuni.*

Financial Support
Iowa State University

Notes:

281 - Using receptor affinity and IFN-inductive propensity of ACE2 to predict SARS-COV2 susceptibility in vertebrates

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Session: DISEASE PATHOGENESIS

Objective

Zoonosis and reverse zoonosis infer a dynamic exchange of pathogens between humans and animals, particularly domestic and wild vertebrates. Using the SARS-CoV2 model, we propose to establish an integrative system for virus-susceptibility determination and antiviral validation. Specific Objectives used to address our hypothesis: (1) Establish a robust procedure to bioinformatically predict the susceptibility of major suspected animal species to a viral infections; (2) Validate virus-susceptibility in vertebrates using in vitro (cell culture) and ex vivo (organoid) models.

Methods

Through integration of key immunogenetic factors, including the existence of S-binding-void ACE2 isoforms and the disparity of ACE2 expression upon early innate immune response, we further refine the SARS-CoV2 susceptibility prediction to fit recent experimental validation.

Results

In addition to showing a broad susceptibility potential across mammalian species based on structural analysis, our results further reveal that domestic animals including dogs, pigs, cattle and goats may evolve ACE2-related immunogenetic diversity to restrict SARS-CoV2 infections. Thus, we propose that domestic animals may be unlikely to play a role as amplifying hosts unless the virus has further species-specific adaptation. Findings may relieve relevant public concerns regarding COVID-19-like risk in domestic animals, highlight virus-host coevolution, and evoke disease intervention through targeting ACE2 molecular diversity and interferon optimization.

Conclusions

SARS-CoV2 evolves to fit well with human (and non-human primates) ACE2 receptor through the structural interfacial affinity, immunogenetic diversity and epigenetic expression regulation, which results in a highly efficient infectivity. Most mammalian animals, especially those that belong to glires, primates and carnivores, have a higher potential for SARS-CoV2 susceptibility but in a species-different manner based on the existence of S-binding-void ACE2 isoforms and the difference of the IFN-inductive propensity of the major ACE2 genes.

Financial Support
USDA National Institute for Food and Agriculture; U.S. National Science Foundation

Notes:

282 - Mandibulofacial dysostosis caused by a recessive mutation in Hereford cattle

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Session: DISEASE PATHOGENESIS

Objective

In spring 2020, six Hereford calves presented with congenital facial deformities attributed to a condition we termed mandibulofacial dysostosis (MD). Affected calves shared hallmark features of a variably shortened and/or asymmetric lower mandible and bilateral skin tags caudal to the commissure of the lips. The tags attached through a connective tissue band to a remnant of Meckel's cartilage that was encased in bone as it extended from the zygomatic process of the temporal bone towards the skin tag. Additional, variable deformities were observed. A single common ancestor was shared by the sire and dam of each affected calf. The objective of the study was to identify the causative variant of MD in these calves. If identified, genetic testing would allow breeders to avoid production of affected calves; this work also has the potential to yield novel information regarding the regulation of craniofacial development.

Methods

Affected calves were obtained to establish a phenotypic characterization of the defect and case definition. DNA from 3 affected calves, 7 family members, and 10 herd mates was collected for whole-genome sequencing (WGS). Candidate mutations fitting a hypothesized recessive mode of inheritance were further evaluated with additional WGS from nearly 5,000 individuals as well as with genotyping of 2 additional affected calves, 5 obligate carriers, 117 individuals related to the suspect founder, and 623 individuals from two of the reporting herds.

Results

143 candidate variants in the original WGS data matched the hypothesized mode of inheritance. Additional WGS and Sanger sequencing data narrowed this list to two remaining variants with only one of these being a novel, missense mutation with a predicted impact on protein function.

Conclusions

We hypothesize that the recessively inherited missense mutation identified impacts the catalytic activity of the encoded enzyme resulting in the observed MD phenotype. Our findings will enable Hereford breeders to identify carrier animals of the disease and avoid production of calves displaying the congenital facial deformities described.

Notes:

283 - Development of a caprine respiratory disease induction model for *Pasteurella multocida* P1063 (type A3).

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Session: DISEASE PATHOGENESIS

Objective

Few models describe induction and effect of experimental respiratory infection on clinical pathology in goats. Previous experimental models of respiratory infection in goats relied on co-administration of an immunosuppressive corticosteroid; however, these models often encounter significant mortality. Our objective was to investigate an infection model that did not trigger immunosuppression, relying instead on multiple inoculations of *Pasteurella multocida* (PM) to induce respiratory infection. It was hypothesized that multiple inoculations of PM would result in significant changes in the indicators of clinical respiratory disease without concurrent mortality.

Methods

Six healthy Boer or Boer- cross goats were inoculated with *Pasteurella multocida* strain P1062 (type A3) via 2 intratracheal injections (10 mL of 10^6 CFU/mL) and 1 nasal administration[MJP[MS1] (30 mL of 10^6 CFU/mL). The goats were monitored over 312 h for clinical and hematologic parameters of infection. Physical examination, respiratory disease scores, hemograms, and acute phase proteins were evaluated. Comparisons were made to pre-induction parameters, for statistical significance.

Results

Following induction of respiratory disease, there was a significant rise in rectal temperature and rales up to 96 h. Lymphocyte counts, serum amyloid A values, and respiratory scores were significantly elevated compared with pre-dose conditions. No mortality was associated with this model, and minimal pathologic changes were noted at study conclusion

Conclusions

The findings of this model suggest a method of establishing clinical respiratory infection in goats without inducing significant mortality. The repeated intratracheal inoculation method of inducing respiratory disease could be further used to produce experimental respiratory disease in goats when the use of corticosteroid (and resulting mortality) is not desirable. With the feasibility of this method established, additional research evaluating the optimal dose and frequency of *P. multocida* administration is warranted.

Financial Support
Iowa State University

Notes:

284 - Metabolism and inflammation predict cardiopulmonary disease outcomes in fattening beef cattle: histopathology

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Session: DISEASE PATHOGENESIS

Objective

Profits from beef production of Great Plains feedlots are increasingly offset by losses from pulmonary hypertension (PH) and co-morbidities. We hypothesize that inflammation combined with elevated metabolic demand from fattening contribute to PH in feedlot cattle.

Methods

Using fattened Angus steers (n = 107) from a moderate (1250 m) elevation cow-calf operation with a history of feedlot heart disease, we are executing three Specific Aims to identify blood biomarkers (inflammation) and understand response to viral challenge (BRSV). Steers were stratified (2 x 2 factorial design (n = 6/group) based on pulmonary arterial pressures (PAP; i.e., indicator trait of PH) and response to viral challenge. Cardiopulmonary tissues were harvested and studied to aid understanding of disease in cattle suffering from PH.

Results

Steers gained 1.5 kg/day for 9 months and were determined to be of High or Low PAP (n = 45) indicative of PH. These groups averaged 87.2 and 44.1 ± 5.3 mmHg, respectively ($P < 0.01$). Death loss was 5.5% with all of these mortalities attributed to PH-induced heart malformations occurring after 6 months of feeding. Despite similar body (600 ± 10 kg) and carcass weight, low PAP steers had better ($P < 0.01$) average daily gain ($1.5 > 1.3 \pm 0.05$ kg/d) and feed efficiency (feed:gain in kg; $2.7 < 3.5 \pm 0.2$) than the High PAP steers. Pathologic alterations in the cardiopulmonary tissues of high PAP steers included striking fatty infiltration of the cardiac perivascular and interstitial compartments with fibrotic and lymphomononuclear inflammation co-localizing in the perivascular spaces and cardiac interstitium. Additionally, coronary arteriosclerotic lesions were visualized, primarily in the left cardiac ventricular free wall and papillary muscle. High PAP steers also exhibited outstanding BALT (bronchus-associated lymphoid tissue) hyperplasia and adverse remodeling of the pulmonary arterial and venous circulations.

Conclusions

Project identified fattening Angus steers that had normal cardiopulmonary physiology versus those experiencing PH leading to cardiopulmonary malformations. These performance and histopathology results revealed unique phenotypes of cattle suffering from feedlot heart disease and will help identify blood biomarkers that predict disease risk leading to improved health of fed cattle.

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

Notes:

285 - Treponeme-associated hoof disease of wild elk in sheep model

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Session: DISEASE PATHOGENESIS

Objective

Treponeme-Associated Hoof Disease (TAHD) is a polybacterial multifactorial disease affecting free-roaming wild elk in the Pacific Northwest. Previous studies have indicated bacterial etiology similar to that of digital dermatitis may be the causative agent(s) of the disease, including several *Treponema* species.

Methods

To evaluate if infectious bacteria were indeed key to the development of TAHD, lesion material from elk TAHD lesions was collected, macerated and used in previously developed experimental digital dermatitis induction model using sheep.

Results

At 14 days post inoculation, sheep inoculated with elk lesion material developed raw erosive lesions, consistent with previous lesion-induction models. Lesions continued to develop to 4 weeks post inoculation and were associated with presence of *Treponema* spp. and other bacterial species present in the elk lesion inoculum as determined by bacterial 16S rDNA sequencing. At 6 weeks post inoculation, approximately one third of the animals spontaneously healed the lesions although *Treponema* were present by PCR. Examination of lesions showed same characteristics of TAHD: neutrophilic cellular infiltrate, ulceration/erosion, hyperkeratosis, and proliferative acanthosis. Additionally, inoculated sheep developed antibody response to *Treponema* antigens, as has been observed in natural infections.

Conclusions

Taken together, these results indicate transmissible infectious bacterial agents are the root cause of TAHD. The mild lesions and spontaneous healing in the sheep model indicate there may be additional host and environmental factors that contribute to the severity and perpetuation of TAHD lesions in wild elk. Understanding the contributing factors both infectious and inherent to host or environment are critical for developing effective evidence based mitigation strategies.

Notes:

286 - Langat Virus Inhibits STAT3 Signaling via Blocking Its Phosphorylation

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Session: DISEASE PATHOGENESIS

Objective

Langat virus (LGTV) is a flavivirus of the tick-borne encephalitis virus (TBEV) serocomplex. TBEV causes neurological infections with an estimated 10,000 to 15,000 cases worldwide per year. LGTV has low pathogenicity and a high level of sequence identify to TBEV. Due to these characteristics, LGTV has been used as a model to study TBEV and was once selected as a vaccine candidate against TBEV. The objective of this study was to determine the effect of LGTV infection on the signal transducer and activator of transcription 3 (STAT3), which is known to play a pivotal role in numerous biological processes including cell survival, proliferation, embryogenesis, differentiation, immunity, and inflammatory responses.

Methods

The LGTV was propagated and titrated in Vero cells. Oncostatin M (OSM) was used to stimulate STAT3 in the cells. Indirect immunofluorescence assay (IFA), Western blotting and co-immunoprecipitation were performed to determine the STAT3 protein level and phosphorylation status.

Results

Upon OSM stimulation, the virus-infected cells have a significantly lower level of phosphorylated STAT3 (p-STAT3) protein than the mock-infected control. The LGTV blocking of STAT3 activation was dose-dependent as the level of p-STAT3 decreased along with incremental LGTV inocula. The p-STAT3 reduction occurred after 24 h post-infection, indicating LGTV replication is needed for the inhibition. IFA showed that the LGTV infection blocked nuclear translocation of STAT3. However, LGTV infection appears to have minimal effect on the STAT3 total protein level. Pre-treatment of Vero cells with OSM led to a significant reduction of LGTV replication.

Conclusions

LGTV inhibits STAT3 signaling via blocking its phosphorylation. Data from this study shed light on the LGTV and TBEV pathogenesis and potentially facilitate future development of antiviral therapeutics and improved vaccine.

Financial Support
University of Maryland

Notes:

287 - LCM-seq analysis of local and systemic responses of mammary epithelial cells in cows locally treated with LPS

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Session: DISEASE PATHOGENESIS

Objective

We aimed to analyze transcriptional changes of mammary epithelial cells (MEC) isolated from bovine mammary glands after intramammary challenge with lipopolysaccharide (LPS).

Methods

Ten multiparous cows were used in the study. Five treatment (T) and five control (C) cows were paired based on days in milk, milk yield and parity. For T cows, both mammary glands on one side of the udder were infused with LPS (50 µg in 10 mL saline); these glands were designated (TL). The contralateral glands received 10 ml saline and were designated (TS). Likewise, for C cows two ipsilateral glands received saline (CS) and the contralateral glands remained uninfused (CU). Mammary tissues were biopsied before (0 h) and at 3 and 12 h post-infusion and processed for laser capture microdissection (LCM). MEC were collected using LCM, total RNA was isolated and subjected to high throughput RNA sequencing.

Results

Among various comparisons, we found 3167 (TL3 vs. TL0), 670 (TL12 vs. TL0), 2555 (TL3 vs. TS3), and 3823 (TL3 vs. CS3) differentially expressed genes [DEGs; FDR<0.05, Log_2 (fold change) ≥1]. The major local responses of MEC in TL glands at 3h included upregulation of ribosome biogenesis, innate immunity and KEGG pathways of TNF, NOD-like receptor and NFKB signaling. Ingenuity pathway analysis showed activation of *TNFR2*, PI3/AKT, iNOS and acute phase protein response. Upstream regulators of these pathways predicted invasion of cell, chemotaxis and cell migration, and showed activated *HIF1A* network. Downregulated genes included network of carbohydrate metabolism, PPAR fatty acid biosynthesis, and several ionic transporters. Major systemic responses of MEC in TS glands showed weak cell mediated immune response, lymphocyte activation, and cytokine production responses. Ingenuity pathway analysis of systemic response genes at 3 h showed p53 senescence pathway with activated upstream regulators as NFKB and TNFA.

Conclusions

These results of comprehensive transcriptome profiling of MEC may explain gene regulation of local and systemic responses of MEC during *E. coli* mastitis.

Financial Support
USDA National Institute of Food and Agriculture

Notes:

288 - Describing outbreaks and identifying vectors of bluetongue and epizootic hemorrhagic disease virus in Louisiana

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Session: EPIDEMIOLOGY

Objective

Bluetongue virus (BTV) and Epizootic Hemorrhagic Disease Virus (EHDV) are arthropod-borne viruses of ruminants transmitted by biting midges in the genus *Culicoides*. The objectives of this study were to characterize the maintenance and transmission of BTV and EHDV epidemiology of these viruses as they relate to white-tailed deer and cattle and to evaluate different trap types for collecting *Culicoides* midges at the Idlewild Research Station in Clinton, La.

Methods

During a 7-year study with yearly outbreaks of hemorrhagic disease in a captive white-tailed deer herd, 15 species of *Culicoides* were captured using CDC black[FLD1] light traps baited with dry ice. Quantitative PCR was performed to screen for BTV and EHDV in pools of midges as well as the tissues of deer. From 2012-2018, 1711 pools representing 24,859 specimens were tested and 5 of the 15 collected *Culicoides* species (*C. debilipalpis, C.stellifer*, *C. venustus, C. haematopotus, crepuscularis,*) were found to be positive for BTV and EHDV.

Results

During the 7 years, 112 white-tailed deer deaths were confirmed for BTV or EHDV: BTV serotypes 10 and 12 were confirmed as well as EHDV-1, 2, and 6. Agar gel immunodiffusion test results showed a significant increase in BTV/EHDV antibodies in white-tailed deer during the 7-year study; antibody positive rates increased from 15% to 78% in the deer herd of approximately100 animals. We compared the number of specimens and species captured using 3 animal baited traps, CDC traps with and without black light. The number of captured midges and their virus infection rates were compared for dry ice baited CDC traps with or without black light. The overall minimum infection rate (MIR) from 5 species of *Culicoides* was 6.2 for BTV and 9.1 for EHDV from traps with light. The MIR for the traps without light was 14.1 for BTV and 2.8 for EHDV.

Conclusions

Notably, this was the first report of EHDV-6 and BTV-12 in Louisiana. We found that animal baited traps did not collect any species that were collected with CDC traps with UV light.

Financial Support
USDA National Institute for Food and Agriculture

Notes:

289 - Description of turnover events of animal caretakers in Ohio swine farms

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Session: EPIDEMIOLOGY

Objective

Attracting and maintaining quality animal caretaking personnel has been one of the major issues the US swine industry has faced in recent years. Turnover rates for caretakers in US swine farms has been reported to be between 20 to 35%, depending on farm size. Turnover events can be costly and may have an impact on productivity and overall animal health. The objective of this study was to describe the frequency of turnover events during a one-year period in eleven farms within two production systems in the state of Ohio.

Methods

A retrospective cohort study was conducted using eleven farms within two production systems located in Ohio. Human resource and production data for the year of 2019 was obtained at the weekly level. The primary outcome of interest was a turnover event, which was defined as when an employee left the farm, and was aggregated at that week level. Information on whether the leave was voluntary (employee decided to leave) or involuntary (the company made the decision) was also collected. All statistical analyses were performed in STATA 15.

Results

Systems 1 and 2 contributed 2 and 9 farms, respectively. Average herd size among the farms ranged from 1,225 to 6,180 pigs with a median of 2,500 pigs and average number of full time employees ranged from 5 to 20 employees with median of 12 employees. There were a total of 152 turnover events during the 52 weeks in 2019, with 4 and 148 leave events in systems 1 and 2, respectively. Of these, 3 and 15 of the turnover events were involuntary. The frequency of turnover events among all the farms over the examined period ranged from one to 32 events, with five of the nine farms in system 2 having more than 20 leave events in a single year.

Conclusions

Preliminary descriptive analysis provided insight into the amount of employee turnover that swine producers can experience, which could lead to disruptions in productivity and have negative impacts on animal health. Next steps include combining health and production data to investigate whether an association exists between turnover events and animal productivity.

Notes:

290 - The presence of persistent bovine viral diarrhea virus infection and a novel bosavirus in a bison herd

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Session: EPIDEMIOLOGY

Objective

Bovine viral diarrhea virus (BVDV) is a significant pathogen of cattle, leading to losses due to reproductive failure, respiratory disease and immune dysregulation. An investigation was conducted in an American bison (*Bison bison*) herd dealing with reproductive issues in 2018-2019 breeding season to determine likely cause of the losses.

Methods

Diagnostic tests including serology and virology (virus isolation and metagenomic sequencing) were conducted on samples collected from the breeding herd and from 4 American bison with failure to thrive and general unthriftiness in March 2020. Two of the these bison (one male and one female) and two "normal" bison (one male and one female) were submitted for pathological examination in September 2020.

Results

Random serology in 2019 indicated 26 of 26 animals had BVDV 1 titers between 512-8192. Type 2 titers were present in 26 of 26 and ranged from 64-8192. In 2020, metagenomic sequencing on pooled nasal swabs and serum from the four "unthrifty" bison identified a BVDV1a strain and bovine bosavirus (BBV). The BVDV genome was most similar to the BVDV type 1a vaccine strain Oregon C24V with 92.7% identity. Sequencing results were confirmed by PCR detection of BVDV and BBV in individual serum samples: BVDV was detected in two bison and BBV was detected in two bison with one bison co-infected with BVDV and BBV. Serum from these same animals collected two months later remained positive for BVDV and BBV, again with one animal co-infected with both BVDV and BBV. Pathological examination of the two BVDV PI bison revealed smaller spleens than the "normal bison". On histopathology, the two BVDV positive animals had lymphoid depletion in the ileo-cecal valve lymphoid region. The female PI bison had decreased primordial/primary follicles in the ovary and there was decreased spermatogenesis in the testes of the PI male.

Conclusions

These results suggest that both viruses can persistently infect bison. While the significance of BBV infection is unknown, the ability of BVDV to persistently infect bison has implications for BVDV control and eradication programs.

Financial Support
South Dakota State University

Notes:

291 - Empowerment of small-holder women in East Africa using poultry production

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Session: EPIDEMIOLOGY

Objective

In East Africa, the day-to-day management of poultry is undertaken by women, often with assistance from their children. Despite carrying primary responsibility for poultry-keeping activities, women do not have complete ownership of the birds or decision-making power, regarding the use of the poultry products and income from sales. This project aimed to: 1. Increase poultry production and income and improve livelihood among women small holder farmers (WSF) in East Africa, 2. Empower WSF in the five Domains of Empowerment (5DE)- namely decisions about agricultural production, access to and decision-making power, control over use of income, leadership in the community and time use, and 3. Improve marketing and strengthen regional trade, and agricultural cooperation in East Africa.

Methods

The Feed the Future Women's Empowerment in Agriculture Index (WEAI) was used to measure WSF's empowerment by collecting data on the five Domains of Empowerment (5DE); namely: decisions about agricultural production, access to and decision-making power, control over use of income, leadership in the community and time use and calculating The Gender Parity Index (GPI).

Results

There was improved livelihood through increased poultry production and improved income generation leading to acquisition of assets such as land; WSF were empowered with ownership of the birds and assets, with leadership and decision-making power; there was improved marketing, and agricultural cooperation among the WSF through formation of a cooperative, and improved engagement in domestic and local decision making.

Conclusions

The WSF were empowered as indicated by variables in the five Domains of Empowerment (5DE) assessed before and after establishment of poultry enterprises and calculation of a modified Gender Parity Index

Financial Support
Mississippi State University

Notes:

292 - Prevalence of *PRRSv, PEDv, PDCoV and TGEv* in pig farm manure pits

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Session: EPIDEMIOLOGY

Objective

Viruses such as Porcine Reproductive and Respiratory Syndrome (PRRS), Porcine Epidemic Diarrhea (PED), Porcine Delta coronavirus (PDCoV) and Transmissible Gastroenteritis (TGE) continue to be present in the United States (US) industry generating important losses [1, 3, 4]. Factors related to the occurrence of these viruses need to be understood as they may have a seasonal trend [2]. As a consequence of pig infection, these viruses can be present and persist in manure pits; however, it is unknown how widespread they are, and what risk they represent to a region. Here, we aimed to assess the prevalence of PRRSv, PEDv, PDCoV and TGEv in manure pits.

Methods

This cross-sectional study was conducted in the midwestern US. Pit manure samples were obtained from Minnesota environmental agencies who receive manure samples on a yearly basis from pig producers. Each sample was accompanied by date of collection and county where the farm is located. Another set of samples were collected by our group by visiting pig farms and collecting manure pit samples directly from the pit. Since the prevalence of these viruses in manure samples is unknown, a default barn level prevalence of 50% with an allowable absolute error of 5% and a 95% level of confidence was used for sample size calculation. Based on this calculation, a total of 385 pig barns were needed to estimate herd level prevalence for these viruses. These samples were submitted to the University of Minnesota Veterinary Diagnostic Laboratory for testing by RT-PCR for PRRSv, PEDv, PDCoV and TGEv. Subsets of samples with positive RT-PCR results were further tested by virus isolation.

Results

At the moment of writing a total of 300 manure samples had been tested. PRRSv was detected in 27 (9%, 95% CI [8.81%, 9.19%]) samples with a median cycle threshold (Ct) value of 37.56. PEDv was detected in 48 (16%, 95% CI [15.8%, 16.2%]) samples with a median Ct value of 33.11. PDCoV was detected in 19 (6.3%, 95% CI [6.14%, 6.46%]) samples with a median Ct value of 34.49. TGEv was not detected in any sample. No viable virus was found in the analyzed samples.

Conclusions

Results from this study confirms the presence of these viruses in manure pits. Although viruses were not isolated, likelihood of virus viability can be high and warrants further investigation. In this study, we believe that virus viability was hindered due to multiple freeze-thaw cycles together with low titer.
PRRSv Ct values tended to be higher compared to PEDv/PDCoV. These differences may be explained by the fact that the latter viruses replicate in the intestinal lining leading to a large number of viral particles being shed into the environment.
This study raises awareness on the presence and concentration of high impact pathogens in manure pits. This raises the question of what are the risk implications of these findings. More studies need to be conducted to further understand the risk implications especially when conducting manure pumping and spreading.

Financial Support
University of Minnesota

Notes:

293 - Isolation and characterization of the emerging pathogen *Escherichia albertii* in broilers in Mississippi and Alabama

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Session: EPIDEMIOLOGY

Objective

Escherichia albertii is an emerging foodborne human enteric pathogen. The prevalence and major animal reservoirs of this significant pathogen are still not clear. Our recent pilot study reported the isolation of *E. albertii* with high rate (30%) in a broiler farm in Tennessee; all isolated chicken *E. albertii* displayed multidrug resistance. Here, we performed a large-scale survey to evaluate the prevalence and features of *E. albertii* in the broilers in Mississippi and Alabama.

Methods

Cloacal swabs were collected from broilers in 3 slaughter plants over a 9-week period with the chickens from 9 farms (30 per farm) in Mississippi (6 farms) or Alabama (3 farms). The samples were grown in tryptic soy broth followed by PCR analysis using highly specific primers targeting *E. albertii cdt* gene. Upon identification of *E. albertii* positive samples, a modified MacConkey agar was used to isolate *E. albertii*. The isolated chicken *E. albertii* strains were further subjected to antibiotic susceptibility assay against 20 antimicrobials, whole-genome sequencing, and comparative genomics analysis.

Results

Of the 270 cloacal swabs, 43 (15.9%) were PCR-positive for *E. albertii*. Twelve *E. albertii* strains were eventually isolated from the PCR-positive samples. The occurrence of *E. abertii* in individual farms exhibited dramatic variations with respect to the rate of PCR-positive samples (from 0% to 73.3%) and percentage of isolated *E. albertii* strains (from 0% to 23.3%). Of the 12 *E. abertii* isolates, all (100%) exhibited resistance to aminoglycoside antibiotics; eight (66.7%) were resistant to the first-generation cephalosporin, cephalothin; seven (58.3%) displayed intermediate resistance to imipenem, a carbapenem antibiotic. Whole-genome sequence-based phylogenetic analysis showed the chicken *E. albertii* strains were diverse but phylogenetically close to those of human origins.

Conclusions

The findings from this large-scale study indicate the importance of chicken as a reservoir for *E. albertii*. The occurrence of *E. albertii* varies greatly across different chicken farms.

Financial Support
USDA National Institute of Food and Agriculture

Notes:

294 - Large-scale survey of prion protein genetic variability in scrapie disease-free goats from the United States.

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Session: EPIDEMIOLOGY

Objective

Scrapie is a slowly progressive neurodegenerative disease of small ruminants caused by an accumulation of an abnormal isoform of prion protein in the central nervous system. Polymorphisms of the prion protein gene (PRNP) strongly modulate scrapie susceptibility and incubation period in goats. The aim of this study was to identify PRNP genetic variability in goats across the United States.

Methods

Blood samples were collected from a total of 6,104 apparent scrapie disease-free goats from 660 operation and 19 different breeds. Genomic DNA was extracted and genetic variability of *PRNP* codons were detected using genetic analyzer. Chi-square test was used to compare PRNP genotypic frequencies across the study variables.

Results

Sequencing of PRNP revealed 26 genotypes with different frequencies based on eight codons. The GG127, QQ222 and RR154 genotypes were predominant and showed a remarkably high frequency across all goats. The QK222 and NS146 heterozygous genotypes, known to be protective against scrapie, were found in 55 (0.9%) and 1158 (18.9%) goats respectively. The QK222 genotype was mostly found in Toggenburg (13.43%) and Oberhasli (6.48%) goats, while NS146 was more common in Savannah (40.91%), Boer (37.93%), Nubian (32.45%) and LaMancha (28.53%) goats. The MM142, IM142, RQ211 and QK222 genotypes showed higher frequency in goats on dairy operations. While, the HR143, NS146, ND146 and RH154 genotypes had a higher frequency in goats on meat operations. The goats from the eastern states had a higher frequency of RH154, RQ211, while the goats from western states had a higher frequency of NS146.

Conclusions

In conclusion, these results showed high genetic variability of PRNP among the U.S. goat population and may serve as a rationale for development of goat breeding programs at the national level to mitigate the risk of scrapie.

Notes:

295 - Surveillance of bovine anaplasmosis Tennessee beef cattle

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Session: EPIDEMIOLOGY - CATTLE

Objective

Bovine anaplasmosis (BA) is an economically significant disease caused by the vector-borne hemobacteria, *Anaplasma marginale*. There are no recent prevalence estimates or known associated factors for BA in Tennessee (TN), making it difficult to account for production losses. The aim of this study was to determine the seroprevalence and associated factors of BA within TN cattle.

Methods

In an active surveillance, 247 blood samples were collected from beef cows at a slaughterhouse during May of 2013. In a passive surveillance, data associated with BA testing (20,004 lab submissions) of cattle in TN from 2012 to 2020 was analyzed. In both methods, serum samples were screened with competitive enzyme linked immunosorbent assay (cELISA).

Results

In the active survey, the apparent seroprevalence of BA in TN was 10.53% (95% CI: 7.29% - 14.98%) and the estimated true seroprevalence was 9.17% (95% CI: 5.63% - 14.11). In the passive survey, the apparent seroprevalence of BA in TN between 2012 and 2020 was 12.22% (95% CI: 11.78% - 12.68%) and the estimated true seroprevalence was 10.99% (95% CI: 10.51% - 11.49%). In the passive survey, age and breed of animals as well as year of sample submission were associated with positive BA test results. The odds of the outcome (an animal seropositive for BA) were 3.325 times higher in adult cattle than in juvenile cattle, 8.845 times higher in Angus cattle than in Holstein, 8.964 times higher in Hereford cattle than in Holstein, and 2.315 times higher in 2015 than in 2019.

Conclusions

Age and breed of animals as well as year of sample submission were associated with positive BA test results. Records from the TN Animal Health Diagnostic Laboratory appear to accurately estimate the seroprevalence of BA in TN and show the counties with the highest seroprevalence are in the western region of TN. With this data, future measures can reduce the spread of BA by addressing the associated factors in high risk areas.

Financial Support
Foundation for Food and Agricultural Research

Notes:

296 - Characteristic climatic patterns that precipitate outbreaks of Rift Valley fever in East Africa

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Session: EPIDEMIOLOGY - CATTLE

Objective

Multiple outbreaks of Rift Valley fever have been reported in East Africa since the virus was identified in 1912. These outbreaks are often associated with persistent and above-normal precipitation. However, not every excessive precipitation event leads to an RVF epidemic. Such sterile precipitation events reduce the accuracy of the available prediction systems and therefore frustrate targeted investments that are made in an anticipation of an outbreak. To support the development of a more efficient prediction system, we analysed rainfall and temperature trends In Kenya, Tanzania and Uganda in RVF outbreak periods in a bid to identify peculiar climatic patterns that could be associated with the outbreaks.

Methods

In Kenya, records on outbreaks reported in 1997/1998, 2006/2007 and 2018 were retrieved used in the analyses. Similarly, outbreaks reported in 1997/1998, and 2007 in Tanzania and in 2016 and 2018 in Uganda were used. Monthly rainfall estimates were downloaded from CHIRPS and temperature estimates were obtained from NOAA National Centres for Environmental Information. Standardised rainfall anomalies were derived for outbreak periods using estimates for 1981-1996 as the reference period. A logistic regression model was used to fit a model for forecasting future outbreaks.

Results

Rainfall rather than temperature provided unique patterns where for almost all the outbreaks, periods of heavy rain were preceded by a dry spell where the average rainfall was lower than the long-term average. These analyses confirmed than while above normal and persistent rainfall was a necessary cause of RVF outbreaks, risk of outbreaks increased substantially when this was preceded by drought or a dry spell lasting for at least 1-2 months.

Conclusions

The dry spell may be necessary to support the epidemiology of the disease in various ways. One plausible explanation is that the increased movement of animals during the dry period, with the utilization of riverine habitats for grazing, could play a major role in exposing livestock to sylvatic transmission cycles. Such infections get amplified when rains set in. The analyses conducted demonstrate the strengths of using an area-wide/regional approach to investigating occurrence patterns of a high consequence pathogen like Rift Valley fever virus.

Financial Support
U.S. Department of Defense

Notes:

297 - Investigating the occurance of transplacental Anaplasma marginale transmission in endemic beef cattle herds

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Session: EPIDEMIOLOGY - CATTLE

Objective

Bovine anaplasmosis is a bacterial disease of cattle caused by the bacterial pathogen *Anaplasma marginale*. Anaplasmosis is endemic in Kansas beef cattle herds with approximately 47% of Kansas beef herds actively infected with *A. marginale*. Transmission of *A. marginale* to naïve animals can occur via ticks (the most efficient and only biological vector) or blood-contaminated surgical instruments and biting fly mouthparts. Anaplasmosis literature states that transplacental transmission between dam and calf may also occur, however, robust data on the frequency of transplacental transmission is lacking. This study was designed to investigate the frequency of transplacental *A. marginale* transmission in highly endemic cow-calf herds. Knowing how and when cattle become infected with *A. marginale* is important when developing anaplasmosis management strategies.

Methods

Two fall-calving cow-calf herds with high incidence of anaplasmosis were identified. Blood samples were collected from dams and calves and were tested for *A. marginale* infection using a molecular test to detect *A. marginale* DNA.

Results

The *A. marginale* infection incidence of the dams was 65.8% and 85.7 % for each cow-calf herd, respectively. The *A. marginale* infection rates of the calves will be examined within one month of birth and again between 5-7 months of age.

Conclusions

. Knowing how and when cattle become infected with *A. marginale* is important when making herd anaplasmosis management strategies, including retention of *A. marginale*-infected dams, use of antibiotics for the purpose of anaplasmosis control, and integration plans for new animals entering an endemic herd.

Financial Support
U.S. Department of Agriculture

Notes:

299 - Ovarian tumors in cattle

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Session: EPIDEMIOLOGY - CATTLE

Objective

Ovarian tumors are common in domestic animals but they are not frequent in cows. This study aimed to identify bovine ovarian tumors in a local abattoir in Algeria.

Methods

Histopathological and hormonal evaluations were performed to characterize the type of ovarian tumor and to determine which hormone was secreted by the animal.

Results

The obtained data after microscopic examination of ovarian tumor tissues indicated that in two cases it was granulosa cell tumor (GCT). The first GCT showed a trabecular pattern with many metastases. The second GCT showed microfollicular pattern and Call Exner Bodies which confirmed the benign aspect of this neoplasm. The third tumor was Sertoli-Leydig cell tumor. In this case, the neoplasm contained tubules lined by Sertoli cells and interstitial clusters of Leydig cells. The last identified tumor was a simple cystadenoma with multilocular aspect. The liquid contained in the two GCT showed high levels of progesterone while estrogen and testosterone concentrations were low. In contrast, cystadenoma was without noticeable functional activity.

Conclusions

In conclusion, this study reports four ovarian tumors in cows with different patterns. To our knowledge, this is likely the first report of Sertoli-Leydig cell tumor of ovary in cows.

Notes:

300 - Contribution to the study of genital tract pathologies of cows in Algeria

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Session: EPIDEMIOLOGY - CATTLE

Objective

The aim of this study was to determine genital organ disorders of cows in the capital of Algeria and to describe the microscopic changes associated with these cases.

Methods

For that, samples were obtained from an abattoir at which the genital tracts of 2025 cows and heifers of various breeds (Holstein, Montbeliard, Fleickveih, native and cross-breeds) were examined. The samples were collected between 01/01/2010 and 29/06/2014 (pregnant specimens were also collected), placed in separate plastic bags and transported to the laboratory as soon as possible. All the macroscopic and microscopic changes were revealed.

Results

The results obtained showed a high incidence of slaughtered pregnant cows (16.49%). Majority of these cases were at the early gestation. The most frequently encountered defects were ovarian cyst, uterine infection, and inflammatory salpinx, which proved that contamination and infection are always present in the cowsheds from where animals were brought. Other observed abnormalities were cervicitis, mucometra, double cervix, unicornis uterus, horn malformation, uterine tumour, triple cervix (which is a new finding in the world), salpinx adhesion, pyosalpinx, hydrosalpinx, ovarobursal adhesion, ovarian inactivity, ovarian inflammatory changes, ovarian tumour and parovarian cysts. Microscopic examination allowed us to specify the types of 240 OC collected in *ex vivo* and subsequently obtaining their real frequencies (follicular cysts: 64.73 %; luteal cysts: 34.85 %).

Conclusions

At the end, our results have confirmed the impact of ovarian cysts, uterine infection and the slaughtering of pregnant cows on the profitability of the national cattle population.

Notes:

301 - Preliminary results from a survey of farm-to-market pathways for Minnesota beef cattle

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Session: EPIDEMIOLOGY - CATTLE

Objective

The objective of this survey was to document the pathways by which beef cattle in Minnesota arrive at market and to determine common management strategies. This information will provide a foundational overview for life cycle assessment by documenting and describing the farm-to-market pathways currently being utilized by Minnesota beef producers.

Methods

An anonymous Qualtrics survey was conducted in an iterative fashion in order to capture all potential pathways and major management strategies currently being utilized by Minnesota beef producers, while also keeping the survey length at an average of 5 minutes to encourage a high completion rate. Prior to release, the survey was tested using a convenience sample of Minnesota beef producers and beef extension agents. Survey dissemination was achieved through social media posts (Facebook and Instagram); fliers with QR codes; emails to lists provided by the Minnesota Beef Council and the Minnesota State Cattlemen's Association; inclusion in beef-related Podcasts; and publication in trade-specific newsletters. In order to appeal to producers, an optional raffle of a Pierce VaxMATE cooler was included.

Results

In order to capture all potential pathways to market, the survey contained multiple subsections, i.e. cow-calf, backgrounder, feedlot, seedstock, showstock. The project is ongoing and the survey continues to be disseminated to beef cattle producers in Minnesota. The survey has currently garnered 183 responses, with at least one response from nearly every county in Minnesota. Currently, 63.4% of respondents identified their primary operation type as cow-calf, 0% as a backgrounder, 13.7% as a feedlot, 3.3% as a showstock operation, 15.3% as a seedstock operation, and 4.3% classified themselves as "Other".

Conclusions

A survey was successfully distributed among Minnesota beef producers to gain insight into how cattle are transported and retained from birth to harvest. Based on initial responses, there are a variety of pathways by which Minnesota-reared cattle reach market and many beef producers were noted to maintain multiple operation styles.

Financial Support
National Cattlemen's Beef Association - Beef Checkoff

Notes:

303 - Measures of passive maternal immunity and the occurrence of negative health events in pre-weaned beef calves

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Session: EPIDEMIOLOGY - CATTLE

Objective

The objective of this study was to determine the relationship between measures of transfer of passive maternal immunity on the post-test probability of negative health outcomes in pre-weaned beef calves.

Methods

Blood was collected from 369 calves, between 2 and 7 days of age, from a ranch with 3 herds. Negative health events were recorded by the producers prior to weaning. Sera were analyzed for IgG using a commercial radial immunodiffusion and 3 refractometry scales: Brix percentage (Brix), total protein (STP), and specific gravity (SG). Immunological values were categorized as low, medium, and high, for each immunological measure with the criteria that each category had to have at least 5 individuals with negative health events. Categories for IgG were <1800 mg/dL, 1800-4300 mg/dL, and >4300mg/dL. Categories for STP were <6.1 g/dL, 6.1-7.4 g/dL, and >7.4 g/dL. Categories for Brix were <8.9%, 8.9-10.5%, and >10.5%. Categories for SG were <1.035, 1.035-1.040, and >1.040.

Results

A negative health event occurred in 7% (26/370) of calves. Positive likelihood ratios (LR+) were 2.6 (2.6, 2.7), 2.1 (2.0, 2.1), 2.6 (2.5, 2.6), and 2.2 (2.1, 2.2) for the lowest categories of IgG, STP, Brix, and SG, respectively. LR+ for other categories were less than 1. Positive predictive values for the lowest categories were only marginally better than pre-test probabilities. Negative predictive values were non-informative.

Conclusions

Immunological measures of passive maternal immunity are only marginally better than using clinical judgment alone to predict which calves will develop negative health outcomes in the pre-weaning period.

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

Notes:

304 - Comparative phylogeny, antimicrobial resistance, and virulence of canine and human uropathogenic *Escherichia coli*

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Session: EPIDEMIOLOGY - COMPANION ANIMAL

Objective

Urinary tract infections (UTI) are one of the most common infectious diseases affecting humans and companion animals. Uropathogenic *Escherichia coli* (UPEC) cause up to 80% of UTIs in dog and humans and can be a zoonotic pathogen. Although humans and dogs share similar epidemiology of UPEC UTI, little is known about the comparative phylogenetic structure, genotypes and phenotypes of spatiotemporally related human and canine UPEC populations.

Methods

We collected 50 human and 53 canine UPEC isolates from clinical UTIs diagnosed between 2018-2020 from Columbus, Ohio. UPEC isolates underwent whole genome sequencing and a maximum likelihood phylogenetic tree was generated based on single nucleotide polymorphisms (SNP) analysis. The frequency of virulence and acquired antimicrobial resistance (AMR) genes, biofilm formation and antimicrobial susceptibility was compared between hosts using whole genome sequence data, a crystal violet assay and a broth microdilution system following the Clinical Laboratory Standard Institute (CLSI) guidelines.

Results

Phylogenetic analysis showed that most clusters were host-adapted, but some clusters, including five pandemic sequence type lineages, contained both human and canine isolates. Human UPEC were at greater odds of being AMR (OR:3.75, P<0.01) and multi-drug resistant (OR:3.37, P=0.02) and their average resistome contained more AMR genes (Human mean=2.76, Canine mean=0.89; P<0.01). UPEC from humans contained 3.5 more virulence genes compared those from dogs (P=0.05), but virulence genes required for UTI pathogenesis did not differ between species. Canine UPEC had a greater capacity to form biofilms (OR:2.89, P=0.01).

Conclusions

Although humans and canines share similar UTI disease epidemiology, human and canine UPEC strains differ in their population structure, antimicrobial resistance and biofilm formation. Despite these difference, potential zoonotic spread should be considered in all patients diagnosed with UPEC UTI as there is significant human-canine isolate overlap among pandemic UPEC lineages.

Financial Support
Ohio State University

Notes:

305 - Prevalence of severe fever with thrombocytopenia syndrome virus in cats

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Session: EPIDEMIOLOGY - COMPANION ANIMAL

Objective

Severe fever with thrombocytopenia syndrome (SFTS) is an emerging tick-borne zoonosis in China, the Republic of Korea, and Japan. The presence of the SFTS virus (SFTSV) in companion, livestock, and wild animals has been reported. Recently, human SFTS-like clinical symptoms in cats and cheetahs have been reported in Japan. Therefore, the prevalence of the SFTSV gene or antibody in cats is important for public health as well as veterinary medicine.

Methods

Sera were collected from 201 feral and house cats in the Republic of Korea in 2017. Samples were analyzed for the presence of the SFTSV gene after RT-nested PCR amplification and for anti-SFTSV antibodies after enzyme linked immunosorbent assay.

Results

Eight (4.0%) and nine (4.5%) of 201 cat sera were found to be positive for the SFTSV gene and anti-SFTSV nucleocapsid protein antibodies, respectively. Specifically, 5.9% feral and 2.0% house cats were positive for the SFTSV gene, and 6.9% feral and 2.0% house cats were positive for anti-SFTSV antibodies. All sequences of the SFTSV S segment obtained were included in Japanese/Korean SFTSV clades, as opposed to the Chinese clade.

Conclusions

This study constitutes the first serological study of SFTSV in house and feral cats in the Republic of Korea. Evidence of SFTSV in companion animals indicates that SFTSV can circulate in homes and that more intensive precautions and education measures are needed for companion animal guardians and veterinarians.

Notes:

306 - Canine severe fever with thrombocytopenia syndrome in Republic of Korea

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Session: EPIDEMIOLOGY - COMPANION ANIMAL

Objective

Severe fever with thrombocytopenia syndrome (SFTS) is a tick-borne disease transmitted by *Dabie bandavirus* belonging to the *Phenuiviridae* family in China, Republic of Korea (ROK), and Japan. In clinical cases of SFTS, the most common symptoms were severe fever, myalgia, and diarrhea. Although canines do not generally present with clinical symptoms of SFTS, SFTSV has been reportedly isolated from this species in ROK. This study reports on the development of clinical symptoms associated with SFTS, in a companion dog, after being bitten by ticks.

Methods

To determine the hematological data and serum chemistry, blood was collected by venipuncture. Blood samples were prepared in tubes coated with 0.5 M EDTA and used for the analysis of hematological data. Reverse transcription (RT) – polymerase chain reaction (PCR) and Quantitative RT-PCR were performed to amplify viral RNA. Antibody titer was evaluated by immunofluorescence assay IFA.

Results

A total of three blood samples were collected from an 8-year-old Maltese dog admitted to a local animal hospital with fever and anorexia. Laboratory analysis showed increased values of white blood cells, lymphocytes, mean platelet volume, alkaline phosphatase, and C-reactive protein. On the other hand, the values of red blood cells, platelet, hematocrit, and hemoglobin were decreased below the respective reference ranges. After the disease onset, the number of copies surged in 2 days and reduced in 7 days. However, viral antigen was not detected in 13 days. IgG antibody against SFTSV was not detected in serum on day 2, whereas it was on days 7 and 12 by IFA.

Conclusions

This clinical case report is the first for animal SFTS, presenting virus isolation, laboratory findings, and clinical symptoms. Although in the present study dog to human transmission was not identified, the status of SFTSV infection in pets is critical with respect to public health, and thus, veterinarians must be vigilant in observation and testing for SFTSV as currently there is no available guideline for SFTS infection in animals.

Financial Support
Korea Institute of Planning & Evaluation for Technology

Notes:

307 - The impact of cannabis legislation, socioeconomic and dog characteristics on cannabis poisoning reports of US dogs

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Session: EPIDEMIOLOGY - COMPANION ANIMAL

Objective

With current trends in cannabis legalization, large efforts are being made to understand the effects of less restricted legislation on human consumption, health, and abuse of these products. However, there is little known about the effects of cannabis legalization and increased cannabis use on vulnerable populations, such as dogs. The objective of this study was to examine the effects of different state-level cannabis legislation, county-level socioeconomic and demographic factors, and dog-level characteristics on dog cannabis poisoning reports to an animal poison control center.

Methods

Data were obtained concerning reports of dog poisoning events, county characteristics, and state cannabis legislation from the American Society for the Prevention of Cruelty to Animals' (ASPCA) Animal Poison Control Center (APCC), the US Census Bureau, and various public policy-oriented and government websites, respectively. A multilevel logistic regression model with random intercepts for county and state was fitted to investigate the associations between the odds of a call to the APCC being related to a dog being poisoned by a cannabis product and the following types of variables: dog characteristics (e.g., age and sex), county-level socioeconomic and demographic characteristics (e.g., income disparity), type of state-level cannabis legislation, and source of the call (i.e., owner vs. veterinarian).

Results

There were significantly higher odds of a call being related to cannabis in states with lower penalties for cannabis use/possession. The odds of these calls were higher in counties with greater levels of income variance, higher percentage of urban population in a given county, and among smaller, male, and intact dogs. The odds of a call being related to cannabis were higher when a veterinarian called rather than a dog owner. Furthermore, the odds of a cannabis intoxication call increased throughout the study period.

Conclusions

Reported dog poisonings due to cannabis appear to be influenced by dog-level characteristics and community-level factors.

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:

308 - A system dynamics model of shelter capacity for care

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Session: EPIDEMIOLOGY - COMPANION ANIMAL

Objective

Shelter animal welfare concerns lead to the development of capacity for care calculators, which produce an optimal animal inventory based on average number of daily adoptions. Their applicability to municipal, open-admission shelters is limited, as these shelters are often tasked with accepting all unowned animals in the community. System dynamics modeling captures a system's behavior resulting from its structure. The objective of this study was to develop a system dynamics model to observe the effects on the shelter system in response to changes in exogenous parameters, given fixed resources.

Methods

A causal loop diagram of factors contributing to animal welfare, length of stay, and animal outcomes was developed. A shelter management stock-and-flow model was then constructed to demonstrate endogenous shelter system dynamics by tracking changes in length of stay, shelter population, population health, and proportion of live/dead outcomes. Endogenous model parameters were drawn from previously collected shelter survey datasets. Exogenous parameters included intake and outflow rates, resource use, initial illness incidence, and initial inventory size.

Results

Operating above capacity for care for a set time resulted in an increased number of healthy animals that were euthanized due to resource disparity. Inventory size and animal health oscillated in relation to operating capacity and increasing illness incidence due to increased cost-to-treat ill animals.

Conclusions

The shelter model showed that variables beyond adoption rate, intake, and capacity for care may impact shelter conditions and proportion of live animal outcomes. This model may be used to understand complex relationships within shelters and to evaluate management strategies.

Financial Support
Mississippi State University

Notes:

309 - Effect of functional feed additive on digestibility of two grass hays with similar neutral detergent fiber content

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Session: EPIDEMIOLOGY - COMPANION ANIMAL

Objective

The objective of this study was to evaluate the effect of a functional feed additive on voluntary dry matter intake and nutrient digestibility of two types of grass hays with similar neutral detergent fiber content in lightly exercised horses.

Methods

Four mature stock-horse type geldings were used in a 4x4 Latin Square design. Each of the four periods consisted of a 3-d forage acclimation period, a 17-d feeding period, and a 72 hr total fecal collection period. Treatment consisted of: *Cynodon dactylon L.* (Coastal Bermuda) grass hay, with and without functional feed additive, and *Eragrostis tef* (Tiffany) Hay, with and without functional feed additive. During the 17-d feeding period, ad libitum voluntary intake was measured. During the total fecal collection periods, total feces voided was collected via fecal bag harnesses. Feces voided was weighed, and sub-samples frozen for later analysis.

Results

Data is currently being analyzed and results are expected to be back November 2020. According to preliminary analysis, voluntary dry matter intake and digestibility of Tiffany hay was greater as compared to Coastal Bermudagrass hay. Addition of the functional feed additive appeared to have no effect on voluntary dry matter intake or digestibility of either hay species.

Conclusions

Conclusions will be determined finally once results are analyzed. Previous studies on Teff hay have shown that dry matter digestibility and voluntary dry matter intake between Tiffany hay and other more commonly fed forages are not significantly different.

Notes:

310 - Isolation and strain-typing of *Borrelia burgdorferi* from ticks on dogs

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Session: EPIDEMIOLOGY - COMPANION ANIMAL

Objective

Borrelia burgdorferi (Bb) is the causative agent of canine Lyme disease. The vector of Bb in Ontario is the blacklegged tick, *Ixodes scapularis*. Prior research has shown that strains of Bb vary geographically and by host species, and that strain type may play a role in human disease. However, no research has examined the role of strain type in canine Lyme disease. This pilot study aims to describe the strains of Bb that are present in Ontario and explore the potential relationship between Bb strain type and clinical signs observed in dogs.

Methods

21 veterinary clinics were recruited from across Ontario from areas with known Lyme disease risk. Blacklegged ticks were collected from dogs from April 2019 to March 2020. Dog owners were asked to complete questionnaires providing demographic and clinical information about their dogs at the time of tick submission and five months following the tick bite. An optional SNAP4Dx test to detect Bb presence in the dogs was offered. Real time PCR was used to determine if tick samples were positive for Bb, and positive samples were cultured using the pour plate method. Isolates from Bb cultures were sent to be strain-typed using multilocus sequence typing. Statistical analysis, such as Fisher's Exact test, will be conducted to compare strain-typing results to questionnaire responses and geographic data.

Results

185 tick submissions from 134 dogs submitted by 14 clinics were received. 38 ticks were positive for Bb. 7 dogs with positive tick submissions underwent SNAP4Dx testing, and 3 were positive for Bb. 23 dogs with negative tick submissions were tested for Bb, and all were negative. Strain-typing of positive tick samples is currently being conducted.

Conclusions

More information on the relationship between strain-type and its role in canine Lyme disease may be valuable to veterinarians in practice. The results of this pilot study will inform future research in this field, and have an impact on human and animal health relating to Lyme disease and Bb strain typing.

Financial Support
University of Guelph

Notes:

312 - Molecular characterization and phylogenetic analysis of Rabies viruses from Azerbaijan

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Session: EPIDEMIOLOGY - COMPANION ANIMAL

Objective

Rabies virus zoonosis infects many different species of warm-blooded animals. Glycoprotein G plays a key role in viral pathogenicity and neurotrophy, and includes antigenic domains that are responsible for membrane fusion and host cell receptor recognition. The objective of this study was to analyze field samples from Azerbaijan, previously shown positive for rabies virus. The genotyping of the rabies virus isolates was assessed by sequencing. For each sample, the complete nucleoprotein (N) gene was sequenced and compared to those of other referenced *Lyssavirus* species.

Methods

Ten samples were sent to the OIE/WHO/EU National rabies laboratory in France (ANSES). PCR products were sequenced in both directions by Eurofins Genomics with the M3 primers and three internal forward and reverse primers (JW662-684F, JW938-959R, JW938-959F). The sequences were assembled using the ContigExpress of the Vector NTI software, version 11.

Results

The virus species found for the 7 RT-PCR samples was classical rabies virus. A mean of 96% of nucleotide identity was shown in the subclade CA4 constituted by six sequences of this study and one Azeri sequence extracted from Genbank (LN879480). In the subclade CA2 99.3% and 99.7% of nucleotide identity were shown between the cat and the sequences AY352497 from Georgia and AY854583 from Iran

Conclusions

The majority of tested samples of this study and referenced Azeri sequences belong to the subclade CA4. The seventh isolate of the study belong to the subclade CA2 with isolates from Irak, Turkey, Georgia. The tenth sequence extracted from Genbank belong to the Middle - East clade (ME1) formed with isolates from Iran, Jordan and Israel. The tenth sequence was isolated from a horse in 2013 in the region Aghjebedi.

Financial Support
U.S. Department of Defense

Notes:

313 - Control of bovine leukemia virus in dairy cattle

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Session: EPIDEMIOLOGY - DAIRY CATTLE

Objective

Over 20 nations eradicated bovine leukemia virus (BLV) from their dairy cows by culling all BLV ELISA-positive cattle. This method is not economically feasible for most U.S. dairy producers where the average herd prevalence is about 45%. A method is needed to reduce BLV prevalence to where culling all remaining ELISA-positive cattle might be practical.

Methods

Volunteer commercial dairy farms were enrolled in three separate field trials to test BLV control methods. In two trials, BLV ELISA, blood lymphocyte count and BLV proviral load (PVL) were evaluated as diagnostic tests to identify cattle for segregation or culling.

Results

An intervention field trial in three herds followed ELISA-negative cattle which had been randomly assigned to receive either no special treatment (negative controls) or to always receive a new hypodermic needle and new reproductive examination sleeve. The control group experienced a rate of new infection of 16.4% (40/240) and the intervention group had an insignificantly higher rate of 20.2% (53/262). A second intervention field trial on three small dairy herds attempted to control BLV transmission by segregating or culling those cattle with the highest blood lymphocyte count and proviral load. Such cattle are thought to be responsible for most transmission to their BLV-susceptible herdmates. Over the course of 3 years, the ELISA prevalence went from about 65% of the milking cows to 20%. A third intervention study is continuing on a 3,000-cow herd using lymphocyte count, BLV ELISA and BLV proviral load to prioritize cattle for segregation or culling. This farm has reduced the percentage of cows with a lymphocyte count over 10,000/μL of blood from 4.22% to 1.04%.

Conclusions

In contrast to medical hygiene intervention, the removal of the cattle with high proviral load and high lymphocyte count is showing good promise in reducing BLV prevalence and the rate of new infections.

Financial Support
USDA National Institute for Food and Agriculture

Notes:

314 - Impact of bovine leukemia virus on cow longevity and production

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Session: EPIDEMIOLOGY - DAIRY CATTLE

Objective

Enzootic Bovine Leukosis (BLV) caused by the bovine leukemia virus has been eradicated in over 20 countries. In contrast, the U.S. and many other nations are experiencing increased prevalence and economic impact. Our objectives were to determine BLV's effect on immune function and its economic impact on U.S. dairy farms.

Methods

The survey of 4,120 dairy cattle in 11 U.S. states estimated a mean within-herd BLV prevalence of 46.5% with 94.2% (97/103) of herds having at least one infected cow. The resultant database was used to compare ELISA-positive cattle to their herdmates with regard to lifespan and production.

Results

We found that infection with BLV was significantly associated with decreased milk production and decreased cow longevity compared with their ELISA-negative herdmates. Each 10% increase in prevalence was associated with a 430.7 lb (195.8 kg) loss in rolling herd average milk production, which is a greater loss than what was found in our 2010 study of Michigan herds. A survival analysis found that ELISA-positive cows were 30% more likely than their BLV-negative herds mates to die or be culled during the subsequent 32 months. Our Extension projects involved 80 herds attempting to reduce BLV prevalence by increased medical hygiene. The average herd saw no reduction in BLV prevalence. Our laboratory studies demonstrate that BLV infection in dairy cattle has a much greater effect on the host beyond the well-recognized lymphoma development that occurs in <5% of infected cattle. Overall impairment of antibody production and interference with T cell immunity was observed along with decreased total IgA concentrations in milk and saliva of BLV ELISA-positive cows.

Conclusions

Immune disruption is associated with BLV infection in dairy cattle. BLV prevalence in the U.S. continues to increase, and is associated with decreased milk production and increased culling.

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

Notes:

315 - Survey of management and animal health practices on organic dairy herds in California

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Session: EPIDEMIOLOGY - DAIRY CATTLE

Objective

The dairy industry represents the second largest segment of organic agriculture, with California ranked in the top 7 organic dairy producer states. Currently, very limited information is known about husbandry practices related to the prevention and treatment of sick cows and calves on organic farms in California. Thus, our study aimed to characterize the organic cattle dairy farms in California in the aspects of animal health, welfare and prevention, and treatment of disease through a survey.

Methods

A survey was conducted to certified organic dairy farms in California from November 2018 to July 2019. The survey questionnaire was made available to these producers both as in hardcopy form and web-based form. Descriptive statistics were used to summarize the results of the questionnaire, and correlations among the answers were evaluated for some of them.

Results

A total of 136 farms were invited to participate in the survey and 36 of them responded. The average numbers of cattle in each farm were 502 cows, 257 heifers and 132 calves in each age, and the main breed of them was Holstein (58%). Their primary milking parlor was herringbone and parabone (47%) style, and rotational grazing (57%) was mainly used for pasture management. In the last 12 months, the most frequent disease impacting cows in the farms was mastitis, which was also the most frequent reason for removal. The most prevalent diseases in heifers and calves were pink eye and digestive problems, respectively. Some of the diseases were highly correlated across the ages, such as digestive diseases in cow and infectious diseases in calves (r=0.81). For medications or supplements, iodine products (55.6%), vitamins A,D,E, (52.8%) and selenium (52.8%) were commonly used among the twelve listed options in the survey. Respiratory problems (40%) and mastitis (25%) accounted for the largest proportion of reasons for antibiotics for treatment in the past year.

Conclusions

This preliminary study allows us to identify areas for improvement of animal health, management, welfare, and farm productivity of organic dairies.

Financial Support
USDA Animal Health Formula Funds

Notes:

316 - Biomarkers measured at arrival associated with morbidity, mortality and average daily gain in male dairy calves

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Session: EPIDEMIOLOGY - DAIRY CATTLE

Objective

There is a strong need for management strategies that help reduce the incidence of disease and subsequent antimicrobial use in the veal industry. Biomarkers could allow producers to identify high-risk calves and take preventive measures before clinical problems occur. The objective of this prospective cohort study was to identify biomarkers measured upon arrival associated with morbidity, mortality, and average daily gain (ADG) in male dairy calves.

Methods

Upon arrival at a grain-fed veal facility in Ontario, calves were health scored, weighed, and blood was collected. Several metabolites were measured including creatine kinase (CK), cholesterol, haptoglobin, manganese, serum total protein, iron, cobalt, zinc, selenium, molybdenum, and IgG. Farm personnel treated calves according to their protocol and weighed them 78 d after arrival. Performance, treatment, and mortality records from the facility were used for analysis. Multivariable Cox proportional hazard models were created to evaluate metabolic biomarkers associated with morbidity and mortality. A mixed linear regression model was created to determine biomarkers associated with ADG.

Results

A total of 992 male dairy calves were evaluated at arrival from January to December 2017. Of the calves evaluated, 74 calves (7.5%) died and 877 (88.4%) were treated for illness over the 11-week observation period. Higher levels of haptoglobin and molybdenum were associated with a greater hazard of morbidity, whereas, higher weight upon arrival, and higher levels of both CK and IgG were associated with a reduced hazard of morbidity. For mortality, higher weight upon arrival, and higher levels of cholesterol and IgG were associated with a lower hazard of mortality occurring over the 78 d period of observation. Higher weight, cholesterol, copper, CK, iron, and IgG were associated with increased ADG, whereas, increased zinc and haptoglobin were negatively associated with ADG.

Conclusions

These results demonstrate that certain biomarkers could be used to identify high-risk calves when measured upon arrival at a veal facility.

Notes:

317 - Impact of Bovine Leukemia Virus infection on disease incidence and severity in dairy cattle

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Session: EPIDEMIOLOGY - DAIRY CATTLE

Objective

Bovine leukemia virus (BLV) is a delta-retrovirus which infects cattle, decreases milk production, longevity, and immune system function. BLV primarily infects B lymphocytes and ~46% of all dairy cattle in the U.S. are estimated to be infected. This study aims to determine the impact BLV has on predisposing dairy cattle to common diseases. The objectives of this study are to 1) Determine the effect of BLV infection status 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 ~125 animals will be enrolled from each of 10 farms. Animals will be tested for BLV status and monitored for disease incidence over a lactation cycle.

Results

For objective 2 there are currently 875 cows on 7 farms enrolled. 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 ($p>0.05$).

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.

Financial Support
USDA National Institute of Food and Agriculture

Notes:

318 - Evaluation of environmental and comfort enrichment on lying behaviours in heifer calves on smallholder dairy farms

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Session: EPIDEMIOLOGY - DAIRY CATTLE

Objective

We aimed to determine the effects of environmental and comfort enrichment on lying behaviours in heifer calves on Kenyan smallholder dairy farms.

Methods

The trial involved 187 heifer calves from 150 farms in Kenya. Intervention farms received enrichments in the calf pen that included placement of rubber mats on the lying area; and fixing gaps/holes in the flooring and roofing. Up to 6 bimonthly farm visits over the first year of life were used to collect data on lying time (using accelerometers) and other animal- and farm-level factors. Multilevel mixed-effects linear regression was used to model daily lying times and frequency of lying bouts.

Results

Over the visits, daily lying times and lying bout durations averaged 12.6-16.9 hr/d and 67.9-86.7 min/bout, respectively, while the median for the frequency of lying bouts was between 30-46/day. In a final daily lying time model, superficial lymph node enlargement, body condition score and use of wood shaving/ sawdust/ crop waste as beddings had positive associations. In contrast, group housing and rubber mat use had negative associations. In an interaction term, lying time was significantly higher for calves on clean versus dirty floors if the age was <190 days but this difference diminished significantly in older animals. In a second interaction term, lying time was lower for calves with leaking versus non-leaking roofs, regardless of the pen floor level, but lying time was higher on elevated than non-elevated floors if the roof was intact. In the final model of frequency of lying bouts, use of a rubber mat, years of experience in dairy farming, and calf body weight had negative associations. In contrast, body condition score had a positive association. In an interaction, frequency of daily lying bouts was lower on clean floors than dirty floors, irrespective of tethering status, but when the floor was dirty, lying bouts were higher for calves not tethered than calves sometimes tethered.

Conclusions

The comfort enrichments improved the welfare and lying experience of heifer calves on smallholder dairy farms.

Notes:

319 - Effects of housing improvements on the growth of heifer calves on Kenyan smallholder dairy farms

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Session: EPIDEMIOLOGY - DAIRY CATTLE

Objective

We evaluated effects of housing improvements on average daily gain (ADG) of heifer calves (< 1 year old) on smallholder dairy farms.

Methods

This trial involved 187 heifer calves from150 farms in Kenya. Intervention farms received calf housing improvements that included placement of rubber mats on the lying area; and fixing gaps/holes in the floors and roofs. Up to 6 bimonthly farm visits over the first year of life were used to measure weight and other animal- and farm-level factors. Multivariable linear regression was used to model ln ADG and ADG during pre-weaning and post-weaning periods, respectively.

Results

Median pre-weaning and post-weaning ADGs were 0.307 (interquartile range (IQR): 0.227-0.398) and 0.487 (IQR: 0.354-0.675) kg/d, respectively. In the final pre-weaning model ($p<0.05$), factors positively associated with ln ADG were calf age at first acaricide application, and total number of calf pens, while factors negatively associated with ln ADG included calf mortality risk over the last 5 years and calf age at first ad lib access to water. In an interaction term, for calves from parity 3+ dams, ADG was lower when milk was fed twice/day than thrice/day, with no difference in calves of lower parity dams. In the final post-weaning model, housing improvements increased ADG by 5.6%. Other factors positively associated with post-weaning ADG were feeding of calf pellets, wheat bran, maize bran, and hay. Calf age at first introduction of concentrate and calf mortality risk over the last 5 years were negatively associated with ADG. In an interaction term, ADG was high when there were fecal coccidia oocysts and when calves had visual or physical contact with their dams, but low when fecal coccidia cysts were present and these dam-calf connections were absent. In a second interaction term, ADG increased with more calf pens for female principal farmers, while remaining low for male principal farmers.

Conclusions

While controlling for other factors of ADG, making affordable calf housing improvements enhanced ADG, particularly during the post-weaning period.

Notes:

320 - Incidence, risk factor, and impact of metritis in dairy cows housed in dry-lots.

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Session: EPIDEMIOLOGY - DAIRY CATTLE

Objective

Most of the literature reporting the incidence, risk factors, and impact of metritis have described metritis epidemiology in dairy cows housed in free stalls barns. Hence, the objective of this study was to conduct similar epidemiological analysis of metritis in cows housed in an open dry-lot herd.

Methods

A total of 498 cows that calved from January 16th, 2020 to March 12th, 2020 were examined for metritis using the Metricheck device at 4, 7, and 10 days in milk (DIM). Additionally, cows with signs of systemic illness were also examined for metritis. Data regarding parity, dystocia, calf type (male, female, twins), retained placenta (RP), stillbirth culling, pregnancy per AI at first service, and milk yield during the first 10 wk of lactation was extracted using the farm's database software. The assessment of potential risk factors for metritis was done with univariate and multivariate analysis. Multivariate models were fitted to the data to evaluate the impact of metritis on pregnancy odds, hazard of culling, and milk yield.

Results

The incidence of metritis in our study was 28.9%, and the median DIM to diagnosis was 6. Univariate analysis revealed that parity, dystocia, calf type, RP, and stillbirth were associated with occurrence of metritis ($P < 0.05$). Multivariate logistic model revealed that primiparous cows were at 2.2 times higher odds of being diagnosed with metritis than multiparous cows ($P < 0.01$) and the odds of metritis being 4.2 times higher for cows that had stillbirth ($P < 0.01$). Metritis decreased the odds of pregnancy at first service (OR = 0.56, $P = 0.02$) and milk yield by 2.7 kg/d ($P < 0.01$). However, culling was not affected by metritis (HR = 1.51, $P = 0.16$).

Conclusions

The incidence of metritis observed in our study cows is within range of metritis incidence reported by others. Multiparity and stillbirth were the most important risk factors for metritis. Milk yield and pregnancy risk at first service were impaired in cows diagnosed with metritis.

Financial Support
USDA National Institute of Food and Agriculture

Notes:

321 - The association of cow related factors with metritis cure risk, fertility, milk yield, and culling

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Session: EPIDEMIOLOGY - DAIRY CATTLE

Objective

The first step for the development of selective therapy for metritis (MET) is the identification of factors associated with increased risk of cure and economically important outcomes. Hence, the objective of this study was to assess the association of cow-related factors with MET cure risk, milk production, fertility, and culling.

Methods

A subset of cows (n= 315) enrolled in a randomized clinical trial that aimed to evaluate an alternative MET therapy were included in our study. Metritis was defined as fetid and watery discharge, and cure was defined as absence of MET signs 12 days after diagnosis. Cows either remained untreated (CON) or received subcutaneous injections of ceftiofur crystalline-free acid 3 days apart (CEF). A subset of 150 healthy cows that did not developed MET was included to compare performance and survivability. Factors evaluated included plasma concentrations of metabolites, and haptoglobin (Hp), parity, rectal temperature and DIM at MET diagnosis, vaginal laceration (VL), BCS, dystocia, twins, and retained placenta.

Results

Among CON cows, DIM at MET diagnosis was positively associated with cure, while plasma Hp concentration tended to be negatively associated with cure. The CON cows that were diagnosed with MET after 8 DIM or had plasma Hp concentration ≤ 0.54 mg/mL had milk production, pregnancy and culling risk comparable to healthy cows. Among CEF cows, DIM at diagnosis and dystocia were positively associated with cure, while VL and Hp were negatively associated with cure. Among CEF cows, Hp, DIM at diagnosis, dystocia, and VL were associated with cure. Lactational performance losses are more pronounced among CEF cows when MET was diagnosed at ≤ 5 DIM, Hp > 0.78 mg/mL, or if they had VL or dystocia.

Conclusions

In conclusion, these results indicate that timing of the onset of MET and inflammatory biomarkers could be used for the development of a selective therapy strategy for MET, but more research is needed to identify more accurate predictors of MET spontaneous cure and treatment failure.

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

Notes:

322 - Randomized control trial examining the effects of xylazine sedation in calves disbudded with a cautery iron

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Session: EPIDEMIOLOGY - DAIRY CATTLE

Objective

While there is a large body of research on pain control strategies for cautery disbudding, there is little research examining the effects of sedation on calves for this procedure. This study evaluated the effects of xylazine sedation in conjunction with a local anesthetic and nonsteroidal anti-inflammatory drug (NSAID) in calves following cautery disbudding on outcomes associated with pain and inflammation (pressure sensitivity, feeding behaviors, and struggling behaviors).

Methods

This trial was conducted from December 2018 to June 2019. One hundred and twenty-two female and male Holstein calves aged 13 to 44 days were enrolled over 9 blocks and randomly allocated to 1 of 2 treatments: 1) sedated: lidocaine cornual nerve block, meloxicam and xylazine, or 2) non-sedated: lidocaine cornual nerve block and meloxicam. Data were analyzed using mixed models with a fixed effect for baseline values and calf nested within trial block as a random effect. Linear regression was used to assess continuous outcomes and logistic regression to assess binary outcomes.

Results

Sedated calves had reduced average drinking speed at 0 to 24 hours and 24 to 48 hours following disbudding compared to non-sedated calves (24 to 48 h; -40.9 mL/min, 95% CI -76.8 to -4.9, $P = 0.03$) but there was no difference between groups in total amount of milk consumed daily ($P = 0.86$). Sedated calves had reduced pressure sensitivity (measured using an algometer) ($P < 0.01$) at 0, 60- and 240-min post-disbudding (0 min; -0.37 kgf, 95% CI -0.49 to -0.25; 60 min; -0.69 kgf, 95% CI -1.03 to -0.35; 240 min; -0.72 kgf, 95% CI -1.16 to -0.28) but there was no detected differences between groups 24 h after disbudding ($P = 0.42$). During the disbudding procedure, non-sedated calves had 4.5 (95% CI 1.5 to 13.2, $P < 0.01$) times the odds of struggling more than twice compared to sedated calves.

Conclusions

These results indicate that xylazine sedation can reduce behavioral indicators of pain in calves disbudded with a cautery iron, but also appears to impact suckling behavior for at least 48 h following sedation.

Financial Support
Boehringer Ingelheim Animal Health; Dairy Farmers of Ontario; Ontario Agri-Food Innovation Alliance

Notes:

323 - Milk characteristics, health and reproduction of dairy cows based on the temporal diagnostic of hyperketonemia

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Session: EPIDEMIOLOGY - DAIRY CATTLE

Objective

Hyperketonemia (HYK) monitoring programs commonly measure beta-hydroxybutyrate (BHB) from 3 to 14 d postpartum in dairy cattle. We aim to evaluate whether the week postpartum when HYK is diagnosed is associated with a different response on milk production and composition, herd removal, and reproductive performance in multiparous cows.

Methods

Blood samples were collected from multiparous Holstein cows (n=383) in a commercial dairy herd in Minnesota at 7±2 and 14±2 d postpartum for the measurement of blood BHB for diagnosis of HYK (BHB ≥ 1.2 mmol/L). We obtained monthly DHIA test results on milk yield and composition for a complete lactation (10 mo), and pregnancy and herd-removal records. Milk outcomes were analyzed using a Generalized Estimated Equation with exchangeable correlation structure. To evaluate pregnancy rate by 150 d and herd removal by 60 d we performed Cox proportional-hazard models. Potential confounder variables were offered to the models (i.e., body condition score, parity, and calving ease).

Results

Hyperketonemic cows (HYK+) in week 1 produced 1,067 kg (95%CI: 305, 1,830) less milk per lactation and more fat in milk than HYK- cows (Mean = 0.27%, 95%CI: 0.07, 0.46). After adjustment by HYK in week 1, HYK+ cow sin week 2 showed no difference in milk yield (0.05 kg, 95%CI: -2.48, 2.58) nor fat content in milk (0.14%, 95%CI: -0.07, 0.36) with HYK- cows. The risk of early removal was 3.9 times (95%CI: 1.66, 9.30) higher among HYK+ cows in the first than in the second week, while no difference was observed on week 2 (HR= 1.09, 95%CI, 0.74, 1.62). Similarly, the risk of becoming pregnant by 150 d was lower among HYK+ cows in the first than in the second week postpartum (HR week 1= 0.70, 95%CI: 0.48, 1.01; HR week 2= 1.09, 95%CI, 0.74, 1.62)

Conclusions

Cows diagnosed with HYK during the first week postpartum had lower milk yield and milk fat content, higher risk of being removed early in the lactation, and of becoming pregnant than HK- cows. However, these parameters were not affected among cows diagnosed with HYK in the second week postpartum.

Notes:

324 - Disbudding and dehorning practices for pre-weaned dairy calves by farmers in Wisconsin, USA

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Session: EPIDEMIOLOGY - DAIRY CATTLE

Objective

The objective of this study was to understand common dehorning and disbudding practices in Wisconsin to encourage adoption of best practices through targeted extension education programming.

Methods

A survey, comprised of 79 questions about calf health management and dehorning practices, was administered online and at extension events to a convenience sample of dairy farmers and calf raisers in 2019. Prior to survey administration, the survey was pre-tested by producers who were contacted by educators from UW-Madison. No significant survey changes were required after pre-testing, and the pilot responses were included. The results of the dehorning questions were tabulated using Qualtrics software.

Results

Wisconsin producers (n = 188) reported milking a mean of 486 cows and had a mean of 110 heifer calves being fed milk. Respondents could select multiple methods of disbudding or dehorning if used on their calves. A total of 126 producers (67%) reported using caustic paste for disbudding, most commonly on the day of birth (64; 51%) or between 1 d to 1 wk of age (52; 41%). Hot iron disbudding was reported by 112 producers (60%) and most commonly done at 8 wks of age or older (39; 35%), between 4 to 8 wks of age (38; 34%), and between 1 to 4 wks of age (30; 27%). A total of 85 (45%) respondents reported using pain control, with 28 (33%) using a combination of medications. After evaluation of individual medications, 40 respondents reported using a local anaesthetic (21%), 65 reported using an NSAID (35%) and 10 reported using a sedative (5%). The majority of respondents (96/170) reported that their use of disbudding medication had changed in the last decade, most commonly due to the influence of their veterinarian (66; 68%), and public perception/consumer demand (28; 29%).

Conclusions

Results demonstrate a proportion of surveyed farmers continue to perform disbudding without pain mitigation, suggesting a need for educational programming to encourage best practices for pain-control and disbudding at an earlier age.

Financial Support
Dairy Farmers of Ontario; Ontario Agri-Food Innovation Alliance; University of Guelph

Notes:

325 - Effects of serum total protein on health measures, average daily gain, and metabolites in the Holstein dairy calf.

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Session: EPIDEMIOLOGY - DAIRY CATTLE

Objective

Adequate passive transfer of maternal antibodies directly affects calf health. This study was conducted to determine if the association is repeatable in the production setting; by assessment of serum total protein (TP), with serum metabolites, average daily gain, morbidity, and mortality in Holstein male calves (n=1,631).

Methods

Calves were purchased from dairy farms in the western United States; individually identified, and placed in a calf ranch as one day old. Cause-specific morbidity and mortality was recorded for each calf daily from entry to exit or death. A 5-mL blood sample was collected from each animal at 48 ± 6h post-arrival, centrifuged at 2000 *g* for 10 min and serum was stored at -20°C. Serum total protein (TP) was measured using a digital refractometer. Calves were categorized based on proposed AVMA serum TP guidelines into poor (TP < 5.1 g/dL, n=159, mean ± SD 4.68 ± 0.31 g/dL), fair (5.1 < TP ≤ 5.7 g/dL, n=399, 5.45 ± 0.19 g/dL), good (5.8 ≤ TP ≤ 6.1 g/dL, n=322, 5.96 ± 0.11 g/dL) and excellent (TP > 6.1 g/dL, n=751, 5.96 ± 0.11 g/dL). Samples were analyzed using a reverse-phase HPLC using a C18 column for lipid soluble vitamins and a colorimetric assay for glucose. Morbidity and mortality data were analyzed using GLIMMIX and logistic regression models with significance declared at $P \leq 0.05$.

Results

Differences between poor and excellent TP categories were observed in mortality and otitis disease treatments ($P < 0.05$ for both). Differences were observed when comparing poor and fair to excellent categories for serum glucose ($P < 0.05$). Differences were also detected when comparing poor with all other groups for serum retinol ($P = 0.001$). Serum β-carotene , and α-tocopherol were different when comparing all categories to excellent ($P < 0.05$ for both).

Conclusions

A higher proportion of calves with adequate passive immunity were shipped from the calf ranch and fewer of them received treatments for otitis disease. Overall, serum metabolites were different among TP categories, suggesting an associative relationship with health and the immune system development and functionality.

Notes:

326 - Forecasting foot and mouth disease outbreaks using multi-model ensembles

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Session: EPIDEMIOLOGY - MODELING

Objective

Foot and mouth disease (FMD) is highly infectious transboundary livestock disease that causes significant economic and agricultural losses for infected countries. Mathematical models are often used as tools to inform preparedness and response plans for potential FMD outbreaks in countries where the disease is not endemic. It can be challenging to select a single model to base high-stakes decisions on, particularly when quality models give different, sometimes inconsistent results. Ensemble modeling methods provide a standardized and transparent way of producing a single, interpretable projection from multiple model outputs. This methodology is frequently used in weather forecasting and climate change projections and has increased the accuracy of predictions in those fields, but it has rarely been applied in a veterinary epidemiological setting.

Methods

We have developed the Bayesian Reliability Ensemble Average method (BREA) for use in epidemiological forecasting. Using a suite of FMD simulation models and data from the initial weeks of multiple FMD outbreaks, we explored whether the BREA multi-model ensemble methodology improves the accuracy of forecasts early in FMD outbreaks before the outcome is known. The FMD models included in these analyses are well established and have all been used in a policy context around the world.

Results

Our results show that the BREA method is capable of capturing the observed data from multiple different FMD outbreaks and performs better than any single model alone. We also find that this result holds even when the models are provided with only the first two weeks of outbreak data.

Conclusions

These results suggest that the BREA ensemble modeling method could be a powerful tool for epidemiological applications where outbreak data are often limited, and can reduce the confusion caused by multiple models with differing predictions by presenting a single, interpretable prediction. Ensemble modeling therefore has the potential to improve our ability to make epidemiological predictions, which would be a great benefit for animal health globally.

Financial Support
USDA National Institute of Food and Agriculture

Notes:

327 - Use of an integrated modeling approach to assess risk of windborne transmission of Foot and Mouth Disease

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Session: EPIDEMIOLOGY - MODELING

Objective

Windborne spread of Foot and Mouth Disease (FMD) requires specific epidemiological and meteorological conditions, thus modeling the risk of windborne spread involves integrating epidemiological and meteorological models. The objective of this study was to investigate the potential risk of windborne spread of FMD from an infected US feedlot using an integrated modeling approach.

Methods

We integrated a within-herd epidemiological model, an advanced atmospheric dispersion model, and calculation of infection risk dependent on exposed herd size. A previously developed epidemiological model was used to simulate the spread of FMD through a typical US feedlot, while the National Oceanic and Atmospheric Administration's HYSPLIT atmospheric dispersion model, which has been validated for FMD modeling, was used to model virus dispersion. Infection risk for exposed herds was calculated as a binomial probability accounting for dose and exposed herd size. We modeled risk of windborne spread from a typical 4000 head feedlot in IA, and a typical 48,000 head feedlot in KS during winter and summer seasons.

Results

Risk of windborne spread of FMD varied based on weather/season conditions, estimated average per head viral shedding rate, size of infected herd, and size of exposed herd. In the baseline winter scenario at peak shedding day for the infected feedlot, the median of the maximum daily risk for a 1000 head exposed herd located downwind of a KS feedlot ranged from 89.88% at 3km to 8.37% at 10km, and from 48.38% at 3km to 1.13% at 10km for an IA feedlot. Risk for larger exposed herds was greater.

Conclusions

The minimum control area recommended by USDA APHIS in an FMD outbreak is 10km from the infected premise. Our results indicate that significant risk of windborne spread may extend beyond 10km in some situations, which could be a concern, particularly in areas with large herds in relatively close proximity. Our model may be useful as a research tool in the absence of an outbreak, and to help direct surveillance and response efforts in the event of an outbreak.

Financial Support
Kansas State University

Notes:

328 - Modelling *Pasteurella multocida* dynamics within free living amoeba in soil in the Kazakhstan steppe

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Session: EPIDEMIOLOGY - MODELING

Objective

Pasteurella multocida causes several economically-important diseases of production animals and is a threat to wildlife e.g. Haemorrhagic septicaemia (HS) of ruminants. HS has caused a series of mass mortality events affecting the critically-endangered saiga antelope in the Kazakhstan steppe. Free living amoeba (FLA) have been shown to facilitate environmental survival and transmission of bacteria. *P. multocida* has been shown to invade, multiply within and lyse FLA in laboratory conditions. This project aims to explore the potential for FLA to prolong environmental survival of *P. multocida*, and thus provide a potential source of bacteria for outbreaks for grazing species.

Methods

A deterministic, discrete time-step spreadsheet model was developed to predict the dynamics of FLA and *P. multocida*in soil in the Kazakhstan steppe during 01-May to 14-June 2015-2018. Dynamics were simulated following 5Log10 CFU/g *P. multocida* being shed from a carrier animal into the environment and predictions were compared for 2 model structures (i) FLA-*P.multocida* interaction occurs and (ii) FLA-*P.multocida* interaction does not occur. The impacts of variability and uncertainty was explored using sensitivity analysis.

Results

Under the assumption FLA-*P.multocida* interaction does not occur, *P.multocida* underwent exponential decay to < 1 CFU/g within 13 days. Comparatively, under the assumption the interaction occurs, within the same 13 day time frame, *P.multocida* reached growth of > 3.18 x 105 CFU/g for all years and locations. Model predictions showed year to year variation in timing of peak *P.multocida* growth, occurring earlier in 2015 in comparison to subsequent years. Sensitivity analysis suggests the findings are robust, despite uncertainty in parameter values.

Conclusions

To our knowledge this is the first mathematical modelling study to demonstrate that FLA have the potential to significantly increase environmental survival and growth of *P. multocida*. Further field studies and experimental studies are needed to validate the model and test the hypothesis that FLA contribute to *P.multocida* outbreaks.

Notes:

330 - Leveraging evidence from meta-analyses to determine the sample size of subsequent trials

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Session: EPIDEMIOLOGY - MODELING

Objective

A critical step in trial design is determining the sample size and treatment allocation ratio to ensure the study has sufficient power to document either superiority, equivalence, or non-inferiority over a compactor(s). Currently, the approach to determining the sample size and treatment allocation ratio assumes no other data are available to answer the question. Such an approach might not maximize prior research utility if a large amount of evidence is available in the trial network. Here we investigate the increased power of studies if the design and analysis incorporate evidence from a network meta-analysis.

Methods

We illustrate the results from a network meta-analysis, which involves the reference drug product and the placebo to identify the optimal sample allocation strategy for a new clinical endpoint study. In this example, we assume resources set the sample size for the new endpoint trial. The goal is to identify the optimal allocation ratio for the new 3-arm endpoint trial, which involves the reference drug product, test product, and the placebo. The hypotheses to be tested are that the reference product and test product are equivalent, and both are superior to the placebo. Without prior evidence, an equal allocation strategy is the most powerful approach, but such an approach ignores prior evidence about some of the hypotheses of interest that is available. The optimal treatment allocation strategy minimizes the standard error of the test's log odds ratio and the reference drug products compared with other sample allocation strategies. Using simulations, we document that we can borrow information from a network meta-analysis to modify the treatment allocation ratio and increase the power of testing bioequivalence.

Results

The outcomes establish that conditioned on using prior evidence in the final analysis, the optimal allocation strategy for the placebo, reference, and test product should be 3, 26, 31 rather than 20, 20 20.

Conclusions

This methodology could improve the power of trial design, reduce the cost of trials, and maximize the utility of prior investments in research.

Notes:

331 - Exploring state-level risks for introducing chronic wasting disease into wild cervid populations

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Session: EPIDEMIOLOGY - MODELING

Objective

Chronic Wasting Disease (CWD) is a fatal, transmissible spongiform encephalopathy affecting members of the deer family, Cervidae. The prion agent can remain in the environment for extended periods of time and use multiple routes of transmission. The disease has caused population declines in species such as the whitetail deer (*Odocoileus virgnianus*) and mule deer (*Odocoileus hemionus*). Since the initial introduction of CWD into wild cervid populations in 1981, twenty-four states have diagnosed CWD in wild cervid herds. There is still a knowledge gap regarding the state-level risk factors for introducing CWD into wild cervid populations. The objective of this study was to conduct a nationwide analysis of state-level risk factors for CWD transmission in the United States.

Methods

Causal loop diagrams were developed to visualize the complex relationships concerning CWD transmission to non-endemic states. A Cox proportional hazard survival analysis model was used to test a variety of state-level characteristics to determine risks associated with the time until the introduction of CWD into a state. Significance was defined at alpha=0.05.

Results

Four factors remained significant in the multivariable model. Carcass bans (HR=0.3098), distance from the CWD epicenter (HR=0.1367), and the presence of alligators (HR=0.3117) were protective of CWD introduction into wild populations. Harvest density indicative of cervid populations per hectare was positively associated with CWD introduction (HR=1.0832 for every 1,000 cervids/h).

Conclusions

Understanding risk factors for CWD is essential for continuing efforts to prevent state cervid populations from becoming infected.

Financial Support
U.S. National Institutes of Health

Notes:

332 - Use of rehabilitation data to monitor spatiotemporal trends of wildlife health in Minnesota

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Session: EPIDEMIOLOGY - MODELING

Objective

Wildlife health surveillance is challenging. An alternative is the use of wildlife rehabilitation data as sentinels. We used rehabilitation data from two major wildlife rehabilitation centers in Minnesota with the objective to detect spatiotemporal patterns and anomalies of the submissions. We hypothesize that detecting multiple wildlife 'species groups' submitted due to similar 'circumstances', such as entrapment and habitat destruction, from the same area during the same period may indicate a major natural or manmade incident threatening the health of wildlife.

Methods

A subset of the electronic database consist of 70,295 primary care records pertaining to 266 species, collected between 2015 and 2019, were analyzed. The multivariate multinomial space-time permutation of the scan statistic and time-series analysis were used to determine the spatiotemporal clusters, seasonality, and the relatedness between species groups.

Results

The multiple spatiotemporal clusters indicated areas with more submissions of certain 'species groups' due to specific 'circumstances' than the expected threshold. The clusters were defined as anomalies and the process enabled generating hypotheses and further investigation to identify potential drivers of these anomalies including flash floods, road constructions, or diseases. The time-series analysis showed distinct seasonality and facilitated the understanding of potential temporal associations between species groups. The detected spatiotemporal patterns were compared across the study years and further discussed with clinicians of the rehabilitation centers and relevant stakeholders.

Conclusions

The strength and novelty in the approach is the use of wildlife rehabilitation data as a sentinel to detect anomalies in multiple 'species groups' submitted due to several 'circumstances' simultaneously. Identification of anomalies and the potential drivers in a near real-time manner may support informed intervention decisions to protect the health of wildlife as well as to determine disease threats to livestock and human populations.

Financial Support
Agricultural Competitiveness Improvement Project - Azerbaijan

Notes:

333 - Role of animal movements in PRRS spread in the U.S. swine industry

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Session: EPIDEMIOLOGY - MODELING

Objective

Animal movements have been linked to the spread of Porcine Reproductive and Respiratory Syndrome (PRRS) in swine populations. Recent studies have associated animal movements with the emergence of highly virulent PRRS viruses classified as RFLP-type 1-7-4, which mostly belong to the phylogenetic family known as Lineage 1A (L1A) and causing outbreaks with more severe clinical disease. This study aimed to quantify the contribution of animal movements to the risk of outbreaks with PRRS viruses of the 1-7-4 family (here, broadly referred to as L1A).

Methods

Temporal network autocorrelation modeling was performed using 3,369 animal movements and 1,761 PRRSV ORF5 sequences linked to 494 farms from a dense pig production area in the US, between 2014-2017. Farms were defined as L1A-positive in a given 6-month period if at least one L1A sequence was recovered from the farm. A farm's current and past exposure to L1A and other PRRS variants was assessed through its primary and secondary contacts in the animal movement network.

Results

Both primary and secondary farm contacts with an L1A positive farm increased the likelihood of L1A occurrence on a farm by 19% (P=0.04) and 23% (P=0.03) respectively. The likelihood of an outbreak also increased for farms that engaged in more outgoing shipments; a 3.0% (P=0.01) increase in risk was observed for every additional outgoing shipment, while the use of vaccines or field virus inoculation on sow farms a year prior reduced the risk of L1A occurrence in downstream farms by 36% (P=0.04).

Conclusions

The importance of outgoing shipments suggests that even sending animals from a farm presents infection risks, possibly through breaches in the biosecurity of the farm. Moreover, secondary farm contacts via animal movements can also be risky. The use of vaccines or field virus inoculation on sow farms a year prior reduced the risk of L1A occurrence in downstream farms, suggesting that control measures that reduce viral circulation and enhance immunological protection in sow farms have a carry-over effect on L1A occurrence in downstream farms (nurseries and finishers).

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

Notes:

334 - Tracking the dispersal of Foot-and-Mouth Disease Virus in Uganda using novel phylodynamic methods

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Session: EPIDEMIOLOGY - MODELING

Objective

The endemicity of Foot and Mouth Disease Virus (FMDV) in East Africa has significant implications for livestock production and poverty reduction, and intensifies the threat of reintroduction to areas from which FMDV has been eradicated. Despite significant impacts of the disease, its complex epidemiology in endemic settings remains poorly understood. Identifying dispersal routes and drivers of FMDV transmission is key to improved control strategies.

Methods

We harnessed the information contained in FMDV VP1 genetic sequences collected during recent robust sampling in Uganda. Using sequences from 48 locations in Uganda, we estimated FMDV spatial diffusion dynamics using a continuous phylogeographic method implemented in BEAST (Bayesian Evolutionary Analysis Sampling Trees). Next, we investigated the impact of underlying environmental and anthropogenic factors on viral dispersal using a combination of *seraphim* and a novel regression tool: a resource gradient regression model. The aim of this model is to identify differences between areas from which the virus was isolated relative to areas from which it was not isolated but could have accessed under the same space-time constraints.

Results

Mean FMDV dispersal velocity was 262.5 km/year (95% highest posterior density = 138.1 - 915.4). Utilizing a resource gradient regression model, we determined FMDV serotype O in Uganda tends to disperse from areas in close proximity to livestock markets, areas with high cattle density, and areas with infrequent droughts (viral source areas) to areas further from markets, with lower cattle densities, and frequent droughts. We speculate these trends reflects the implications of animal movements in Uganda related to animal sales and migration for pasture and water access.

Conclusions

Understanding how resource gradients determine the direction of viral dispersal allows disease response plans to anticipate viral movement and more strategically tailor interventions. Additionally, the introduction of this novel regression method will enable new lines of questions about the ecology of infectious disease transmission.

Financial Support
USDA National Institute of Food and Agriculture; U.S. Department of Defense

Notes:

335 - Predicting spatial distribution of anthrax and identifying the influencing factors in southern Kenya

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Session: EPIDEMIOLOGY - MODELING

Objective

Anthrax is a global important zoonotic disease affecting livestock, wildlife and humans with high economic impact. Anthrax recurrent outbreaks in Kenya are recorded as far back as 1957 and the knowledge of its potential risk areas and the key contributing factors is still limited. The overall objectives of this study were to predict the spatial distribution of anthrax outbreaks in Kenya as a proxy for anthrax risk and identify the key contributing factors.

Methods

Our study applied ecological niche model (ENM) of boosted regression trees (BRT) to predict the spatial distribution of anthrax in Southern Kenya. The input data included confirmed historical anthrax occurrences between 2011-2017 as presences, and spatially explicit environmental and socioeconomic data collected from public repositories. Equal number of pseudo-absences as presences data were randomly generated at each model run. Multiple model runs (n = 100) were executed and the most important predictors compared to limit potential bias from poorly derived pseudo-absence data.

Results

Our model predicted potential risk areas for anthrax outbreaks with areas covering distribution predictions at $p > 0.6$ predominantly in Lake Victoria basin, Western highlands, South-eastern region and Central highlands of Kenya. The model predictions achieved area under curve (AUC) of 0.8 indicating acceptable to excellent accuracy. The key important influencing factors with BRT relative influence > 6 were found as cattle density, rainfall of the wettest month, soil clay content, soil pH, soil organic carbon and length of longest dry season.

Conclusions

The identified potential anthrax risk areas present an early warning opportunity to attract response actions on anthrax outbreak control and management through One Health approach. The approach should target formulating sound strategies for better control of anthrax including the use of annual vaccinations of livestock and outreach programs, to reduce mortalities in livestock and minimize spill over to humans. The findings should also inform future epidemiological research in Kenya and beyond.

Financial Support
U.S. Defense Threat Reduction Agency

Notes:

336 - Estimating farm-level reproductive numbers for PRRSV using sequence-based transmission tree analysis

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Session: EPIDEMIOLOGY - MODELING

Objective

The reproductive number (R) is a fundamental value characterizing a pathogen's ability to spread within populations and is defined as the number of individuals that are infected by a single case. Many disease monitoring programs, however, archive data at the farm-level rather than individual-level. Using transmission tree analysis, we quantify the farm-level R of porcine reproductive and respiratory syndrome (PRRS), which is more compatible with the scale at which disease dynamics are analyzed.

Methods

We constructed a time-scaled phylogeny from 96 closely related ORF5 sequences from 70 farms from 2014 to 2017. Using the resulting tree, average generation time, and Bayesian inference, transmission networks were built to infer pig-to-pig infection chains, including inference on the number of unsampled individuals within the chain. Farm-level transmission events were inferred for any pair of sequences for which the infection chain transitioned between farms. We then summarized infection chain length according to movement pathlength (how many steps apart farms were in the animal movement network). The median length of infection chains for farms directly connected by movements was then used as a threshold for defining direct farm-to-farm transmission. The number of inferred direct transmission events originating from each source farm was used to calculate farm-level R.

Results

Infection chain length was significantly correlated with movement network pathlength (rho=0.59, $p< 0.05$). For farms with directly connected by movements, the median infection chain length was 35 transmission events. Overall farm-level R had a median of 1 and a maximum of 4 (IQR = 1-2). Among different farm types, finishing farms had the highest R, while sow farms were the most common recipient of transmission and had relatively low R values.

Conclusions

For this PRRSV variant, a farm is expected to infect a median of one other farm. This approach provides supportive information for epidemiologic assessments and a clearer depiction of transmission risk than solely interpreting evolutionary history or animal movement data.

Notes:

337 - Forecasting PEDV outbreaks at the farm-level in the U.S. swine industry

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Session: EPIDEMIOLOGY - MODELING

Objective

Porcine epidemic diarrhea virus (PEDv) was introduced in the US swine herds in 2013 and is considered to be endemic. Given the multi-faceted and rapidly changing nature of infection risk to farms, it is challenging for producers to estimate and respond to spatial and temporal variation in risk. Our objective is to develop a platform to predict PEDv occurrence at farm level two-weeks in advance.

Methods

Using a spatio-temporal dataset containing weekly PEDV infection status for ~30% of the U.S. sow herd, we developed a machine learning pipeline that forecasts the probability of PEDv infection in those farms. Participating stakeholders in a swine-dense region of the U.S. shared weekly information on PEDv status of farms and animal movements for the past week and scheduled movements for the upcoming week. Environmental (average temperature, humidity, among others) and land use characteristics (hog density, proportion of area with different land uses) in a 5 km radius around each farm were summarized. Using the Extreme Gradient Boosting (XGBoost) machine learning model with Synthetic Minority Over-sampling Technique (SMOTE), we developed a near real-time tool that generates weekly PEDv predictions to farms of participating stakeholders.

Results

Based on retrospective data collected between 2014 and 2017, the sensitivity, specificity, positive and negative predictive values of our model were 19.9, 99.9, 70.5 and 99.4%, respectively. Overall accuracy was 99.3%, although this metric is heavily biased by imbalance in the data (less than 0.7% of farms had an outbreak in each week). Forecasts began being sent to stakeholders in December 2019. The forecast platform also has a built-in feature to periodically re-train the predictive model in order to remain as relevant as possible to current epidemiological situations, or to expand to a different disease.

Conclusions

These dynamic forecasts, which account for recent animal movements, present disease distribution, and environmental factors, will promote data-informed and targeted disease management and prevention within the U.S. swine industry.

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

Notes:

338 - Modeling avian influenza virus transmission dynamics in migratory waterfowl to assess risk to pig populations

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Session: EPIDEMIOLOGY - MODELING

Objective

Infectious avian influenza has the potential to influence more than just bird health; this disease can have rippling effects on biodiversity, livestock, public health, and create significant economic losses worldwide (Henaux et al., 2013). Migratory waterfowl (MWF) are the wildlife reservoir for avian influenza virus (Krause et al., 2004) and the source of spillover events that can infect other wildlife and susceptible livestock. However, influenza dynamics in MFW remain unquantified and routes of transmission to livestock remain unidentified. Here, we use a long-term dataset that describes influenza seroprevalence in avian wildlife in Ohio from 1976 to 2015 to quantify transmission among mallards (*Anas platyrhynchos*) and other MWF. We also quantify the risk of transmission from avian wildlife to swine.

Methods

We fit age-structured catalytic and reverse catalytic transmission models to serological data to estimate the time-varying force of infection and the rate of waning immunity.

Results

The models indicated varying transmission rates based on the sample year, sample month, and mallard age (mature or immature). Additionally, they suggest that immunity can wain, allowing for possibility for re-infection of avian influenza within the same year. From this, we simulated a model to determine the probability of swine influenza infection from MFW during the fall and validated our final model with data that describes swine influenza infections from 2015 to 2019.

Conclusions

Our computational tools, calibrated with timeseries prevalence data, quantify reservoir dynamics of influenza and identify how migration contributes to cross-species transmission. This work can improve disease surveillance in both bird and swine populations and contribute to more accurate disease risk assessment among swine production.

Henaux et al., J Appl Ecol. 2013; Krauss et al., Vector Borne Zoonotic Dis. 2004

Financial Support
Boehringer Ingelheim Animal Health

Notes:

339 - Modeling swine movement patterns and disease surveillance at the U.S. national scale

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Session: EPIDEMIOLOGY - MODELING

Objective

Introduction and spread of transboundary animal diseases (TADs) are a major threat to the US agricultural system. A variety of tools that incorporate data from multiple sources aim to support science-based decision-making, but these tools must be developed in advance of an outbreak in order to provide timely response. Thus, our objective is to develop data driven swine shipment and disease surveillance models for the US that can be used to better understand the surveillance for TAD and other swine diseases.

Methods

Understanding swine shipment is a critical component to managing long-distance livestock disease spread, but because all animal shipments are not recorded in the US, models that accurately predict animal shipments below the state level are needed. Our earlier work created the US Animal Movement Model (USAMM) and the US Disease Outbreak Simulation Model (USDOS) based on cattle shipments. We have collected swine movement data that allows development of USAMM and USDOS for swine. We also combine swine movement and slaughter data in order to understand the geographic coverage of slaughter surveillance.

Results

Here we show preliminary results for USAMM-Swine to provide the first predictions of the numbers and sizes of swine shipments at the national scale 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:

340 - Assessing randomized controlled trial designs and statistical analyses for the comparative efficacy of treatments

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Session: EPIDEMIOLOGY - MODELING

Objective

The designs of feedlot trials are often reported as a workflow for allocating animals to treatment. The workflows lead to designs with different type I and type II error rates due to differing control of clustering i.e., arrival cohort or pen. We evaluated the impact of the workflows on the type I and type II errors, and assess the effect of missing information about clusters on possible statistical models.

Methods

We evaluated 5 workflows, 4 treatment fixed effect sizes, 2 outcomes (continuous/binary), and 3 statistical models with different structures of random effects. The workflows differ in the order and unit of allocation of animals to pen and treatment. In design 1, treatments are randomly assigned to individual animals, and individual animals are independently randomized to pens. In design 2, animals are allocated to pens by arrival cohort, while treatments are assigned randomly to animals within each pen/arrival cohort block. In design 3, animals are allocated to pens by arrival cohort and pens are randomly allocated to treatments. In design 4, animals are randomly allocated to pens, and pens are randomly allocated to treatments. Design 5 is a cluster randomized trial by a 2 -pen block. The models were a model with no random effects, a model with a pen-level random effect only and a model with pen-level and arrival cohort random effects.

Results

For binary data, for model 1 the type I error rates of design 3, 4 and 5 are larger than 5% and less than 5% for design 1 and 2. As expected, type I error decreased when model 3 is applied in design 3, 4 and 5. When the difference between the two treatment groups increased, the power increased. Design 1 and 2 have higher power compared to the others when appropriate analyses are used to control random effects. For the continuous outcome, the patterns of power and type I error change were reflected the binary situation.

Conclusions

The full model with all random effects should always be used for analyses. Design 1 and 2 are superior as they control type I and type II errors.

Notes:

341 - An ice-cold independent competent cell preparation for gene editing by CRISPR-Cas9

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Session: GENOMICS

Objective

The CRISPR-Cas9 system involves a genome-spying tool (i.e. gRNA) and the powerful DNA cutter Cas9 enzyme. It has revolutionized biology to enable us delete, edit, insert or substitute genes for modifying the phenotype of an organism. Electroporation is one of the efficient CRISPR-Cas9 delivery method into a competent cell, but competent cell (i.e. fragile cell and prone to death) preparation and handling need ice-cold.

Methods

This study presented a simple competent cell preparation method at room temperature (24°C) without dependency on ice-cold followed by delivery of CRISPR-Cas9 and editing of the *tol*C gene of *E.coli* O157:H7 (EDL933). I used DH5a as a cloning host for quality control. Both *E.coli* grew in 10 ml LB broth overnight and transferred 1ml into 100ml LB broth to grow further (OD_{600}=0.3-0.6). Then centrifuged 1.5ml of the culture at 9000rpm at 24°C for 2 min, washed twice with 1ml buffer (water or 300mM sucrose), and resuspended the pellets in 30µl respective buffer. pCas, pTargetF or pUC19 plasmids (50ng/µl each) were added to the 30µl EDL933 or DH5a, electroporated, resuspended in 1ml LB broth, incubated for 1h, and plated on LB agar with kanamycin or spectinomycin (50µg/ml each). Afterward, the method was used for the edition of EDL933 *tol*C gene by CRISPR-Cas9. I designed the gRNA primers against *tol*C gene using CRISPRseek command in R software. First, pCas (also encoded lamda red) was electroporated into EDL933. Next, the gRNA primers were inserted into pTargetF by Q5®site-directed mutagenesis PCR followed by treating it by KLD enzymes. pTargetF and HiBiT luminescence tagged donor DNA fragment (300ng/µl) for homology directed repair were electroporated into the pCa9 harboring EDL933.

Results

Over 10^7 cells were transformed per µg of plasmid DNA used; water seemed more efficient than 300mM sucrose. 42.9-87.7% of CRISPR-Cas9-HiBiT donor DNA exposed cells (n=28) emitted luminescence than the nonexposed wild-type cells (0%; n=7) by GloMax discover.

Conclusions

The current competent cell preparation method is simple, saves resources, and suitable for gene edition by CRISPR-Cas9.

Notes:

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

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Session: GENOMICS

Objective

Animal health is a critical component of dairy cattle productivity; however, current genomic selection genotyping tools have a paucity of genetic markers within key immune gene clusters (IGC) involved in the cattle innate and adaptive immune systems. We sought to assemble IGC haplotypes, identify single nucleotide polymorphisms (SNP) that distinguish each haplotype, and quantify their effect on animal health phenotypes.

Methods

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

Results

Alignment of whole genome shotgun data from 125 Holstein bulls to these alternative haplotypes revealed 55,410 SNPs; however, many of these variant sites were unsuitable for use on custom genotyping arrays. Using model-based and machine-learning approaches, we selected 124 of these markers for custom genotyping. We found that 105 (~85%) of our markers had genotype call-rates greater than 80% in the Holstein and beef cohorts. We identified two markers from a preliminary analysis of the BVD PI cohort with significant effects when one or two copies were present (relative risks of 4.26 and 0.13, respectively).

Conclusions

We demonstrate that our approach is suitable for identifying genetic markers in highly polymorphic regions of the cattle genome. Furthermore, our machine learning models can be applied to other datasets for the selection of informative genetic markers in subsequent genomic selection studies.

Financial Support
USDA National Institute for Food and Agriculture

Notes:

344 - Investigation of the host genetics role in PCV2 infections

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Session: GENOMICS

Objective

Porcine circovirus 2 (PCV2) is an etiological agent that impact production efficiency and can lead to mortality. The majority of pigs in a typical farm infected with PCV2 do not display clinical symptoms and there is no test to predict susceptibility. The objective of this study was to identify genes, DNA variants and pathways that could predict susceptibility to PCV2.

Methods

A subset of the samples (n=215) representing the tails of the distribution for PCV2 viral load from a PCV2 dataset (n=974) were genotyped with *SowPro91,* a custom genotyping array (105,601 SNPs). Since the majority of the PCV2 dataset (78%) was genotyped with the limited Porcine SNP60 BeadArray (53,529 SNPs), a Bayesian method (BayesIM) that utilizes haplotypes instead of individual SNPs in association analysis was implemented to infer *SowPro91* haplotypes to the entire PCV2 dataset. Haplotypes were assigned to individuals based on similarity using a hidden Markov model and used as covariates in GWAS.

Results

GWAS of experimentally infected pigs (n=974) uncovered QTLs (SSC7 and SSC12) associated with PCV2 viremia and immune response. The QTL on SSC7 was near SLAII locus, involved in antigen recognition and immune response. Since the SNP60 BeadArray is scarce in SNPs located at this locus (45 SNPs), *SowPro91* was designed to saturate SLA region with 4,432 SNPs. A BayesIM approach, which is alternative to imputation, inferred *SowPro91* haplotypes to the entire PCV2 dataset. The position of the SSC7 QTL was dependent on the prior haplotype size (12 to 250 kb), with QTL locations ranging from 22.1 Mb to 24.65 Mb. For both QTLs (SSC7 and SSC12), the largest model frequency, an indicator of the QTL effect, was observed at the largest prior haplotype size (250 kb). We expect that an increase in the size of the data will improve the consistency of the QTL location, providing potential genes and DNA variants associated with PCV2 susceptibility.

Conclusions

This research aids in the development of mapping approaches to identify DNA variants associated with PCV2 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:

345 - Identification of BoLA alleles associated with BLV provirus levels in US beef cattle

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Session: GENOMICS

Objective

The most recent USDA survey estimates that 38% of beef cattle in the United States are seropositive for Bovine Leukemia Virus (BLV). Cattle that develop bovine leukosis as a result of BLV infection have impaired immune function, reducing productivity and lifespan likely due to increased susceptibility of opportunistic infections. Additionally, nearly 5% of infected cattle develop lymphomas leading to slaughter condemnation, a substantial economic burden to beef operations. Several lines of evidence have suggested that specific Bovine Leukocyte Antigen (BoLA) alleles are associated with BLV resilience in dairy cattle. The BoLA-DRB3 allele encodes for a heterodimeric cellular receptor responsible for antigen recognition and presentation to T cells. The objective of this study is to assess host BoLA-DRB3 haplotypes in the context of BLV proviral load (PVL) to determine whether these genetic associations exist in beef populations.

Methods

Our group has developed a next-generation sequence-based typing approach to determine BoLA haplotypes. Blood samples from 3,146 beef cows from 27 different US cow/calf herds were analyzed for BLV antibodies via ELISA. A subpopulation of 648 animals that were ELISA-positive were tested for BLV PVL, 177 animals had detectable levels of BLV provirus. Endpoint PCR was used to amplify and barcode the second exon of the BoLA-DRB3 gene per animal. Resulting amplicons were sequenced to identify each animal's BoLA-DRB3 alleles.

Results

This data will be combined with animal metadata to determine associations between BoLA-DRB3 haplotype to PVL in Midwest beef cattle populations and associations with longevity, co-morbidity incidence.

Conclusions

This dataset will be one of the first studies to determine BoLA-DRB3 allelic frequency in the context of BLV infections within Midwestern beef cattle.

Financial Support
Michigan State University

Notes:

346 - Response of the lung transcriptome to antiviral and ibuprofen in calves with respiratory syncytial virus infection

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Session: GENOMICS

Objective

Bovine respiratory syncytial virus (BRSV) plays a major role in etiology of the bovine respiratory disease complex. In this work calves also served as a model for understanding human respiratory syncytial virus infection due to a similar pathogenesis and clinical manifestation in infants. The goal of this study was to evaluate *in vivo* the effect of ibuprofen and fusion protein inhibitor (FPI) on gene expression in lung tissue of calves infected with BRSV.

Methods

Calves were divided to 6 treatment groups: 1) ibuprofen day 3-10, 2) ibuprofen day 5 -10, 3) placebo, 4) FPI day 5-10, 5) FPI and ibuprofen day 5-10, 6) FPI and ibuprofen day 3-10 and were infected with BRSV (day 0). At necropsy (day10) lung tissue with lesions (LL) and non lesion (NL) was collected, total RNA extracted and library prepared. TagSeq data (Illumina) were acquired and differential gene expression (DE) analysis was conducted using limma-voom. Gene ontology (GO) and KEGG pathway enrichment analysis was performed using Bioconductor/GO.db-TopGO-KEGGREST and Cytoscape/ClueGO.

Results

DE analysis between LL and NL tissue in each treatment group showed highest number of DE genes in the placebo group. GO enrichment demonstrated that stress-related terms were specific to the placebo. Terms of innate and adaptive immune functions were common to all groups. GO enrichment analysis with whole transcriptome showed negative regulation of viral genome replication in groups 1 and 2 and elevated activity of the immune protection in groups not treated with FPI. GO and KEGG pathway enrichment analysis in LL vs LN tissue in all treatment groups in comparison with the placebo group demonstrated that immune response function terms and pathways associated with defense against pathogens were the most abundant in NL tissue in group 6.

Conclusions

Therefore, gene expression profile in lungs demonstrated that damage- and stress-associated mechanisms were shown in the placebo group and immune response mechanisms were common to all groups. In comparison to the placebo, combined FPI and ibuprofen treatment, started on day 3, was most distinguishable.

Financial Support
USDA National Institute for Food and Agriculture

Notes:

347 - Early splenic responses to avian pathogenic *Escherichia coli* infection identified in downstream RNA-seq analyses

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Session: GENOMICS

Objective

Poultry growth, egg production, and even survival can be severely decreased when infected with avian pathogenic *Escherichia coli* (APEC). Increased understanding of how immune tissues, like the spleen, respond to APEC would facilitate efforts to improve veterinary and animal breeding strategies for controlling this pathogen. This study used RNA-sequencing (RNA-seq) to identify transcriptome-wide gene expression changes due to infection and downstream analyses to reveal the roles of regulatory networks.

Methods

RNA-seq was performed on spleen tissues collected from a total of 46 F_1 chickens (reciprocal Fayoumi and broiler crosses) at 1 or 2 days post inoculation (DPI) with APEC O1:K1:H7 or sterile PBS. After read mapping to the chicken genome and detection of genes with differential expression, these datasets were further utilized to investigate alternative splicing using transcript-level responses, co-expression patterns among genes with shared regulators, and unequal allelic expression (known as allele specific expression; ASE) as evidence for *cis*-acting regulation.

Results

Significant splenic responses to APEC included 580 genes at 1 DPI and 157 at 2 DPI, with pro-inflammatory genes (such as *IL22*, *IL17A*, and *PTX3*) highly up-regulated by infection. Responses of transcript variants were related to but distinct from the gene-level analysis. Ten sets of co-expressed genes were significantly associated with APEC infection, revealing regulatory networks of the immune response and cell division. Over 8,000 single-nucleotide polymorphisms in the transcriptome data had significant ASE; those ASE loci observed uniquely in the APEC-challenged samples could provide markers for regulatory elements impacted by APEC infection.

Conclusions

Transcriptome analysis provided insight on early responses to APEC in the chicken spleen, with added knowledge from downstream applications like co-expression and ASE. Support: USDA-NIFA Agriculture and Food Research Initiative Competitive Grant #2015-67015-23093 as part of the joint NIFA-BBSRC Animal Health and Disease program and by Hatch project #5424 and #5620.

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

Notes:

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

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Session: GENOMICS

Objective

We have been pursuing the underlying genetics of ascites in broilers. We used whole genome resequencing (WGR) in our ascites experimental research lines to identify 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. WGR in the commercial lines used individually barcoded DNAs for better resolution.

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 highly variable and dependent on the specific genetic background and gender. We used MAS to generate a breeding flock that is homozygous for the non-reference genotypes for both CPQ and LRRTM4. Progeny from this flock were evaluated for ascites phenotype in a hypobaric challenge, and separately evaluated for changes in production traits. Ascites incidence was reduced by 25-40% and there were no significant losses in production traits.

Conclusions

Our goal is to define the most significant loci for breeding against ascites in broiler chickens. The MAS work shows that we can change the incidence of ascites without significant production loss. 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
USDA National Institute of Food and Agriculture

Notes:

349 - Effect of parturition and lactation on adipose tissue transcriptomic profiles in dairy cows

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Session: GENOMICS

Objective

Periparturient cows rely on adipose tissue (AT) fatty acid reserves released by lipolysis to offset negative energy balance. However, lipolysis induces inflammation and structural remodeling in AT that in excess predisposes cows to disease. Currently, transcriptomic profile changes induced by lipolysis in periparturient AT are poorly characterized. The objective of our study was to perform next generation sequencing of bovine AT during the periparturient period.
Subcutaneous AT samples were collected from Holstein cows (n=12) at 11 ± 3.6 d before calving date (PreP) and at 6± 1 d (PP1) and 13 ± 1.4 d (PP2) after parturition. RNA was extracted and sequenced by Illumina NextSeq. Data was filtered and mapped to bosTau7 genome following by detection of differential expressed genes (DEG, fold changes > 1, False Discovery Rates (FDR) < 0.05). DEG enrichment and network analyses were performed in Ingenuity Pathways and Metascape under hypergeometric distribution.
Differential expression analysis showed 1524 and 1946 DEG at PP1 and PP2 respectively compared to PreP. Functional enrichment analysis revealed functions grouped in categories related to lipid metabolism, molecular transport, energy production, inflammation, and free radical scavenging affected by PP1 and PP2. Inflammation related genes *P38 MAPK*, *TLR4*, *IL1*, and *IL6* were categorized as upstream lipolysis triggers. In contrast, *FASN, ELOVL6, ACLS1,* and *THRSP* were identified as upstream inhibitors of lipid synthesis. *CHUK*, an activator of *NFKB*, was identified as an inductor of reactive oxygen species generation through inflammatory pathways that included genes such as *C3*, *CXCL2*, and *HMOX1*.
Our results offer a comprehensive characterization of gene expression dynamics in periparturient AT, identify upstream regulators of AT function, and demonstrate complex interactions between lipid mobilization, inflammation, extracellular matrix remodeling, and redox signaling in the adipose organ.

Methods

Subcutaneous AT samples were collected from Holstein cows (n=12) at 11 ± 3.6 d before calving date (PreP) and at 6± 1 d (PP1) and 13 ± 1.4 d (PP2) after parturition. RNA was extracted and sequenced by Illumina NextSeq. Data was filtered and mapped to bosTau7 genome following by detection of differential expressed genes (DEG, fold changes > 1, False Discovery Rates (FDR) < 0.05). DEG enrichment and network analyses were performed in Ingenuity Pathways and Metascape under hypergeometric distribution.

Results

Differential expression analysis showed 1524 and 1946 DEG at PP1 and PP2 respectively compared to PreP. Functional enrichment analysis revealed functions grouped in categories related to lipid metabolism, molecular transport, energy production, inflammation, and free radical scavenging affected by PP1 and PP2. Inflammation related genes *P38 MAPK*, *TLR4*, *IL1*, and *IL6* were categorized as upstream lipolysis triggers. In contrast, *FASN, ELOVL6, ACLS1,* and *THRSP* were identified as upstream inhibitors of lipid synthesis. *CHUK*, an activator of *NFKB*, was identified as an inductor of reactive oxygen species generation through inflammatory pathways that included genes such as *C3*, *CXCL2*, and *HMOX1*.

Conclusions

Our results offer a comprehensive characterization of gene expression dynamics in periparturient AT, identify upstream regulators of AT function, and demonstrate complex interactions between lipid mobilization, inflammation, extracellular matrix remodeling, and redox signaling in the adipose organ.

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

Notes:

350 - Multi-tissue transcriptomic analysis of BRSV-infected cattle using machine learning and statistical approaches

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Session: GENOMICS

Objective

Bovine respiratory syncytial virus (BRSV) is a viral pathogen that replicates in upper and lower airways, contributing to bovine respiratory disease (BRD) in spite of available vaccines. Transcriptomic evaluation of the host response to BRSV across multiple tissues may elucidate underlying mechanisms involved in BRD. Our objective was to identify genes and enriched pathways associated with BRSV infection through machine learning (ML) and statistical applications.

Methods

Raw sequencing reads from 65 bovine samples across six tissue sites (n=36 BRSV, n=29 control) were assembled with the ARS-UCD1.2 genome assembly in a HISAT2/Stringtie2/prepDE pipeline. Raw gene counts for ML analysis were normalized, transformed, and analyzed with MLSeq, utilizing six ML algorithms. Cross-validation parameters (5-fold, repeated 10 times) were applied in a 70:30 training/testing ratio. Statistical analysis of raw counts was performed with edgeR likelihood-ratio testing (glmLRT) with a q-value (FDR) cutoff of 0.05. WebGestalt, Reactome, and String were utilized for downstream analysis.

Results

ML analysis revealed 150 genes that classified individuals with 80.0-100.0% accuracy. EdgeR analysis identified 188 differentially expressed genes; 53 genes were shared across ML and edgeR analyses. Pathways involving type I interferon signaling, IL-10 signaling, DDX58/IFIH1 regulation, and MHC-I antigen processing/presentation were increased in BRSV-infected cattle. ATP synthesis via the citric acid cycle/respiratory electron transport and body temperature regulation pathways were decreased in BRSV-infected cattle.

Conclusions

Using machine learning and statistical approaches, we described distinct genes and pathways important for understanding clinical BRD associated with BRSV infection. This approach provides new information regarding genomic mechanisms relevant to BRD pathogenesis and immunity.

Notes:

351 - Distinct gene expression patterns associated with BRD identified by statistical and machine learning approaches

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Session: GENOMICS

Objective

Bovine respiratory disease (BRD) is a multifactorial disease complex involving host immune interactions with several distinct bacteria and viruses. Advancements in RNA sequencing support improved molecular understanding associated with BRD. Machine learning (ML) approaches with transcriptomic data can identify novel genes in molecular research. Our objective was to apply ML models to classify gene expression related to clinical BRD across multiple RNA-seq datasets and compare results to statistical methodology.

Methods

Raw sequencing reads from 170 bovine samples (4 studies; n=136 BRD, n=34 control) were downloaded from NCBI-GEO. Quality filtered reads were assembled in a HISAT2/Stringtie2/prepDE pipeline. Raw gene counts for ML analysis were normalized, transformed, and analyzed with MLSeq, utilizing six ML algorithms. Cross-validation parameters (5-fold, repeated 10 times) were applied in a 70:30 training/testing ratio. Statistical analysis was performed with edgeR likelihood-ratio testing (glmLRT) with a q-value (FDR) cutoff of 0.05. WebGestalt, Reactome, and String were utilized for downstream analysis.

Results

The top ML algorithm (PLDA2) revealed 35 genes, primarily representing type I interferon signaling, that classified individuals with 80.8% accuracy. EdgeR analysis discovered 203 DEGs, representing extracellular matrix/collagen degradation, striated muscle development/organization, innate and humoral immunity, and proinflammation. Five genes were identified in both ML and edgeR analyses, representing type I interferon signaling and antiviral defense.

Conclusions

Genes identified in each analysis represent distinct genomic events relevant to understanding and predicting clinical BRD. The few genes shared across analyses may be reliably associated with BRD. Our analysis demonstrates powerful techniques for discovering functional information to predict and understand BRD acquisition.

Notes:

353 - Study of virulence factors and antimicrobial resistance in *S. suis* isolates using whole genome sequencing

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Session: GENOMICS - BACTERIOLOGY

Objective

Streptococcus suis is closely associated with meningitis in pigs. There are 29 known *S. suis* serotypes and they are ubiquitous in swine and, yet only a small percentage of pigs become clinically ill. The objective of this study was to observe the difference in genetic virulence determinants carried by *S. suis* isolates recovered from systemic and non-systemic sites of sick and healthy pigs. Whole genome sequencing (WGS) was used to serotype the isolates and determine the distribution patterns of known/putative virulence associated factors (VAFs) and antimicrobial resistance (AMR) genes in *S. suis* isolates recovered from nursery pigs on 17 Ontario farms.

Methods

In total, 273 *S. suis* isolates recovered from 112 pigs (47 systemic and 139 non-systemic isolates from 65 sick pigs and 90 non-systemic isolates from 47 healthy pigs) were subjected to WGS in this study. The isolates were serotyped *in silico* using the WGS data. The sequences were then annotated, and gene presence/absence data of VAF and AMR genes were determined.

Results

The most frequent serotypes detected were serotypes 2, 9 and untypables. Over all, the presence of VAF genes in *S. suis* isolates recovered from systemic (Kruskal-Wallis (KWT) X^2= 30.386, p = 3.5e-08) and non-systemic (KWT X^2 = 34.315, p = 4.6e-09) sites in sick pigs was higher compared to isolates recovered from non-systemic sites in healthy pigs Although many VAFs were abundant in all isolates, *apuA*, *dltA, pgdA, guaA, AgI/II, luxS, dpr, and troA* genes were more prevalent in the isolates recovered from systemic sites of sick pigs. At least one AMR associated gene was carried by 99% (276/277) of the isolates. The most frequent detected AMR genes were tetracycline (*tetO*), and macrolides, lincosamides and streptogramin (MLS) (*ermB*).

Conclusions

In general, it was observed that distribution of VAFs correlated with *S. suis* disease. Contrarily, the distribution of AMR genes was positively associated with isolates from healthy pigs. The results of this study may contribute to the development of pathogen control measures for *S. suis* in pigs.

Financial Support
Ontario Pork; University of Guelph; Government of Canada

Notes:

354 - Exploring mechanisms of accessory genome evolution the clonally evolving *Mycobacterium tuberculosis* complex

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Session: GENOMICS - BACTERIOLOGY

Objective

Variability in bacterial gene content can arise through gene addition via horizontal gene transfer (HGT) or gene deletion. HGT is known to generate genetic diversity in prokaryotic pangenomes; however, there is no known mechanism for HGT in the Mycobacterium tuberculosis complex (MTBC). Here we investigate the evolutionary forces that shape the *Mycobacterium bovis* pangenome, and describe a mechanism for accessory genome variability in the absence of HGT. Using a combination of detailed epidemiologic data and whole genome sequencing data, we describe the evolution of the *M. bovis* pangenome both over the course of an outbreak and over distant evolutionary time. We suspect that gene deletion is the major driver of pangenomic diversity in the MTBC and hypothesize that over the course of an outbreak the *M. bovis* accessory genome will either remain unchanged or will decrease in size. Furthermore, we expect to observe a reduction in accessory genome diversity over spatiotemporal distance from the ancient source MTBC population.

Methods

The *M. bovis* pangenome was constructed using panaroo from over 2000 genomes assembled *de novo*. Population structure was evaluated, and maximum likelihood phylogenies were created for core genes. Outbreak-specific ancestral recombination graphs were created to evaluate pangenome evolution over the course of well-traced outbreaks. Accessory gene variation as a function of evolutionary time and geographic location from the ancestral MTBC location was assessed using linear regression.

Results

Accessory gene content differences were negligible over the course of single-introduction outbreaks. Accessory compositional diversity was highest in genomes from Africa, consistent with the hypothesis that variation in gene content decreases as a function of time and distance from the source population.

Conclusions

M. bovis accessory gene variation is driven by gene deletion. This provides support against ongoing HGT in the MTBC, which has important implications in studying the evolution of disease phenotypes and in epidemiolocal tracing.

Financial Support
USDA National Institute for Food and Agriculture

Notes:

355 - Analytic approach impacts pathogen population structure when analyzing whole-genome sequence data

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Session: GENOMICS - BACTERIOLOGY

Objective

The genotyping of bacterial pathogens is critical for outbreak investigations and whole-genome sequencing (WGS) is increasingly employed as a novel tool for characterizing bacterial genomes due to unprecedented levels of precision now possible. However, the best approach for determining "sequence relatedness" between WGS samples is still unclear, with many options available and new tools being consistently developed. The overall goal of this project is to support an accurate, reproducible, transparent, and uniform approach to WGS analysis for purposes of outbreak detection and pathogen surveillance.

Methods

To achieve our overarching objective, we plan to utilize a systematic analytic experiment to generate 15 different datasets grouped by sample type, sequence quality, geographical source, host species, and a random selection of genomes for 3 bacterial pathogens in NCBI's pathogen database; Salmonella spp., Escherichia/Shigella, and *Listeria monocytogenes*. Each dataset will be analyzed using both the core- and pan-genomes, in addition to each of the following four comparative approaches; based on single nucleotide polymorphisms (SNP-based); k-mer-based; gene-by-gene allelic comparison (also termed a core genome or whole-genome MLST comparison), and finally a novel comparison based on functional domains.

Results

In this presentation, we provide preliminary results based on analysis of 37 *S. enterica* genomes identified by the Centers for Disease Control and Prevention (CDC) as being associated with a multi-state outbreak. We report that outbreak clusters can be misidentified depending on the WGS software used to analyze sequencing data and explore how this can impact outbreak investigations.

Conclusions

We highlight ongoing computing challenges in analyzing large genomic datasets and also present evidence that choices in analytic methods can impact the results garnered from WGS analysis. The results from this study will serve as a guideline to help inform other research teams with decision making around selecting available tools for WGS analysis in outbreak investigations.

Notes:

356 - Distinct transcriptional profiles of *Leptospira borgpetersenii* serovar Hardjo strains JB197 and HB203

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Session: GENOMICS - BACTERIOLOGY

Objective

Leptospirosis is a global zoonotic, neglected tropical disease. Symptoms range from asymptomatic to fatal multi-organ failure in severe cases. *Leptospira* colonize the kidneys of their host and are shed in urine into the environment. Complex species-specific interactions exist between hosts and the species, serovar, and strain of *Leptospira*. *Leptospira borgpetersenii* strains HB203 and JB197 have a high level of genetic homology and are serologically identical as serovar Hardjo. However, in the hamster model, HB203 colonizes the kidney presenting with chronic shedding while JB197 causes severe organ failure and mortality. This study examines the differential gene expression profiles of HB203 and JB197 cultured *in vitro* at 29°C (environment) and 37°C (host) reflecting common temperatures *Leptospira* would be exposed to during their natural life cycle.

Methods

L. borgpetersenii serovar Hardjo type bovis strains JB197 and HB203 were isolated from the kidneys of experimentally infected hamsters and cultured at 29°C and 37°C. RNA-seq was performed on cultured Leptospires. Supportive pathway analysis, RT-PCR, and protein blots were also performed.

Results

Specific contrasts of interest were investigated. Between JB197 29°C vs 37°C 135 genes were significantly differentially expressed, 42 genes between HB203 29°C vs 37°C, 440 genes between 29°C JB197 vs HB203, and 179 genes between 37°C JB197 vs HB203 comparisons. Differentially expressed genes include known virulence factors such as *ligB*, as well as candidate genes for further investigation with uncharacterized protein products. Confirming *Leptospira*'s sensitivity to their environment, pathway analysis suggests important roles for metabolic parameters.

Conclusions

Significant differential expression variation exists between strains of *Leptospira* as well as is evident between culture conditions within strain. Characterization of the strain-specific behavior of Leptospires, as well as the mechanisms behind environmental sensitivity, are critical to the development of targeted preventative and treatment therapies.

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

Notes:

357 - Genomic islands analysis of *Klebsiella pneumoniae* associated with bovine mastitis

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Session: GENOMICS - BACTERIOLOGY

Objective

Genomic islands (GIs) are gene clusters in bacterial genome that appear to be obtained by horizontal gene transfer, granting bacteria extra phenotype features to either better occupy a new niche or to be a pathogen. GIs or specifically pathogenicity islands (PAIs) are widely present in the genomes of many bacterial pathogens including mastitis *E. coli*. However, research on GI has rarely been done in *Klebsiella pneumoniae* (Kp), one of the most common bacterial pathogens causing the costly disease bovine mastitis in dairy industries worldwide. We hope our current and future studies will lead to a better understanding of epidemiology and pathogenicity of virulent Kp strains causing bovine mastitis.

Methods

We performed a bacterial genomic epidemiological study with 81 strains of mastitis Kp previously. To better explore the distribution and function of GIs in mastitis Kp strains, 8 out of 81 (7 strains from bovine mastitis milk and 1 from retail meat) genomes were assembled into complete circular chromosomes and putative GIs were predicted by using a combination of several methods. A GI-like region pool was constructed based on 7 mastitis strain sequences and screened later through three different Kp strain collections: 81 bovine mastitis strains, 26 human pathogenic strains, and 24 dairy environmental strains. Comparisons on each GI's prevalence and structure were conducted.

Results

As our result presents, each mastitis strain contained 25-27 GI-like regions (mean = 26, SD = 0.81), while the meat strain contained up to 35. A GI-like region pool was constructed based on 7 mastitis strain chromosomes, 63 GI-like regions were predicted as putative GIs. Existence of 24 potential integrases were identified in the 63-GI-pool which were often neighbored by one or two IS family transposases. Comparison outputs revealed 6 GIs with relatively higher prevalence in mastitis Kp strains, with either metabolic, fitness, symbiosis or putative virulence gene clusters harbored. Notably, One GI was found with putative pyocin coding genes, which might be one of the virulence factors in assisting Kp strains to compete with other bacterial pathogens. Several other GIs were further identified with complete gene clusters encoding carbohydrate-specific enzyme II (EIIs), which are essential components of bacterial phosphotransferase system functioning in obtaining carbohydrates to adapt different environment. Besides, genes associated with type VI secretion system as well as phages were also identified within some GIs. Moreover, some other GIs were found either prevalent in human/environmental strains or in all three sourced Kp groups rather than only prevalent in mastitis strains. The phenotypic and functional assays of selected GIs are under conducting in our lab.

Conclusions

As a widely existing environmental bacteria, bovine mastitis Kp is active in communicating within and beyond species at genetic level in its living niche. Featured GIs as parts of mobile acquired genes play a crucial role in forging Kp strains into a bovine mastitis pathogen.

Notes:

358 - Evolution of Mexican-lineage low pathogenic avian influenza (H5N2) viruses in Dominican Republic, 2007-2019

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Session: GENOMICS - VIROLOGY

Objective

The first outbreak of Mexican-lineage H5N2 low pathogenic avian influenza virus (LPAIV) in Dominican Republic was reported in 2007. However, limited information and genetic sequence have been reported since the initial detection. Here, we sequenced the H5N2 LPAIV identified in Dominican Republic during 2007-2019 and investigated the genetic diversity and evolutionary history using phylogenetic approaches

Methods

We sequenced the complete genome of 19 H5N2 LPAIV with Illumina Miseq system. The Maximum-likelihood and Bayesian relaxed clock phylogenetic analysis of the 8 segments were performed and the most recent common ancestor (tMRCA) of each clade was estimated. The potential N-glycosylation sites on hemagglutinin (HA) were predicted and visualized

Results

Our data support a single introduction of Mexican-lineage H5N2 LPAIV into poultry in Dominican Republic and subsequent divergence into three distinct genetic subgroups during 2007-2019. The inferred tMRCA for each gene ranged from February 2005 through August 2006, suggesting that ancestors of these viruses emerged from Mexican-lineage H5N2 LPAIV during this period. The changes in N-linked glycosylation pattern, particularly at antigenic sites, in HA protein has been identified which may impact protection when using historic Mexican-lineage H5N2 vaccine strains. The acquisition of an additional basic amino acid in the HA cleavage site also raises a concern regarding the increased risk of mutation to a highly pathogenic form

Conclusions

This study highlights the need for enhanced surveillance of poultry and genetic/antigenic characterization of avian influenza viruses in Dominican Republic

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

Notes:

359 - The role of host genetics on transmission of Marek's disease virus in poultry

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Session: GENOMICS - VIROLOGY

Objective

Marek's disease (MD) is currently controlled through biosecurity, widespread vaccination, and selection for genetic resistance. Throughout history, Marek's disease virus (MDV) field strains have undergone multiple shifts of increased virulence that required introduction of new vaccines. This cycle of virus evolution followed by introduction of new vaccines is not sustainable in this large, expanding, and highly concentrated industry. The specific aim of this reporting period was to assess how host genetics affects pathogen transmission and subsequent disease development in infected contact individuals.

Methods

We used a shedder-sentinel challenge model to determine when, how much, and how long MDV was transmitted. We performed 2 biological replicates with shedder birds from 2 laboratory chicken lines and 1 commercial layer line of differing MD susceptibility. After challenge with MDV, shedder birds were transferred to new isolators of naïve sentinel birds on days 10, 12, 14, 16, 18 and 20. Shedder birds were sampled at each transfer and sentinel birds were bled and feathers collected at 14 days post-exposure to shedder birds, then monitored for 8 weeks and necropsied to determine if they developed MD.

Results

We demonstrated higher virus load in feathers between the laboratory MD-susceptible compared to MD-resistant shedder chickens, but minimal downstream effect in sentinel chickens. The commercial layer line had significantly better survival for shedder chickens compared to the laboratory lines following challenge, but there was no correlation with feather virus load in shedders. Only marginal differences were seen for sentinel feather virus load, infection status, disease status and survival at each time point based on host genetics of the 3 shedder chicken lines.

Conclusions

Our transmission experiments revealed that host genetics is not nearly as significant compared to vaccination on reduction of virus transmission and resulting effect of disease symptoms in naïve unvaccinated sentinel chickens.

Financial Support
USDA National Institute of Food and Agriculture

Notes:

360 - Transcriptomic profiling of equine and viral genes during equine herpesvirus-1 viremia

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Session: GENOMICS - VIROLOGY

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 transports EHV-1 to the vascular endothelium of secondary sites of infection. Subsequent immunopathology at these locations results in secondary disease such as abortion or EHM. Because of the central role of PBMCs in EHV-1 pathogenesis, our goal was to establish gene expression analysis of host and equine herpesvirus genes during EHV-1 viremia.

Methods

RNA sequencing was used to perform a comprehensive and unbiased gene expression analysis of host and equine herpesvirus genes in PBMCs collected prior to infection and during peak EHV-1 viremia

Results

Fifty one differentially expressed equine genes (48 upregulated and 3 downregulated) were identified during peak viremia. After gene ontology analysis, processes such as the interferon defense response to virus, response to chemokines, and the complement protein activation cascade were overrepresented based on the genes upregulated during viremia. Further, genes involved in cell adhesion as well as coagulation were differentially expressed during viremia. Finally, 278 known equine miRNAs and 855 novel equine miRNAs were identified in these samples. These included miRNAs that mapped to the EHV-2 and EHV-5 genomes and 4 equine miRNAs that were differentially expressed in PBMCs during viremia compared to pre-infection PBMCs.

Conclusions

In conclusion, we performed a comprehensive characterization of host and viral genes during viremia and found a significant upregulation of the interferon pathway, induction of antivirals and cell adhesion molecules, as well as activation of complement and coagulation. This work expands our current knowledge about the role of PBMCs during EHV-1 viremia and will inform the focus on future experiments to identify host and viral factors that contribute to clinical EHM.

Financial Support
USDA National Institute for Food and Agriculture

Notes:

361 - Host genome profiles of human and pig intestinal epithelial cells during porcine delta coronavirus infection

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Session: GENOMICS - VIROLOGY

Objective

Porcine deltacoronavirus (PDCoV) is an emerging infectious disease of the swine industry. Phylogenetic analysis suggests PDCoV originated recently from a host-switching event between birds and mammals. Human cell lines are susceptible to PDCoV infection, however, there is no direct evidence that PDCoV causes human disease. Little is known about how PDCoV interacts in novel host environments. In this study, we explored the gene expression profiles of the primary host organism (swine) to a potential novel host (humans) after infection with PDCoV.

Methods

We utilized cell lines derived from intestinal lineages to reproduce the primary sites of viral infection in the host. 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-sequencing (RNA-seq).

Results

We found that there are more differentially expressed genes (DEGs) in humans, 7,486, in comparison to pigs, 1134. On the transcriptional level, humans appear to exhibit a more aggressive response to PDCoV infection in comparison to pigs. Our research shows key immune associated DEGs are shared between humans and pigs during PDCoV infection. These included genes related to the NF-kappa-B transcription factor family, the interferon (IFN) family, the protein kinase family, and signaling pathways such as the apoptosis signaling pathway, JAK-STAT signaling pathway, inflammation/cytokine – cytokine receptor signaling pathway, Toll-like receptor signaling pathway, NOD-like receptor signaling pathway, Ras signaling pathway and cytosolic DNA-sensing pathway.

Conclusions

While similarities exist between humans and pigs in many pathways, our research suggests that adaptation of PDCoV to the porcine host required the ability to downregulate many response pathways including the interferon pathway. This is the first report of transcriptome analysis of human cells infected by PDCoV and provides an important foundation that contributes to an understanding of the mechanisms of PDCoV infection across different hosts.

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

Notes:

362 - Virome analyses in commercial batches of bovine serum from North America and New Zealand

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Session: GENOMICS - VIROLOGY

Objective

Bovine serum is a key supplement for research and vaccine production. The vast worldwide trade of this product is a matter of concern regarding introduction and dissemination of pathogens among countries. Quality control of bovine serum mostly rely on virus isolation procedures. Here we evaluate the virome in commercial bovine serum lots from North America and New Zealand using high throughput sequencing (HTS).

Methods

Seven non-irradiated commercial lots of bovine serum from New Zealand (2 lots), Mexico (1 lot) and U.S. (4 lots) were obtained. The samples are composed of lots of fetal bovine serum (FBS), newborn calf serum (NBCS) and calf serum (CS). Nucleic acid enrichment procedures were conducted, and samples sequenced using an Illumina platform. Sequence contigs were assembled, and the similarity to viral sequences screened using MegaBLAST.

Results

Contigs closely related to eukaryotic viruses including: *Adenoviridae, Parvoviridae, Flaviviridae, Picornaviridae, Pneumoviridae, Reoviridae* and *Retrovirid*ae families were retrieved. Additionally, sequences of circular Rep-encoding single-stranded ssDNA (CRESS-DNA) viruses from the *Circoviridae, Smacoviridae* and *Genomoviridae* families were found. Parvoviruses related sequences were abundant and mostly composed by bovine parvovirus 3, bovine parvovirus 2, bosavirus, bovine hokovirus 1, and bovine bocaparvovirus 2. Furthermore, sequences of important cattle pathogens including bovine viral diarrhea virus, bovine respiratory syncytial virus, bovine kobuvirus, and bovine rotavirus were identified. The majority of the CRESS-DNA viral sequences were related to the *Genomoviridae* family. Overall there was a trend of decreased number of viral species found in FBS whereas the highest number of viral species were retrieved from NBCS.

Conclusions

The elevated level of viral sequences retrieved further supports the potential role of bovine serum on the dissemination of viral pathogens worldwide. The use of HTS in quality control protocols of biologicals may be an important tool toward early detection and limit dissemination of new or emerging pathogens.

Notes:

363 - Virome characterization of show pig serum raised in Oklahoma demonstrated great diversity of ssDNA viruses

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Session: GENOMICS - VIROLOGY

Objective

An important activity in the U.S is raising show pigs, which are usually raised in small non-commercial settings, and often have close contact to humans, and other animal species. Non-commercial operations may play a role in disease maintenance, and hamper control efforts. This study characterized the viral populations in show pig serum.

Methods

Two pools (P1 and P2) each composed of 200 serum samples were prepared from healthy show pigs between 4 and 7 months-old originated from multiple locations in Oklahoma. P1 was composed from samples collected in 2018 and P2 in 2019. Specific viral nucleic acid enrichment was conducted before library preparation. Enriched samples were sequenced using an Illumina platform. Sequence contigs were assembled using metaSPAdes and examined for similarities to viral sequences using MegaBLAST.

Results

High throughput sequencing of 2018 and 2019 samples generated 17,793 and 6,426 *de novo*-assembled sequence contigs, respectively. Sequences related to viruses belonging to the *Anelloviridae, Circoviridae, Parvoviridae, Arteriviridae*, *Flaviviridae, Herpesviridae,* and *Retroviridae* families were retrieved. Twenty-two viral species were detected, including the important swine pathogens: porcine reproductive and respiratory syndrome virus 2, atypical porcine pestivirus, and porcine circovirus 2 (PCV-2). Moreover, complete coding genomes of PCV-2, porcine parvovirus 2 (PPV-2), PPV-4, PPV-5, PPV-6, PPV-7, porcine bocavirus (*Ungulate bocaparvovirus 4*), torque teno sus virus 1 (TTSuV-1), TTSuV-2, and porcine bocaviruses (*Ungulate bocaparvovirus 3*) were recovered.

Conclusions

The study of the viral community present in sera of show pigs demonstrated a vast range of viruses, including important swine pathogens. Moreover, several new genomes were retrieved, including the first full coding genome of a US *Ungulate bocaparvovirus 3*. Here we demonstrate that the show pig population may play a role in disease maintenance and spread, imposing a hurdle toward disease surveillance and control.

Notes:

364 - VP1 sequence based genetic diversity of SAT2 foot-and-mouth disease virus circulating in ethiopia from 1990 to 2015

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Session: GENOMICS - VIROLOGY

Objective

Molecular epidemiology is a tool to link and trace the source and origin of virus to specific outbreak. This can be done by sequence based genetic level analysis of the highly variable viral protein one (VP1) of foot-and-mouth disease virus (FMDV). The objective of this study was to compare, on a genetic level, differences of SAT2 VP1 sequences of FMDV circulating in Ethiopia from 1990 to 2015.

Methods

Nucleotide substitution, distance matrix, amino acid variability and phylogenic analysis were done using MEGA (molecular evolution genetic analysis). The nucleotide and amino acid sequence length analyzed were 648 base pairs and 216 amino acid residues representing the Viral protein 1(freely accessible from the genbank).Universal VP1region targeted primers were used for PCR and dedoxysanger sequencing was used.

Results

In the present study 76 and 73% similarities were identified on nucleotide and amino acid sequences respectively. The overall nucleotide group mean distance and inter-population diversity were 19.1% and 2.14% respectively. Paired sequences from the year 2007, 2009/10, 2014/15 and 1990/91 showed 5% variation. Nucleotide sequences from 2014/15 showed on average 29 % differences with that of 2007,2009/10 as well as 1990/91. This pointed that SAT2 virus circulating in Ethiopia were genetically having four groups: 1991 (one sequence alone), 1990/91, 2007/2009/10 and 2014/15 with west to east and south to north possible incursion routes. Amino acid positions 21-28, 43-51, 81-88, 135-142, 155-160 and amino acid positions 76-99 incorporates the D-E loop were seen to be variable among sequences. Positions 144-146 within the G-H loop is completely conserved as 'RGD' cell attachment site of the virus.

Conclusions

In conclusion, it was observed that four topotypes (IV, XIV, XIII, VII) were circulating in Ethiopia in fifteen years period. The genetic diversity within the serotype, production system, livestock trade, border control for live animal or their product smugglers seems to overcomplicate the epidemiological dynamics of foot and mouth diseases in Ethiopia.

Financial Support
Genome Alberta

Notes:

365 - The G-CSF produced by sentinel immune cells induces neutrophil mobilization during *Streptococcus suis* infection

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Session: IMMUNOLOGY

Objective

Streptococcus suis serotype 2 is an important porcine bacterial pathogen and emerging zoonotic agent. Infections induce an exacerbated inflammation that can result in sudden death, septic shock and meningitis. Although neutrophil infiltration characterizes *S. suis* lesions, their activation dynamic during infection is poorly understood. Importantly, the production and role of the granulocyte colony-stimulating factor (G-CSF), a key neutrophil regulator, have never been addressed. This project aims to describe the mechanisms involved in G-CSF production and neutrophil mobilization in the context of *S. suis* infection.

Methods

Using a mouse model of infection, we assessed the proportion of neutrophils in blood and organs of infected animals using flow cytometry. The levels of G-CSF in the plasma and organs were quantified by ELISA. We used primary cultures to evaluate dendritic cell and macrophage capacity to produce G-CSF in response to *S. suis* stimulation and dissect the mechanisms involved in G-CSF production using bacterial strains deficient in key virulence factors and knock-out cells for important cellular receptors. Biological replicates were included, and data analyzed by student's t-test and ANOVA.

Results

The results showed that neutrophil numbers increased in blood of mice 12 h after *S. suis* infection while simultaneously dropping in the bone marrow. The peak of blood neutrophils correlated with the peak of G-CSF production in plasma and organs. We demonstrated that both dendritic cells and macrophages produce G-CSF as a consequence of *S. suis* lipoprotein recognition by cellular Toll-like receptor 2 (TLR2).

Conclusions

In conclusion, sentinel immune cells produce G-CSF following recognition of *S. suis* lipoproteins by the TLR pathway. The G-CSF then induces the release of neutrophils from the bone marrow to the blood at early stages of infection. It remains to be elucidated if those mechanisms are beneficial or detrimental for the host, through an improved bactericidal effect of neutrophils or the exacerbation of the inflammation, respectively.

Financial Support
Natural Sciences and Engineering Research Council of Canada; Swine and poultry infectious diseases research center; Fonds de recherche du Québec

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

Notes:

T366 - oo much of a good thing: antiviral response and tissue damage during respiratory infections in the porcine lung

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Session: IMMUNOLOGY

T

Objective

he antiviral response leads to host protection through expression of interferon-stimulated genes (ISGs) that clear viruses through mRNA degradation and inhibition of transcription, translation and assembly. Additionally, ISG expression causes activation of an antiviral state in nearby cells. Genes highlighted in this study will help with understanding establishment of the antiviral state in relation to tolerance, susceptibility, and lung damage which has implications in treating infections in livestock and humans. P

Methods

igs were split into 4 treatment groups (control, porcine reproductive and respiratory syndrome virus (PRRSV) infected, influenza B virus (FluB) infected, and FluB/PRRSV coinfection). Lung tissue was collected at 3, 5, and 7 days post infection (dpi) for control, PRRSV and FluB/PRRSV coinfection, and 3 and 5 dpi for FluB. Transcriptomic analysis was performed against S.scrofa 11.1 reference. Differential gene expression (DEG) analysis was carried out using DeSeq2 based on the model treatment + dpi + treatment:dpi + E. Downstream analysis examined the interaction of DEGs across time for over-enrichment. I

Results

nfected groups vs the controls yielded a total of (N=1412) DEGs for the PRRSV group and (N=1578) for the FluB/PRRSV group across all timepoints. The FluB group had a total of (N=64) across 3 and 5 dpi. Expression data was considered statistically significant based on FDR ⩽ 0.1. Venn diagram comparisons of the DEGs across dpi showed groups shared only 16 DEGs at 3 dpi and none at 5 dpi. For 7 dpi, only the PRRSV and FluB/PRRSV co-infected groups were compared and shared a total of 43 DEGs. Upregulation was observed in antiviral genes such as IRF1, MX1, and OAS2, while downregulated genes clustered around pathways effecting lung development and cellular integrity. I

Conclusions

nfected pigs showed upregulation of ISGs with antiviral and neutrophil degranulation pathway activity possibly related to symptomatic lung lesion pathology, although early expression of host interferon and antiviral genes may lead to viral mRNA degradation and assembly clearance.

Notes:

367 - Use of the old horse model to identify host factors contributing to EHM pathogenesis

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Session: IMMUNOLOGY

Objective

Equine Herpesvirus 1 (EHV-1) is the cause of respiratory disease, abortions and sporadic but devastating outbreaks of Myeloencephalitis (EHM) in horses worldwide. Interestingly, EHM affects only ~ 10% of infected horses but host factors that predict whether a horse will get EHM are largely unknown. It has however been shown that EHM incidence increases to ~70% in horses >20 years of age. Based on this evidence, we hypothesized that the "old horse model" can be utilized to clinically induce EHM and identify specific host factors contributing to EHM.

Methods

Horses >18 years old (n=10) or 2 years old, (n=9) were infected with EHV-1 strain Ab4 and studied for 21 days post infection (p.i.). Blood and nasal swabs were analyzed for viremia and nasal shedding by qPCR. Nasal Secretions and cerebrospinal fluid (CSF) were collected for cytokine analysis and EHV-1 specific IgGa, IgGb, and IgG(T) antibody (Ab) isotype ELISA was used to compare Ab responses in serum.

Results

EHM was observed in 9/10 horses >18 years compared to young horses, where we observed mild ataxia in only 1 horse. In contrast, respiratory disease and a classical bi-phasic fever was only present in the young horse group while old horses responded with a single fever peak during the onset of viremia and did not exhibit respiratory disease. In addition, significantly higher viral load during viremia was detected in the old horse group. Further, INF-α/IL-17 induction was lower, but IL-10 induction was higher in nasal secretions of old/EHM horses p.i.. In addition, overall IL-10 levels were higher in CSF of old /EHM horses. Consistent with this cytokine response, increased EHV-1 specific IgGT titers could be detected in old/EHM horses pre- and post-infection compared to young horses.

Conclusions

These findings highlight that EHV-1 disease manifestation and equine immune response differ with age. Further, an increased IgGT and IL-10 response and a shift toward TH-2 immune responses may be correlated with pathogenesis of neurologic disease.

Financial Support
Grayson Jockey Club Research Foundation

Notes:

368 - Development and characterization of new swine immune reagents to understand immune correlate for vaccines/infection

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Session: IMMUNOLOGY

Objective

The USDA-NIFA Swine Immune Toolkit Initiative has a goal to generate priority immune reagents, based on inputs from veterinary immunology researchers worldwide, and pipeline them for marketing. Our efforts are aimed at expression of soluble proteins and production of panels of monoclonal antibodies (mAbs) using collaborations with commercial partners for protein expression and mAb production.

Methods

Generation of new panels of mAbs reactive with porcine IL-5 and IL-21 has been initiated with the help of commercial partners. Panels of mAbs to IL-6, IL-13, IL-17A, IL-28B, CXCL10, and BAFF are being screened for their reactivity in multiple immune assays. Reactivity tests of labeled anti-IL-6, -IL-17A, -IL-13 and -CXCL10 mAbs for intracellular staining of porcine myeloid and T cells using flow cytometry-based assays are in progress.

Results

A sensitive sandwich ELISA assay is now available for IL-17A, and mAb reagents to IL-13 and IL-6 are being screened for best mAb pairs for such assays. Planning for the generation of SLA-I & -II tetramers to identify swine CD4 and CD8 T cells specific for influenza virus peptides has been initiated. Porcine CD3 antibody (clone PPT7) is being evaluated for testing porcine T cell activation.

Conclusions

Panels of immune reagents are required to perform complex immune studies; those currently available for pigs are limited. For each target, our goal is to provide the veterinary community with the new commercial reagents and standardized techniques in using these new reagents for their research efforts. Tools and reagents generated by this project will undoubtedly advance swine immune, disease, vaccine, and biomedical research efforts.

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

Notes:

369 - Single cell and deep transcriptomic analysis identify common and cell type specific genes in porcine immune cell

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Session: IMMUNOLOGY

Objective

Pigs are important to global agricultural livelihoods and the diet of millions of people worldwide, yet this network is challenged by highly transmissible disease. Additionally, pigs have arisen as a human biomedical model given that the porcine immune system shares many similarities with humans. However, the porcine immune cell transcriptome has not been comprehensively studied. Here, we have performed bulk RNA sequencing on flow-sorted porcine immune cells and single cell RNA sequencing (scRNA-seq) on porcine PBMCs to create a deep understanding of the porcine immune system.

Methods

PBMCs were isolated from nine healthy pigs. Isolated RNA from sorted immune cells (monocytes, neutrophils, NK cells and specific populations of T and B cells) from two pigs were used for bulk RNA-seq, while PBMCs were used for scRNA-seq using the 10X platform on the remaining seven independent samples.

Results

Deep transcriptomes (around 465 million total reads pair-end sequences across samples identifying in average 10,422 genes per cell) from sorted cells were used to determine enriched and specific genes among sorted immune cell populations. Highly enriched genes identified biological processes related to the nature of each cell type using Gene Ontology analysis and comparison with human immune cells was performed. Kmeans cluster analysis was performed to identify co-expression clusters among cell types that were used for transcription factor binding enrichment within clusters and for further integration with scRNA data. On average, scRNAseq of 5479 cells were sequenced and 789 genes per cell were detected. scRNA data allowed the identification of 15 cell clusters. These clusters were annotated using specific cell markers, and differentially expressed genes across clusters were calculated.

Conclusions

Taken together, the gene expression profiles and single cell transcriptomic analysis reported here is the first comprehensive transcriptomic study of circulating porcine immune cell types and provides a valuable resource to elucidate molecular markers for porcine immune cell identity and function.

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

Notes:

371 - Characteristics of canine macrophages post *Mycobacterium intracellulare* infection in relation to Th17 responses

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Session: IMMUNOLOGY

Objective

Mycobacterium avium-intracellulare complex (MAIC) infection causes respiratory disease in immunosuppressed hosts. During the past few decades, the case of infection by members of the MAIC has been reported consistently in dogs. In mycobacterial infections, host immune system induced Th17-type immune response by macrophage-mediated protective immunity. It is well-recognized how MAIC drive host immune system in human, but canine immune system infected MAIC has not been studied. The aim is therefore to identify the T helper cell polarizing by *M. intracellulare* infected macrophages when presenting specific antigen in dogs.

Methods

Canine peripheral blood mononuclear cells (PBMCs) were collected from a total number of 10 healthy beagle dogs, via density gradient centrifugation. $CD14^+$ cells were isolated using MACS system and stimulated with 50ng/ml M-CSF for 5 days. After macrophage differentiation, *M. intracellulare* treated at a MOI of 1. Infected monocyte-derived macrophages (MDMs) were cultured with $CD14^-$ cells at 24h post-infection. RNA was extracted from infected-MDMs and $CD14^-$ cells. Macrophages differentiation was identified by RNA-Seq and real-time PCR. The intracellular abundance of *M. intracellulare* was measured in infected MDMs. T helper cell responses were analyzed by Cytokine ELISA and FACS.

Results

During the course of *M. intracellulare* infection, monocyte-derived macrophages produced inflammatory cytokines, such as IL-1β, IL-6, and IL-23, are essential to induce a Th17 immune profile. RNA-Seq also revealed differentially expressed genes were related to Th17 immune responses. IL-17A was detected in CD14- cells cultured with infected MDMs by ELISA. Flow cytometry analysis showed CD4(+) IL-17(+) cells in canine PBMCs.

Conclusions

These results clearly demonstrate for the first time how canine macrophages present antigen to T helper responses during *M. intracellulare* infection. This study will be helpful in understanding the host immune response to *M. intracellulare* infection and raising the possibility of potential mycobacterial infection in dogs. 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.

Financial Support
Rural Development Administration of Korea

Notes:

372 - IgE-binding monocytes have an enhanced ability to produce IL-8 (CXCL8) in animals with naturally occurring allergy

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Session: IMMUNOLOGY

Objective

Interleukin 8 (IL-8) is a potent chemokine that is known to recruit neutrophils and basophils to promote inflammation in many species. IL-8 is produced by many cell types, including monocytes. Here we report a role for IgE-binding monocytes to promote allergic inflammation through IL-8 production in a horse model of natural IgE-mediated allergy.

Methods

We developed a monoclonal antibody with confirmed specificity for both recombinant and native equine IL-8 for flow cytometric analysis. We compared IL-8 production by peripheral blood mononuclear cells in horses with and without a naturally occurring IgE-mediated skin allergy, *Culicoides* hypersensitivity.

Results

Equine IL-8 was produced by CD14+/MHCII+/CD16- monocytes, including a subpopulation of IgE-binding monocytes, following stimulation with lipopolysaccharide (LPS). In addition, IgE crosslinking induced IL-8 production by both peripheral blood basophils and IgE-binding monocytes. Allergic horses had significantly higher percentages of IL-8+ monocytes after LPS stimulation and significantly higher percentages of IL-8+ IgE-binding monocytes after IgE crosslinking. In contrast, frequencies of IL-8+ basophils after IgE-crosslinking were similar in all horses, regardless of allergic disease.

Conclusions

We concluded that IgE-binding monocytes from allergic individuals have an increased capacity for IL-8 production and likely contribute to the recruitment of innate immune cells and inflammation during IgE-mediated allergy.

Financial Support
USDA National Institute for Food and Agriculture

Notes:

373 - Circulating foamy macrophages found in the blood of the golden Syrian hamster model of leptospirosis

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Session: IMMUNOLOGY

Objective

Leptospirosis is a world-wide zoonotic disease caused by pathogenic *Leptospira*. Symptoms can range from asymptomatic, to flu-like, to multi organ failure and death in severe cases. Species and strain specificity play a large role in disease presentation. While manually studying the blood differentials from hamsters challenged with different species and strains of *Leptospira*, our group identified a circulating population of large, monocytic, lipid-filled cells, most similar in the literature to foamy macrophages (FM) which are associated with infectious disease as well as with chronic inflammation.

Methods

Syrian Golden hamsters were intraperitoneally challenged with various *Leptospira* including *L. interrogans* serovars Copenhageni strains IC:02:001 (chronic infection) and L1-130 (severe acute infection), and *L. borgpetersenii* serovar Hardjo, strains HB203 (chronic) and JB197 (severe acute infection). Blood was collected at euthanasia by cardiac puncture. Whole blood smear slides were Giemsa stained and evaluated for manual differential analysis. Whole blood samples were also evaluated with scanning electron microscopy (SEM) and transmission electron microscopy (TEM).

Results

FM were clearly identified by Giemsa stain, SEM, and TEM. FM were identified in hamsters from all *Leptospira* challenges, showing their appearance is not species or strain specific. However, higher percentages of FMs were found in hamsters from the acute infections suggesting an association with disease severity.

Conclusions

Leptospirosis is a world-wide threat that suffers from difficult diagnosis and a lack of cross protective vaccine strategies. By understanding the host immune response, especially in a strain and species-specific manner, we enable the discovery and characterization of potential therapeutic targets that are critical to the control of the disease. The identification of circulating FM in the blood may also add to the diagnostic tools available for Leptospirosis patients. Lastly, the identification of FM in the leptospirosis hamster model provides a unique source for continued study of FM during infection.

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

Notes:

374 - Reserpine increases *Salmonella* resistance in chicken intestinal explants via norepinephrine release

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Session: IMMUNOLOGY

Objective

The overall goal of this study was to investigate whether reserpine, a drug that releases intracellular catecholamines, could improve *Salmonella enterica* clearance in the chicken intestine. Given that reserpine reduces immunosuppressive properties in human circulatory regulatory T cells (Tregs), we hypothesized this drug could reduce *Salmonella* via interrupting intestinal Treg function.

Methods

Chicken ceca explants were first washed with antibiotics, then treated with 0 or 1 uM reserpine for 6 or 12 hr at 41ºC. Catecholamine release was confirmed via U-HPLC. *Salmonella* resistance was assessed by incubating explant supernatants with 10^2 CFU/ml *Salmonella* for 6 hr at 41ºC and enumerating on MacConkey. To source catecholamines, $CD4^+CD25^-$ (naive T cells) and $CD4^+CD25^+$ (Tregs) cells were isolated from ileo-cecal-colic junctions, sorted via flow cytometry, and incubated with 0 or 1 uM reserpine for 1 hr at 41ºC. Cells were then pelleted, and catecholamines were measured via U-HPLC. Significance ($P < 0.05$) was assessed via student's t-test.

Results

Reserpine-treated explants had greater *Salmonella* reduction, greater antimicrobial peptide and IL-2 expression, and reduced CTLA-4 expression versus controls ($P < 0.05$). Furthermore, reserpine treatment increased norepinephrine release in explants and individual Tregs versus controls ($P < 0.05$).

Conclusions

Our findings illustrate a neuroimmunological axis in the gut in which reserpine induces norepinephrine release, resulting in an increased, antibacterial immune response. Furthermore, this study discovered that chicken intestinal Tregs not only have intracellular norepinephrine stores, but reserpine treatment releases these stores. This suggests intestinal Tregs depend on intracellular norepinephrine for its immunosuppressive function. This is supported by decreased expression of CTLA-4, a surface bound, immunosuppressive protein constitutively expressed in Tregs, upon reserpine treatment. Overall, we find that reserpine treatment is a novel means of increasing *Salmonella* resistance in the chicken intestine.

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

Notes:

375 - Combined effects of stressors across ages on blood chemokine profiles in the pig

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Session: IMMUNOLOGY

Objective

The levels of cytokines in the blood can be useful biomarkers of the effect of stressors that trigger immune response or inflammation and corresponding repair system in the pig. The type of stressor and the age of the pig during the stress can modulate the magnitude and direction of the effect. Moreover, the exposure to multiple insults may cancel or augment the changes in blood cytokine levels. The objective of this study was to investigate the impact of multiple stressors across ages on the blood cytokine profiles of pigs.

Methods

The blood cytokine levels from one-hundred and twenty female and male pigs, offspring of Camborough gilts and PIC boars were analyzed. Half of the offspring experienced the stress of maternal immune activation during the last third of gestation triggered by the inoculation with porcine reproductive and respiratory syndrome virus of gilts while the remaining gilts were inoculated with saline solution. The pigs were weaned at three weeks of age and grouped housed. At two months of age, one-third of the pigs were exposed to a second immune challenge using the viral mimetic polyinosinic:polycytidylic acid, another third were assigned to fasting stress for one day, and the remaining third served as control. The level of serum pro- and anti-inflammatory cytokines were measured using a multiplex assay.

Results

The effects of immune stress during development, and fasting or immune stress were tested across sexes using a linear mixed effects model. The levels of interleukin 6 and tumor necrosis factor alpha were lowest among pigs not exposed to stressors, and highest among pigs exposed to immune stress at two month of age. The pigs exposed to immune stress during gestation presented the highest levels of interleukin 2 and interleukin 4.

Conclusions

The combined effects of first and second stress augmented the interferon gamma variability. Our findings support the understanding of the cumulative effects of stressors across ages on immune indicators. This study is supported by USDA NIFA AFRI, grant number 2018-67015-27413.

Financial Support
USDA National Institute of Food and Agriculture

Notes:

376 - Effect of gestational viral infection on offspring immune signaling pathways in the amygdala

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Session: IMMUNOLOGY

Objective

The immune response of a gestating pig to infection can affect the developing fetus. The effect of this immune activation on the offspring brain can be long-lasting, and interfere with postnatal physiology and behavior. Moreover, brain regions that modulate sexual dimorphism are differentially impacted by alterations in immune signals during development. The objective of the present study is to characterize the impact of the immune response of gilts to infection on the immune pathways of the piglet's amygdala, a brain structure that is sexually dimorphic and regulates behaviors.

Methods

The experimental design encompassed twenty-four piglets that were evenly distributed across sexes. Half of the piglets were born from gilts inoculated with porcine reproductive and respiratory syndrome virus on gestational day 76, while the remaining piglets were born from control gilts. The amygdala of the 21 day-old piglets was profiled using a RNA-sequencing platform. The gene expression levels were tested for the effects of gilt viral challenge and sex. The prevalence of differentially expressed genes from pathways annotated in the Kyoto Encyclopedia of Genes and Genomes was explored using the Gene Set Enrichment Analysis approach.

Results

Among the genes impacted by immune activation during gestation, two interconnected pathways exhibited distinct patterns between sexes. The T helper cell differentiation pathways were enriched among the genes over-expressed in females relative to males from virus-challenged gilts. These pathways participate in pathogen recognition and cytokine production. The adipocytokine signaling pathway was enriched among the genes over-expressed in males relative to females from virus challenged gilts. The genes in this pathway modulate cytokine signaling and feed intake.

Conclusions

Our results indicate that viral infection during gestation affects multiple pathways that can influence health and growth and advance the understanding of the effects of maternal infection during gestation. This study is supported by USDA NIFA AFRI, grant number 2018-67015-27413.

Financial Support
USDA National Institute of Food and Agriculture

Notes:

377 - Genomic organization and expression of the swine WC1 multigenic array of hybrid coreceptor/PRR molecules

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Session: IMMUNOLOGY

Objective

γδ T cells can respond to a variety of non-proteinaceous molecules independently of MHC presentation, making them an attractive target for next-generation vaccines. However, little is known about γδ T cells in swine. WC1, a member of the group B Scavenger Receptor Cysteine Rich (SRCR) superfamily, as is the PRRSV receptor CD163A, is expressed exclusively on bovine γδ T cells from a multigenic array (WC1-1 to WC1-13). Bovine WC1 functions as hybrid co-receptor and pattern recognition receptor for the γδ TCR. WC1+γδ T cells share a restriction in their TCR gene usage, yet respond to different pathogens based on which WC1 molecule(s) they express, which bind to whole pathogens via their SRCR domains. Because WC1 genes are encoded as a multigenic array with bacterial binding and signaling capacity, we hypothesize that each WC1 gene has co-evolved with pathogens. The objective of this study is to characterize the porcine genomic WC1 multigenic array, WC1 transcripts, and WC1 protein reactivity with porcine WC1 (SWC5) antibodies.

Methods

We prepared RNA from PBMC from York x Duroc piglets and used 5'/ 3' RACE PCR and RT-PCR to obtain cDNA clones. The cDNA sequence was mapped to swine genomic contigs using Maker and JBrowse. Primary PBMC and transfected Expi293 cells expressing individual WC1 genes were stained with SWC5 mAbs and analyzed by flow cytometry.

Results

We obtained nine WC1 full-length cDNAs and mapped the exon-intron structure of corresponding WC1 genes. Amino acid differences between cDNA and annotated genes may occur because of sequencing errors or may indicate either variation between individuals or the presence of more WC1 genes. In PBMC, WC1 mAbs stained overlapping subpopulations of CD2-γδ T cells. WC1 mAbs differentially recognize multiple transfected and immunoblotted swine WC1 proteins. Swine WC1 SRCR domains bind to Leptospira spp and contain conserved residues for PRRSV binding.

Conclusions

Taken together, these results suggest that there may be porcine γδ T cells that are differentially responsive to pathogens based on their WC1 expression.

Financial Support
USDA National Institute for Food and Agriculture

Notes:

378 - Development of equine immune reagents

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Session: IMMUNOLOGY

Objective

The analysis and detailed understanding of immune responses of the horse is essential for infectious disease research, vaccine development, and testing new treatments for acute and chronic inflammatory diseases. Immunological research requires specific antibodies, typically monoclonal antibodies (mAbs). Our objectives are to develop and characterize new immune reagents for the horse and to make them available to the equine research community.

Methods

Immune marker targets are expressed as recombinant proteins and used for immunization of mice. Afterwards, fusions are performed to produce mAbs using hybridoma technology. The specificity of the mAbs is tested against various recombinant equine immune targets by ELISA and/or flow cytometric analysis. Only mAbs that are solely recognizing the target of interest are further characterized using native protein detection. mAbs against secreted immune molecules are also used for multiplex assay development.

Results

We have developed new mAbs against inflammatory cytokines including equine IL-1b, TNF-a and IL-8. The mAbs were characterized for detecting the native cytokines in equine PBMC by flow cytometry. All of them specifically detect their native equine cytokines counterparts. In addition, a chemokine 6-plex assay was developed and validated for detection of secreted IL-1b, TNF-a, CCL2, CCL3, CCL5 and CCL11 in horse samples, including supernatants from cultured and stimulated PBMC, serum, and other samples.

Conclusions

The new IL-1b, TNF-a and IL-8 mAbs and the chemokine 6-plex assay are sensitive new tools for immunological research and will 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:

379 - Single-cell RNA sequencing reveals pig intestinal innate lymphoid cells and unique activation profiles of T cells

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Session: IMMUNOLOGY

Objective

Intestinal lymphocytes play an important role in balancing immune tolerance and activation to promote animal health and prevent disease. To better understand intestinal lymphocytes in pigs, we utilized single-cell RNA-sequencing (scRNA-seq) to assess gene expression at single-cell resolution, specifically focusing on analysis of T cell and innate lymphoid cell (ILC) lineage lymphocytes.

Methods

Cells were harvested from porcine ileum, enriched for lymphocytes, and scRNA-seq was performed. Gene expression was quantified, cells were clustered, and cell types were identified by gene expression profiles.

Results

Analysis yielded 14,773 T/ILC lineage lymphocytes grouped into 25 clusters. Expression of *CD4, CD8B*, or *TRDC* identified 20 clusters as CD4 αβ T cells, CD8 αβ T cells, or γδ T cells, respectively. Five remaining clusters were identified as ILCs by transcriptional similarities to T cells, expression of *PTPRC* and *CD2,* and lack of *CD3E* expression. One ILC cluster expressed *IL22* and *RORC* and was identified as ILC3s, while the remaining 4 ILC clusters expressed genes encoding NK receptors (*KLRK1, KLRA8*) and cytotoxicity-related proteins (*NKG7, PRF1*) and were identified as ILC1s. γδ and CD8 αβ T cells had two distinct gene expression profiles: some clusters had high expression of cytotoxicity-related genes (*GZMB*, *GZMA)*, while others had high expression of MHC II-related genes (*HLA-DRA, SLA-DQB1*).

Conclusions

We described gene expression profiles of porcine ILCs and similarities to ILC subsets of other species, but cell function in pigs remains to be defined. Two distinct gene expression profiles for intestinal γδ and CD8 αβ T cells were characterized by inverse expression of cytotoxicity- or MHC II- related genes; however, the significance of this phenomena as it relates to activation state and cell function is unclear. Overall, the data provide new insight into subsets and gene expression dynamics of ILCs and T cells in the porcine intestinal tract, though further research is required to validate findings at the protein level and determine biological significance.

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

Notes:

380 - *Bacillus*-based microbials lower the pathogenic synergy of a *Salmonella* Choleraesuis and PRRS virus co-infection

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Session: IMMUNOLOGY

Objective

This study examined the impact of direct-fed microbials (DFM) on the synergistic syndrome resulting from co-infecting of pigs with *Salmonella enterica* serotype Choleraesuis and porcine reproductive and respiratory syndrome virus (PRRS) virus, namely septicemia and enhanced pneumonia.

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 the ileocecal LN of all challenged pigs regardless of DFM treatment. The pathogenic synergy of the two microbes was exhibited by a higher rate of colonization of the lung by *Salmonella*, a more extensive and severe interstitial pneumonia, and a more intense systemic inflammatory response, than what resulted from *Salmonella* infection alone. In the dually challenged pigs, provision of DFM reduced *Salmonella* colonization of the lung and associated lymphoid tissue (60 vs 91%; $P<0.05$), and reduced the extent and severity of gross lung pathology (55 vs 84%; $P < 0.05$). DFM provision also reduced viremia (3.08 vs 4.09 $\log_{10}$ $TCID_{50}$/ml; $P<0.05$) and exudative inflammation, as indicated by a lower frequency (50 vs 82%; $P<0.05$) of pigs with fibrinous ascites. As compared to non-DFM treated pigs, lung lavage fluids collected from dually-challenged DFM-treated pigs had increased concentrations of IL-1 (287 vs 140 pg/ml; $P<0.05$) and IL-8 (1,700 vs 600 pg/ml; $P<0.05$). This was accompanied by increased expression in white blood cells of the immunoreceptor TREM-1 (25- vs 18-fold-change; $P<0.05$).

Conclusions

These data suggest that *Bacillus*-based DFM can exert a beneficial effect on health by mitigating microbial pathogenicity in the respiratory system as well as the systemic spread of enterically derived bacteria. Changes in inflammatory cytokine production and increased expression of TREM-1 suggest that the *Bacillus*-based DFM have a modulating effect on cells of the innate immune system.

Financial Support
USDA National Institute for Food and Agriculture

Notes:

381 - Impact of Oxidative Stress on Vaccine Responsiveness in Neonatal Dairy Calves

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Session: IMMUNOLOGY - CATTLE

Objective

The overall goal of this project is to identify the extent to which reducing oxidative stress (OS) in calves can improve their response to vaccination.

Methods

We have developed and validated an in-vitro model to evaluate lymphocyte functions under OS conditions. Briefly, peripheral blood mononuclear cells (PBMCs) isolated from neonatal calves were exposed to the free radical-generating substances hydrogen peroxide and 2,2'-Azobis(2-amidinopropane) dihydrochloride at concentrations that created oxidative stress -measured by isoprostane concentrations, an indicator of lipid peroxidation- while maintaining cell viability. Subsequently, the PBMCs were stimulated with bovine herpesvirus-1 (BHV-1) and phorbol myristate acetate (PMA) and various immune functions key for vaccine responsiveness were measured, including the production of BHV-1 antigen-specific antibodies, cytokine mRNA and protein abundance, and activation capacity measured via CD69 expression. Data were compared statistically among treatments PBMCs using Student's t-test. Statistical significance was declared at $P < 0.05$.

Results

Oxidative stress negatively influenced in vitro PBMC immune functions that are relevant to vaccine responsiveness such as activation, immunoglobulin production, and cytokine production. Given that dairy calves experience a high degree of pro-oxidant redox balance throughout the first months of life, OS during this stage might contribute to decreased immune responsiveness in neonatal calves.

Conclusions

Oxidative stress decreased some neonatal immune functions relevant to vaccine responsiveness in vitro. Future in vitro and in vivo studies will focus on the extent to which supplementation with anti-oxidative micronutrients assists in restoring the immune functions affected by OS.

Financial Support
USDA National Institute for Food and Agriculture

Notes:

382 - Immunological response to naturally occurring bovine respiratory disease in stocker cattle during early management

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Session: IMMUNOLOGY - CATTLE

Objective

Cytokines and acute phase proteins indicate disease and infection severity. We aimed at determining the temporal changes in cytokines and acute phase proteins related to naturally occurring Bovine Respiratory Disease (BRD) in commercial stocker cattle.

Methods

Weaned crossbred beef steer calves (n=40) were purchased from auction markets and housed at a commercial stocker operation in Crossville, TN in winter 2020. Calves were monitored daily by the producer to visualize clinical signs of BRD and were treated with antibiotics accordingly. Researchers collected blood samples and performed thoracic ultrasonography weekly (day 0, 7, 14, 21) to identify lung consolidation. Haptoglobin (HP), IL-8, IL-1β, and TNFα concentrations were determined using commercial ELISA kits. To test if fixed effects of treatment for BRD, day of treatment, or the interaction impacted serological measures, responses were log-transformed prior to performing mixed model analysis of variance (Proc GLIMMIX; SAS 9.4). Animal measured within day was a random repeated measure ($\alpha = 0.05$). Thoracic ultrasonography scores ranged from 1 to 3. A score of 2 or greater on any given day was termed "consolidated". To test the association of lung consolidation with ever receiving treatment, a Chi-square test (Proc FREQ) was used.

Results

Calves treated for BRD had significantly increased ($P = 0.02$) serum concentration of HP (1.9 mg/mL ± 0.5) compared to non-treated (0.7 mg/mL ± 0.2). There was a significant day effect ($P = 0.02$) on TNFα regardless of treatment status. At d7, TNFα was significantly higher (14.4 ng/mL ± 10.1) than d21 (9.2 ng/mL ± 7.5). However, concentrations were not different at d14 from either d7 or d 21. We did not observe any significant relationship for IL8 and IL1β in between the treated and non-treated cattle. An association between the presence of lung consolidation and treatment was observed ($P = 0.004$).

Conclusions

Acute phase response and cytokines change in response to infection could indicate BRD onset. This study will aid in early BRD diagnosis and better management decisions in commercial stocker calves.

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

Notes:

383 - Comparing colostral natural antibody IgM titers in high, average, and low immune responder dairy and beef cows

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Session: IMMUNOLOGY - CATTLE

Objective

Natural antibodies (NAb), present in the absence of exogenous antigen stimulation, provide immediate protection and forge an important link between innate and adaptive immunity. NAb are highly cross-reactive and are present in bovine colostrum which is of interest due to the importance of maternal antibodies in neonatal health. Previously, cows selected based on the patented University of Guelph-High Immune Response (HIR™) technology, High responders were shown to have greater amounts of colostral specific antibodies, ß-lactoglobulin, and less disease compared to their herd-mates. However, NAb in colostrum has not been examined in the context of HIR™ phenotypes.

Methods

Colostrum was collected from beef (n=172) and dairy (n=263) cows within the first 12 hours after calving. Cows were previously classified as High, Average, or Low immune responders based on their estimated breeding values of specific antibody- (AMIR) and cell-mediated immune responses. Colostral NAb immunoglobulin M (IgM) titers were measured by an indirect ELISA against the keyhole limpet hemocyanin and reported on a log2 scale. Data were normally distributed and analyzed using mixed and general linear models.

Results

Beef cows ranked as high AMIR had significantly greater LS Means of colostral NAb IgM titers (12.14± 0.12 SEM) than low AMIR cows (11.62 ± 0.15 SEM, $p<0.01$) and greater than average AMIR cows (12.07 ± 0.09 SEM); however, this difference was not significant ($p=0.61$). Dairy cows ranked as high AMIR produced colostrum with significantly greater LS Means of colostral NAb IgM titers (12.38 ± 0.3 SEM) than average AMIR cows (11.98 ± 0.25 SEM $p=0.04$), and greater than low AMIR cows (12.11 ± 0.26 SEM) however this difference was not significant ($p=0.21$). Dairy cows had significantly greater LS Means of colostral NAb IgM (11.96 ± 0.07 SEM) than beef cows (11.78 ± 0.05 SEM, $p=0.04$).

Conclusions

Colostral NAb may help provide essential protection to the neonate. Selecting for cattle that produce high-quality colostrum with higher specific and NAb may provide a strategy for improving neonatal health and colostral products.

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:

384 - Optimization of curcumin solubility and encapsulate loading to improve gastrointestinal absorption in the cow.

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Session: IMMUNOLOGY - CATTLE

Objective

Optimize a formulation of curcumin supplement for stability in the rumen and controlled release.

Methods

Ultrasonication and rapid shifts in pH were used to form concentrated curcumin microcrystals. The microcrystals were encapsulated in hydrocolloids including; alginate, carrageenans (κ-, ι-, λ- derivatives), and high and low methoxyl forms of pectins. The amount (or loading) of curcumin after processing was verified by spectrofluorometric and spectrophotometric methods. The lead formulation was administered to a group of 4 cows alongside 4 cows given empty capsule in a crucial validation study. Serial blood and urine samples were collected and analyzed by high perfomace liquid chromatography for key metabolites of curcumin.

Results

A maximum loading of 200 mg/ml curcumin was acheived and spectral analysis documented a highly stable formulation. Aggregation was avoided by a high concentration (0.1%) of chitosan in the outer coating. This product was formulated in a spray-dry manner and adminstered as a top-dressing. In sample analysis, 414 metabolites were identified and annotated. Principal component analysis revealed significant concentrations of known curcumin metabolites in urine samples from cows fed the supplement. Known curcumin metabolites were not identified in serum samples. However, the serum samples collected 7 hours post feed administration contained a number of compounds unique to supplemented cows.

Conclusions

We have developed a microencapsulated curcumin feed supplement that is highly concentrated and rumen-protected. Absorption of the product was verified by identification of curcumin metabolites in urine samples. In the upcoming year 2 of our study, we will test the hypothesis that dietary supplementation with curcumin will increase the concentration of high density lipoprotein (HDL) and improve its antioxidant and immunomodulatory properties in dairy cows. We will also test the impact of the dietary supplement on the cow's inflammatory response to bacterial mastitis.

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

Notes:

385 - Linking Bovine Leukemia Virus pro viral load with single-nucleotide polymorphisms in the bovine MHCII DRB3 locus

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Session: IMMUNOLOGY - CATTLE

Objective

Bovine leukemia virus (BLV) causes the most common neoplastic disease in cattle, enzootic bovine leukosis. BLV Infects 83% of dairy farms in North America. Progression of BLV is linked to Bovine leukocyte antigen (BoLA). 233 DNA samples collected from a previous study were used to analyze linkages between the BoLA-*DRß3* Exon 2 alleles, proviral load (PVL) and immune phenotypes. Linking BLV PVL with the MHC II DRß3 locus can confirm associations between alleles, BLV status and immunological effects of BLV.

Methods

PVL was determined for all samples. DNA for the DRß3 exon 2 was PCR amplified and analyzed by Sanger sequencing. Alignment and consensus creation was done via MacVector version 17.5.5. Two methods are being used for alignment to confirm haplotypes. One method uses Fastphase to align sequences with the 327 known BoLA-*DRß3* Exon 2 alleles after running a ClustalW analysis of the sequences. We also use Halpofinder, a script designed for detection of BoLA-*DRß3* Exon 2 alleles. Statistical analysis will be via Prism Graph Pad version 8 to compare BLV status, allele haplotype and PVL.

Results

BoLA-*DRß3* Exon 2 influences progression of BLV, measured by PVL. The *DRß3**1501 allele is linked to high BLV PVL and *DRß3**0902 allele is associated with low PVL. It is expected these alleles will trend with previous studies. To date, 24 homozygous samples have been aligned with allelic haplotypes via Haplofinder. Fastphase will be used for haplotype confirmation.

Conclusions

All PCR reactions, purifications, and Sanger sequencing are complete. Allele calling in uncloned PCR products is complicated in heterozygotes. Further visual inspection of electropherograms will identify SNPs, which can then be used to define haplotypes after trimming and alignment to the reference database. An initial analysis of 24 homozygous samples identified the following BoLA-*DRß3* Exon 2 alleles within our sample group (in order of frequency): *0101, *1501, *1101, *1201, *1601, *2703*, and *1001. As expected via previous studies, haplotypes *0101 and *1501 are already high in frequency among samples.

Financial Support
Michigan Alliance for Animal Agriculture

Notes:

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

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Session: IMMUNOLOGY - CATTLE

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) were housed at the University of Florida dairy unit. Blood was collected at calving using sodium heparin evacuated tubes. Parity, gestation length, days in close-up pen (DCU), body condition score (BCS), and rectal temperature (RT) were recorded. Cows having dystocia, twins, stillbirth, vaginal laceration, or retained placenta were classified as having a risk factor (RF) for metritis. Cows were examined for signs of metritis at 3, 7, 10, and 13 days in milk, and 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. The absence of L-selectin (CD62L) and the presence of CD11b on cells were used as markers of myeloid and lymphoid cell activation. Data were analyzed by logistic regression, and the model included parity, GL, DCU, BCS, RT, RF, and immune markers with $P \leq 0.2$ in the univariate analysis.

Results

The proportion of activated B cells and the proportion of monocytes were predictors of metritis, in addition to parity, RF, GL, and BCS. Each 1-unit increase in the percentage of CD62L- B cells increased the odds of metritis by 13% (OR=1.13; 95% CI=1.1-1.2; $P<0.01$). Each 1-unit increase in monocyte proportion increased the odds of metritis by 34% (OR= 1.34; 95% CI=1-1.9; $P=0.07$). The full model had a sensitivity of 85%, specificity of 67 %, positive predictive value of 71%, negative predictive value of 81%, and area under the curve of 83%.

Conclusions

In summary, markers of B cell activation and monocyte proportion were significant predictors of metritis.

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

Notes:

387 - Viperin expression in Bovine Respiratory Syncytial Virus infected calves

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Session: IMMUNOLOGY - CATTLE

Objective

Bovine respiratory syncytial virus is an important pathogen in bovine respiratory disease. Viperin is an innate immune protein induced by interferon in response to viral infections; interferon independent induction by LPS has been reported. We investigated the expression of viperin in BRSV infection. Our group had previously shown in vitro that a concentrated culture supernatant of *Histophilus somni* induced viperin production in vitro and was associated with reduced BRSV replication. We sought to evaluate the ability of avirulent *H. somni* intranasal inoculation to stimulate viperin production in the upper respiratory tract prior to BRSV infection with the goal of decreasing viral shedding and improving clinical outcome.

Methods

Holstein calves received intranasal *H. somni* 129pt or placebo on 3 days prior to mock viral infection. Nasal swabs were taken daily for 7 days. Then intranasal administration of *H. somni* 129 or placebo was repeated twice two weeks later followed by aerosol infection with virulent BRSV. Nasal swabs were taken daily, and clinical signs were recorded. We developed a bovine viperin qRT-PCR and evaluated viperin expression in daily nasal swab samples as well as in lung lavage cell pellets taken at necropsy on day 7 post viral infection.

Results

Viperin expression was very low for pre virus infection samples but beginning on day 3 after BRSV infection viperin expression increased to a peak of over 20-fold baseline on days 5/6 post infection. Mixed effects analysis showed a significant time effect ($p<0.0001$) but no difference between *H. somni* and placebo treated calves. Also, on day 5 in both groups virus shedding peaked to over 1000 pfu/ug RNA isolated from nasal swabs. Despite viperin production clinical signs were severe and not significantly different between *H. somni*129 treated and placebo treated calves.

Conclusions

Intranasal administration of *H. somni* 129pt did not induce in vivo viperin expression. However, BRSV infection strongly induced expression of viperin.

Financial Support
USDA National Institute for Food and Agriculture

Notes:

388 - Phenotypic and functional characterization of mucosal associated invariant T cells from bovine

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Session: IMMUNOLOGY - CATTLE

Objective

Mucosal associated invariant T (MAIT) cells are innate T lymphocytes that recognize and respond to riboflavin metabolites, derived from bacteria and fungi, which are presented by the highly conserved MHC-related protein-1 (MR1). MAIT cells have been described in humans and rodents, and are known to play a role in the response to a variety of viral and bacterial infections. MAIT cells have been described in the bovine. However, very little is known about the phenotype or function of this population in the calf during disease conditions. Our goal was to characterize the phenotype and functions of MAIT cells in the calf, and to determine their role in the context of a respiratory infection.

Methods

Peripheral blood mononuclear cells (PBMCs) and bronchoalveolar lavage (BAL) cells were isolated from healthy calves or animals infected with bovine respiratory syncytial virus. MAIT cells were identified using a 5-OP-RU (a vitamin B2 derivative)-conjugated tetramer that binds to the MR1-restricted T cell receptor. The frequency and phenotype of MAIT cells were evaluated via multi-color flow cytometry.

Results

MAIT cells were detected at low frequencies in the peripheral blood and were present at higher frequencies in the BAL. Two subsets of bovine MAIT cells were defined in peripheral blood based upon their expression of CD8 ($CD3^+CD8^+$ and $CD3^+CD8^-$ T cells). Surface CD4 expression was not detected on bovine MAIT cells. MAIT cells from peripheral blood had the capacity to produce IFN-γ and IL-17 in response to mitogen stimulation. The frequency of MAIT cells in both the blood and BAL increased following respiratory viral infection.

Conclusions

MAIT cells are present in the periphery and mucosal tissues of the bovine, where they have the capacity to produce pro-inflammatory cytokines. MAIT cells expand in response to infection and may have a role in host defense in the respiratory tract in cattle.

Notes:

389 - The role of immunogenic engineered exosomes in bovine respiratory syncytial virus

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Session: IMMUNOLOGY - CATTLE

Objective

Bovine respiratory syncytial virus (BRSV) is a cause of respiratory disease in young cattle and the efficacy of available vaccines for BRSV is limited. Exosomes (30-150nm) are extracellular vesicles derived from cells and have important roles in cellular communication as a cargo. Exosomes secreted by virus infected cells can carry cell-specific antigens to immune cells and activate a specific immune response. Our prior work has shown that host-cell derived exosomes can be purified and antigenically modified to carry peptides of interest using a functional-integrated microfluidic bioreactor array. Here, we aim to define the potency and immunogenicity of antigenically engineered exosomes for eliciting targeted immune responses and preventing BRSV infection in a mouse model.

Methods

Dendritic-cell derived exosomes were isolated using the integrated microfluidic bioreactor array and engineered to present two immunodominant MHC class I epitopes from the matrix (M) and nonstructural protein 1 (NS1) of RSV. The immunogenicity and efficacy of the engineered exosomes were investigated by immunizing C57BL/6 mice subcutaneously with escalating doses of engineered exosomes, and measuring antigen specific T cell responses via ELISA and multi-color flow cytometry.

Results

The immunogenicity of the exosomes was confirmed by measuring antigen-specific T cell clonal expansion and IFN-γ production. The exosomes were confirmed to carry the engineered peptides of interest and to have capacity to induce antigen specific $CD8^+$ T cell responses both *in vitro* and *in vivo*.

Conclusions

Dendritic-cell derived surface engineered antigenic exosomes have the capacity to elicit antigen-specific $CD8^+$ T cell activation *in vitro* and *in vivo*, and may have the potential to be used as a novel vaccine platform for the prevention of BRSV and other viral infections in animals.

Financial Support
USDA National Institute of Food and Agriculture

Notes:

390 - Generating bovine monocyte-derived dendritic cells in serum-free medium for experimental and clinical applications

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Objective

Monocyte-derived dendritic cells (MoDC) are critical in activating immunity and establishing immunologic memory to pathogens. Large numbers can be easily generated *ex vivo,* but existing protocols utilize serum-supplemented culture medium. Serum contains unknown quantities of immune modulating components that can impact experimental results and limit clinical applicability. The objective of this study was to establish standardized methodology for generating and characterizing bovine MoDC under serum-free (SF) conditions.

Methods

MoDC generated from non-pregnant cattle were used for a series of experiments evaluating the following culture conditions: medium type, method of monocyte enrichment, culture duration, and concentration of differentiation additives. Viability and yield were assessed using propidium iodide staining and manual hemocytometer counting, respectively. Using flow cytometric analysis MoDC phenotype (MHC class II, CD86, CD14, and CD205 expression) and T cell activation/proliferation (CD25 and CFSE expression) were assessed. Cytokine secretion was quantified using a multiplex cytokine panel (IL-1α/β, IL-8, IL-10, IL-17A, IFN-γ, MIP-1α, TNF-α, IL-4). Cell metabolism was analyzed using an Extracellular Flux Seahorse Analyzer. Data were analyzed using paired t-tests and repeated measures ANOVA.

Results

Immature MoDC generated in SF medium using magnetic-activated cell sorting with plate adhesion to enrich monocytes and cultured for 4 days have the following phenotypic profile: MHC class II^{+++}, $CD86^{+}$, $CD205^{++}$, and $CD14^{-}$. These MoDC can be activated as noted by a metabolic switch to aerobic glycolysis, induction of T cell activation and proliferation, and increased CD86 and CD40 expression and cytokine secretion (IL-1α, IL-10, MIP-1α, and IL-17A).

Conclusions

Cultivation of bovine MoDC utilizing this culture system offers a well-defined protocol for efficient, reliable, and reproducible generation of highly pure, immature bovine MoDC for experimental and clinical applications.

Financial Support
Midwestern University

Notes:

391 - Pharmacological mTOR inhibition alters phenotype and function of bovine monocyte-derived macrophage subsets

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Session: IMMUNOLOGY - CATTLE

Objective

Nutrient deficit and concomitant reduced activation of the nutrient-sensing mammalian target of rapamycin (mTOR) may alter phenotype and function of bovine innate immune cells. Our objective was to test the effects of pharmacological mTOR inhibition in polarized monocyte-derived pro-inflammatory (M1) and regulatory (M2) macrophages on phenotype, oxidative burst, and phagocytosis.

Methods

Monocytes (CD14+ PBMC) from pregnant cows (n = 12) in late lactation (222-312 DIM) were isolated by density-gradient centrifugation and magnetic bead assisted cell separation. Cells were polarized *in vitro* to M1 (10 ng/mL IFN-γ, 20 ng/mL GM-CSF) or M2 macrophages (IL-4, M-CSF, 20 ng/mL each). On day 2, mTOR inhibitors rapamycin (RAPA) and Torin-1 (TR1) were added at 100 nM/mL, respectively. On day 3, expression of surface markers (MHCII, CD163) was measured with or without stimulation with lipopolysaccharide (E. coli LPS, 100 ng/mL) for 16 h. Oxidative burst and phagocytosis were measured on day 4 in parallel cultures.

Results

Polarization resulted in minimal expression of CD163 in M1 compared to M2 ($P < 0.001$). LPS stimulation decreased MHCII expression in M2 ($P < 0.001$) but not in M1 ($P = 0.79$). The presence of TR1 increased MHCII expression in unstimulated M1 ($P < 0.001$). Expression of CD163 was decreased in unstimulated M2 by RAPA ($P = 0.06$) and TR1 ($P < 0.001$). TR1 increased oxidative burst in M1 ($P < 0.001$) and decreased it in M2 ($P = 0.02$). Both mTOR inhibitors decreased phagocytosis in M1 ($P < 0.01$).

Conclusions

Pharmacological inhibition of the mTOR pathway was associated with macrophage subset-specific changes in phenotype and function. The inhibition favors a pro-inflammatory phenotype and function of bovine M1 macrophages, whereas differentiation of regulatory M2 macrophages is inhibited. Involvement of the nutrient-sensing mTOR pathway in shaping bovine macrophage phenotype may help explain the bias in inflammatory responses and, through a phagocytosis inhibition the reduced ability to eliminate pathogens during times of nutrient deficit such as the postpartum period.

Financial Support
USDA National Institute for Food and Agriculture

Notes:

392 - Changes in biomarkers of metabolic stress during late gestation of dairy cows associated with colostrum volume

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Session: IMMUNOLOGY - CATTLE

Objective

In this study we compared the metabolic status of dairy cows during the last 6 wk of gestation based on colostrum volume.

Methods

Healthy Holstein cows were randomly selected from 2 commercial dairy herds in Michigan. Two cohorts of 21 cows each, one for the summer and another for the fall of 2019, were enrolled in each farm with similar parity distribution. Cows were blood sampled weekly during the last 6 wk of gestation, and biomarkers related to nutrient utilization, oxidant status, and inflammation were quantified in serum. Cows were milked within 6h of calving and the volume of colostrum produced was recorded and an aliquot collected. For this study, only the data from 63 cows producing colostrum with IgG > 50 g/L were considered to avoid confounding due to poor IgG content. Cows were grouped into high colostrum producer (HCP) or low colostrum producer (LCP). For that, we arbitrarily defined 6 L of colostrum (4 L for first and 2 L for second feeding of calves), as the cut-off point. Data were analyzed statistically through mixed models with repeated measures including the fixed effects of group (HCP vs. LCP), time, and their interaction; and the random effects of cow, lactation number, and farm.

Results

The HCP cows had higher β-hydroxybutyrate, blood urea nitrogen, magnesium, and lower glucose serum concentrations throughout the study period compared to LCP cows. Furthermore, HCP cows also showed higher concentration of reactive oxygen species compared to LCP cows, resulting in higher oxidant status index values despite no changes in antioxidant potential. No differences were found for haptoglobin, a biomarker of inflammation.

Conclusions

Collectively, these data show that HCP cows had greater utilization of nutrients compared to LCP cows throughout the study period. Thus, indicating a higher metabolism activity, which might explain the elevation in markers of oxidant status. Nevertheless, the differences observed did not result in changes in biomarkers of inflammation or lipid mobilization, suggesting that physiological homeostasis was not disrupted in HCP cows during late gestation.

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

Notes:

393 - Prophylactic ursolic acid treatment modulates inflammatory responses during *Mannheimia haemolytica* infection

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Session: IMMUNOLOGY - CATTLE

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. These infections are often characterized by an inflammatory IL-17-mediated immune response, which increases neutrophil infiltration and activates other pro-inflammatory immune cells. Therefore, to better understand the immune-induced tissue damage that follows MH infection, this study employed the use of an IL-17 inhibitor, ursolic acid (UA), and determined its subsequent impact on immune responses and disease severity.

Methods

Two independent experiments were performed using 4 week old 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, and flow cytometry was used to compare production of reactive oxygen species and phagocytic potential of circulating and tissue-specific immune cells.

Results

Reduced bacterial isolation from the lungs and decreased pathology suggests that UA treatment may reduce MH dissemination and disease pathogenesis. UA treatment also altered the expression of the inflammatory markers, such as IL-6 and STAT3, and innate immune defenses.

Conclusions

Prophylactic UA treatment appears to reduce the severity of MH infection in calves; however, future studies will elucidate the mechanisms for the treatment's effects.

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

Notes:

394 - β-hydroxybutyrate impaired mammary gland defense during a *Streptococcus uberis* challenge in dairy cows

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Session: IMMUNOLOGY - CATTLE

Objective

β-hydroxybutyrate (BHB) has been associated with disease in early lactation dairy cattle, but such associations do not demonstrate causation. Therefore, the objective of this study was to examine the effects of BHB on inflammation and dairy cattle performance during an intramammary *Streptococcus uberis* challenge.

Methods

Late lactation multiparous dairy cows (n = 12) were continuously infused IV with either BHB to induce hyperketonemia (target concentration: 1.8 m*M*) or with saline (CON) for 72 h during a *S. uberis* intramammary challenge. Body temperature, dry matter intake (DMI), milk production, and milk *S. uberis* CFU were measured daily until one-week post-challenge. Blood samples were collected during infusion to assess metabolic changes (glucose, insulin, glucagon, and NEFA). Mammary biopsies were conducted at 72-h post-challenge to assess transcript abundance of inflammation-associated genes. Linear mixed models were used to assess the fixed effects of treatment, parity, time, and all two- and three-way interactions, and the random effects of block and cow.

Results

BHB-infused cows exhibited a delayed febrile response (trmt*time interaction, $P < 0.01$), noted by a lower body temperature during the final day of infusion ($P < 0.01$), followed by a greater body temperature 6 d post-challenge ($P < 0.01$). Consequently, BHB-infused cows had greater *S. uberis* CFU on d 4 ($P = 0.03$), 6 ($P < 0.01$), and 7 ($P < 0.01$) as compared to CON. Accordingly, BHB-infused cows had a lower DMI ($P = 0.02$) and reduced milk yield ($P = 0.03$). BHB-infusion reduced blood glucose (trmt*parity interaction, $P = 0.04$) in 3+ parity cows as compared to CON ($P < 0.001$), however, only marginal effects were seen on other metabolic parameters. Finally, BHB-infusion reduced or tended to reduce mammary transcript abundance of *IL1β* ($P = 0.04$) and *TNFα* ($P = 0.09$). Diverging responses were found on inflammasome-related genes, as BHB-infusion increased *NLRP3* ($P < 0.01$), but decreased *CASP1* ($P = 0.03$).

Conclusions

These data suggest that BHB altered the immune response promoting tolerance toward *S. uberis* rather than resistance.

Financial Support
USDA National Institute of Food and Agriculture

Notes:

395 - Synthetic mRNA transfection induces expression of antibodies against *Tritrichomonas foetus* surface antigen TF1.17

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Session: IMMUNOLOGY - CATTLE

Objective

The mucosal immune response of bulls to *Tritrichomonas foetus* (TF) is limited and fails to clear the parasite from the urogenital epithelium. Previous research showed that mRNA application can induce antibody expression by epithelial cells. Our objective was to induce expression of antibodies by mRNA treatment of bovine preputial keratinocytes against the TF adhesion-mediating surface antigen TF1.17.

Methods

Bovine primary preputial keratinocytes were transfected with synthetic mRNA encoding expression of bovine IgG1 against two different TF1.17 epitopes. At 24 and 48 h post-transfection, keratinocyte supernatants were collected for indirect immunofluorescence assay (IFA) to identify antibody binding to TF, then keratinocytes were fixed with 4% paraformaldehyde for identification of cytoplasmic bovine IgG1 by IFA.

Results

At 24 h post-transfection keratinocytes expressed IgG1 in their cytoplasm as confirmed by IFA. Cell culture supernatants collected at 48 h post-transfection contained secreted IgG1 directed against TF1.17, as confirmed by demonstration of binding of IgG1 to the surface of TF by IFA.

Conclusions

Synthetic mRNA transfection of bovine urogenital epithelial cells induced expression of antibodies against TF. Antibodies secreted by cells bound to TF as shown by IFA. The results demonstrate the potential of this novel approach to induce passive mucosal immunity against TF at the site of infection.

Notes:

396 - Endocannabinoid concentrations and receptor expression in cultured bovine endothelial cells challenged by endotoxin

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Session: IMMUNOLOGY - CATTLE

Objective

Aberrant inflammation associated with coliform mastitis is a major contributor to the severity and potential lethality of systemic infections. The 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 and 2 (CB1/CB2, respectively) and their ligands including fatty acid ethanol amides and glycerols. The EC arachidonoylethanolamide (AEA) was shown to possess anti-inflammatory properties through CB2 activation and can be metabolized by the cyclooxygenase-2 (COX-2) enzyme into prostamides. In contrast to pro-inflammatory prostaglandins also produced by COX2, several prostamides possess anti-inflammatory activities depending on cell type and species. Fluctuations of plasma EC concentrations were recorded in dairy cattle affected by coliform mastitis. However, the impact of EC expression on vascular endothelial cell function during lipopolysaccharide (LPS) challenge has not been elucidated. The purpose of this study was to elucidate changes in the EC system of cultured bovine aortic endothelial cells (BAEC) challenged with LPS.

Methods

Primary BAEC cell lines were cultured in F12K media containing 10% FBS and 0.05% selenium. Cells were treated with 25 ng/mL of LPS for 8 hours. Taq-Man custom probes were used for QPCR. Quantification of EC was done by LC/MS.

Results

Receptor CB1 was more abundantly expressed after LPS treatment compared to media control, whereas CB2 was only expressed in LPS challenged cells, and was not detected in unchallenged cells. All EC concentrations increased with LPS exposure. Prostamide concentrations were only detectable after LPS treatment.

Conclusions

Elevated AEA concentrations with LPS exposure and increased CB2 expression is indicative of possible involvement of the EC system in inflammatory regulation. Production of prostamides after LPS exposure may also be an inflammatory regulatory mechanism and warrants further investigation.

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

Notes:

397 - Synergistic activation of bovine CD4+ T cells by IL-12 and neutrophils

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Session: IMMUNOLOGY - CATTLE

Objective

Neutrophils migrate into draining lymph nodes within hours of pathogen challenge or vaccination. In healthy humans, mice, and cattle, neutrophils are also present in secondary lymphoid tissues. CD4+ T cell activation requires three signals: T cell receptor (TCR) binding (first signal; 1SI), co-stimulation by other immune cells (2SI), and the presence of inflammatory cytokines, such as IL-12 (3SI). We recently reported that bovine neutrophils are unique in their ability to activate CD4+ T cells (Li et al. 2019), and thereby hypothesized that bovine CD4+ T cell activation occurs synergistically via neutrophils and IL-12.

Methods

Naïve CD4+ T cells were harvested from the lymph nodes of grass-fed Wye Angus cattle and stimulated for 3.5 days with anti-CD3 (1SI), anti-CD28 (2SI), and recombinant IL-12 (3SI). Neutrophils from the same cattle were then co-cultured with CD4+ T cells in the presence or absence of IL-12. Cells were examined by immunostaining for surface expression of CD25 and CD62L, intracellular staining for IFNg, and bovine cytokine array assay of the supernatants of stimulated cells.

Results

The strongest activation was achieved in the presence of all three signals, as demonstrated by CD25 upregulation and IFNg production in CD4+ T cells, and IFNg and IL-2 in cell supernatants. IL-12 function required 2SI from CD28, as in humans and mice, and the presence of CD62L, an adhesion molecule, was positively associated with IL-12. Results also showed that 1SI+neutrophils led to enhanced CD25 expression that was further increased by IL-12 addition, suggesting synergistic action by IL-12 and neutrophils. Neutrophils alone did not affect IFNg levels, but the addition of neutrophils to 1SI+IL-12-stimulated CD4+ T cells significantly increased IFNg production.

Conclusions

Our data suggest synergistic activation of bovine CD4+ T cells by neutrophils and IL-12, a unique mode of action that could assist the development of immune interventions for cattle.

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

Notes:

398 - Porcine macrophage-like cell line CΔ2+ is susceptible to Japanese encephalitis virus infection

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Session: IMMUNOLOGY

Objective

Japanese encephalitis virus (JEV) is a zoonotic arthropod-borne flavivirus that is a leading cause of severe neurologic infection in humans. Pigs have high titers and a lasting viremia upon natural infection making them important reservoirs of JEV. To date, the pathogenesis of JEV infection in pigs is poorly understood. Macrophages are commonly targeted by JEV, but primary macrophages are labor intensive to isolate, so we examined the susceptibility of an established porcine monocyte-derived macrophage-like cell line (CΔ2+) to the attenuated JEV strain SA-14-14-2.

Methods

Monolayers of CΔ2+ and BHK-21 (positive control) cells were infected with SA-14-14-2 for 5 days at a multiplicity of infection (MOI) of 0.1. Culture supernatants and cells were collected at 0, 12, 24, 48, 72, 96, and 120 hours post infection (hpi), and monolayers were observed for cytopathic effects (CPE). The amount of infectious virus in supernatants was quantified using a standard plaque assay. Infected cells were stained with trypan blue to determine viability. An indirect immunofluorescence assay was used to detect the presence of JEV NS1 antigens.

Results

CΔ2+ cells were susceptible to SA-14-14-2 infection and produced infectious virus with a mean peak titer of 7 $\log_{10}$ pfu/ml, comparable to 7.6 $\log_{10}$ pfu/ml by BHK-21 cells which are hamster fibroblasts known to be vulnerable to JEV. Infected CΔ2+ cells proliferated through 48 hpi, compared to 24 hpi for BHK, after which cell numbers and the proportion of viable cells declined. Infected CΔ2+ and BHK cells also showed time-dependent CPE and intracellular localization of the JEV NS1 protein was observed at 24hpi.

Conclusions

These findings demonstrate that the porcine macrophage cell line CΔ2+ may be a relevant cell line for understanding JEV infection dynamics in a natural host species. These data provide a foundation to compare various JEV strains *in vitro* to allow for better understanding of host cell mechanisms critical for viral replication and maintenance in pigs, as well as a model for screening for potential therapeutic targets and mitigation strategies.

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

Notes:

399 - *In ovo* and oral administration of probiotic lactobacilli modulate immune responses in newly hatched chickens

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Session: IMMUNOLOGY - POULTRY

Objective

The objective of the present study was to investigate the effects of *in ovo* and oral administration of a multi-strain lactobacilli mixture on cytokine gene expression, lymphocyte subsets, and antibody-mediated immune response in chickens.

Methods

Lactobacilli were administered through the *in ovo* route at embryonic day 18, oral gavage at days 1, 7, 14, 21, 28, 35 post-hatch, and both delivery routes. Control group was *in ovo*-injected with PBS. On days 5 and 10 post-hatch, spleen, and bursa of Fabricius were collected for gene expression and cellular analysis. On days 14 and 21 post-hatch, birds were injected with sheep red blood cells (SRBC) and keyhole limpet hemocyanin (KLH), and sera were collected on days 7, 14, and 21 post-primary immunization.

Results

The results demonstrated that birds receiving *in ovo* inoculation of lactobacilli had lower expression of cytokines including interferon (IFN)-β, IFN-γ, and interleukin (IL)-8 in the spleen when compared to the PBS-treated control. On the other hand, the expression of *IFN-α,* IFN-β, IL-12, and IL-13 was upregulated in birds receiving 10^7 colony forming unit (CFU) of lactobacilli via both *in ovo* and oral administration post-hatch. Birds receiving 10^7 CFUs of lactobacilli via *in ovo* had a higher percentage of monocyte/macrophage (KUL01) compared to the control group. In addition, groups receiving 10^7 CFUs of lactobacilli, either through *in ovo* alone or both delivery routes, increased the percentage of $CD3^+$ $CD4^+$ T cells and $CD4^+$ $CD25^+$ Treg cells in the spleen. *In ovo* inoculation of 10^7 CFUs of lactobacilli enhanced SRBC-induced antibody response at days 7 and 14 post-primary immunization, whereas birds receiving 10^7 CFUs of lactobacilli via both *in ovo* and gavage routes had higher anti-KLH IgM and IgG titers at days 14 and 21 post-primary immunization, respectively.

Conclusions

In conclusion, these findings suggest that pre-and post-hatch administration of lactobacilli may modulate birds' immune responses, especially during the first few days after hatching.

Financial Support
Canadian Poultry Research Council; 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:

400 - Probiotic lactobacilli limit avian influenza virus H9N2 replication in chicken cecal tonsil mononuclear cells

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Session: IMMUNOLOGY - POULTRY

Objective

Low pathogenic avian influenza virus (LPAIV) H9N2 poses significant threat to animal and human health. The growing interest in beneficial effects of probiotic bacteria on host immune system has led to research efforts studying their interaction with cells of host immune system. However, the role of lactobacilli in inducing antiviral responses in lymphoid tissue cells requires further investigation. The objective of the present study was to examine the antiviral and immunostimulatory effects of lactobacilli bacteria on chicken cecal tonsils (CT) cells against H9N2 LPAIV.

Methods

CT mononuclear cells were stimulated with probiotic Lactobacillus spp mixture either alone or in combination with a Toll-like receptor (TLR)21 ligand, CpG oligodeoxynucleotides (CpG).

Results

Pre-treatment of CT cells with probiotic lactobacilli, alone or in combination with CpG, significantly reduced H9N2 LPAIV replication. Furthermore, lactobacilli alone elicited cytokine expression, including IL-2, IFN-γ, IL-1β

, IL-6, and IL-12, and IL-10, while when combined with CpG, a significantly higher expression of (interferon-stimulated gene (viperin)), IL-12, IL-6, CXCLi2, and IL-1β was observed. However, none of these treatments induced significant changes in nitric oxide production by CT cells.

Conclusions

In conclusion, probiotic lactobacilli demonstrated a modulatory effect on CT cells, and this correlated with enhanced antiviral immunity and reduced H9N2 LPAIV viral replication.

Financial Support
Agriculture and Agri-Food Canada; Egg Farmers of Canada; University of Guelph; Canadian Poultry Research Council

Notes:

401 - Regulatory microenvironment in feathers of chickens infected with very virulent Marek's disease virus

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Session: IMMUNOLOGY - POULTRY

Objective

Marek's disease (MD) is a highly contagious disease of chickens which is controlled by vaccines. Although vaccines provide protection against clinical disease when vaccinated chickens become infected with Marek's disease virus (MDV), the virus can undergo productive replication which leads to shedding of infectious viruses from feathers. Therefore, the present study was conducted to investigate the presence of immunoregulatory microenvironment in feathers of chickens and to examine the effect of vaccines in modifying the immunoregulatory responses in feathers.

Methods

Chickens were administered with Herpesvirus of Turkey/CVI988 vaccine and/or infected with MDV. At 4, 10- and 21-days post-infection (dpi), feathers and spleen were collected to determine the composition of various cells types by flow cytometry and the expression of genes associated with immune regulatory functions.

Results

The results revealed the presence of a high number of CD4+CD25+ and CD4+TGF-b+ T regulatory cells in feathers of MDV infected chickens at 21dpi. The number of these T cells was significantly lower in vaccinated chickens compard to MDV infected chickens. Further, vaccinated and infected chickens had a lower number of CD4+ and CD4+CD8+ T cells compared to MDV infected chickens. The expression of TGF-b and PD-1 was significantly increased in feathers of MDV infected chickens compared to vaccinated chickens. Administration of CVI988 vaccine significantly reduced MDV load in feathers at 21dpi compared to MDV infected chickens.

Conclusions

The findings reveal the existence of an immunoregulatory microenvironment in feathers of MDV infected chickens, which may favour the effective replication of infectious MDV. Although administration of vaccines reduced MDV load in feathers, it does not prevent shedding of the virus. Identifying the molecular factors that facilitate productive replication of MDV in feathers and exploring the immune evasive strategies employed by the virus will enable the development of intervention approaches for prevention of MDV replication and transmission.

Financial Support
Natural Sciences and Engineering Research Council of Canada; University of Guelph

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

Notes:

403 - Toward the discovery of novel serum immune biomarkers for intestinal health monitoring in poultry

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Session: IMMUNOLOGY - POULTRY

Objective

Biomarkers are cellular, biochemical or molecular perturbations that can be objectively measured and evaluated in biological media, and as such can be used as indicator for pathogenic processes and pharmacological responses to therapeutic interventions. However, because of the complexity of biological interactions, the use of a single biomarker is often not feasible and it is rather advised to design a combination of biomarkers to assess a certain process. The main aim of this study was to identify established serum immune biomarkers for intestinal health in poultry that are easily measurable, and to analyze the potential of diamine oxidase (DAO), an intestinal epithelial cell-specific protein, as a serum biomarker for intestinal permeability (IP).

Methods

Firstly, an extensive literature study was performed to identify established biomarkers. Secondly, an *in vivo* experiment was conducted to analyze the potential of DAO as a serum biomarker for intestinal permeability. A full factorial experiment was designed, taking into account commercial age (*minus* two days (D-2), D-1, D1 to D2) and hatching condition (on-farm hatched, hatchery-born, and hatchery-born + antibiotic treatment) as factors. A total of 500 hatched Ross 308 chicks were used. Chicks that hatched in the hatchery did not receive water, nor feed until arrival at the farm on D1.

Results

Literature research showed that not many serum biomarkers for IP have been identified yet. Problems that are mainly encountered are the lack of reliable protocols for analysis (e.g. lipopolysaccharide); values that depend on the concentrations available in the intestinal tract, which are variable (e.g. D-lactate), and the unavailability of reference values. Preliminary tests with DAO showed promising results.

Conclusions

Serum biomarkers can provide a non-invasive method to monitor intestinal health on a flock level. However, not many biomarkers have been established yet and more research is necessary. Although showing promising results, more research is needed to establish DAO as a serum immune biomarker for IP.

Notes:

405 - Chicken gamma delta T cell is one of the IFN-γ and TGF-β^{+} sources against Marek's disease virus infection

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Session: IMMUNOLOGY - POULTRY

Objective

Marek's disease (MD) is a viral lymphoproliferative disease of chickens caused by Marek's disease virus (MDV). MD vaccines reduce the incidence of MD but cannot control virus shedding. Therefore, it is crucial to develop new vaccines and elucidate mechanisms of immunity to MDV. Among the innate responses against MDV, gamma delta (γδ) T cells may play a significant role as the first line of defense due to their high frequency in chicken blood and tissues. We hypothesized that MDV interacts with γδ T cells and that these cells play a role in immunity against MD.

Methods

Spleen, lung, and skin were obtained from control, vaccinated, MDV infected, and vaccinated and MDV infected groups at 4, 10, and 21 days post infection (d.p.i.) and the expression of IFN-γ and TGF-β in γδ T cells was analyzed using flow cytometry.

Results

In all tissues, the frequency of IFN-γ^{+} CD8^{+} γδ T cells in MDV infected group was significantly higher than that of the control group at 10 d.p.i. ,while the high frequency of IFN-γ^{+} CD8^{+} γδ T cells was observed at 4 d.p.i in vaccinated group and vaccinated and infected group. TGF-β^{+} CD25^{+} γδ T cells in MDV infected group was increased in spleen compared to the other groups at 10 and 21 d.p.i.

Conclusions

The results presented here suggest that administration of MDV vaccines can induce γδ T cell which might contribute to protection of young chickens against MD. On the other hand, the delayed activation of γδ T cell in unvaccinated and MDV infected chickens may be a contributing factor to lack of immunity in these chickens. Presence of TGF-β^{+} CD25^{+} γδ T in infected chickens may also be a contributing factor to failure of chickens to mount immunity against the virus. In the next phase of our studies, the activation mechanisms of effector γδ T cell and the inhibitory function of MDV-induced TGF-β^{+} CD25^{+} γδ T cells will be examined.

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:

406 - In ovo inoculation of vitamin A modulates chicken embryo immune functions

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Session: IMMUNOLOGY - POULTRY

Objective

Vitamin A mediates many important biological functions in human and animals. Presence of vitamin A receptor on immune system cells, emphasizes its role in immune functions. The objective of this study was to evaluate the effect of in ovo inoculation of vitamin A on chicken embryo immune functions.

Methods

Eighteen day-old embryonated eggs were divided into 3 sets of 5 groups that each set was considered as one time point. Three different concentrations of retinoic acid, (RA: the active form of vitamin A) 30, 90 and 270 µmol/egg were injected into the embryo via the amniotic sac. After 6, 18 and 24 hours, the spleen and bursa of the embryos were collected for RNA extraction and real-time PCR. The results were dose dependant. After 24 hours, inoculation of 270 µmol/egg showed downregulatory effects on interferon (IFN)-α, IFN-β and IFN-γ, IL-1β, IL-2, CXCLi2 and IL-13 relative expression in the spleen, indicating an anti-inflammatory effect at this concentration. In comparison, 90 µmol/egg of RA induced higher expression of the above genes at the same timepoint.

Results

The results were dose dependant. After 24 hours, inoculation of 270 µmol/egg showed downregulatory effects on interferon (IFN)-α, IFN-β and IFN-γ, IL-1β, IL-2, CXCLi2 and IL-13 relative expression in the spleen, indicating an anti-inflammatory effect at this concentration. In comparison, 90 µmol/egg of RA induced higher expression of the above genes at the same timepoint. The results of this study indicated that *in ovo* inoculation of vitamin A can modulate immune functions of the chicken embryo, which could be beneficial for induction of immune responses by *in ovo* vaccines.

Conclusions

The results of this study indicated that *in ovo* inoculation of vitamin A can modulate immune functions of the chicken embryo, which could be beneficial for induction of immune responses by *in ovo* vaccines.

Financial Support
Canadian Poultry Research Council; 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:

407 - Effect of Intramammary Antibiotics, Internal Teat Sealants, or both at Dry-off in Dairy Cows: I. Health outcomes

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Session: MASTITIS

Objective

Prevention and control of mastitis continue to be among the major challenges facing the dairy industry. Intramammary antibiotic (AB) infusion and internal teat sealants (TS) are commonly used for treatment and prevention of intramammary infections (IMI) during the dry period. Our objectives were to estimate the effect of different dry cow treatments (AB, TS, AB+TS or control [no treatment]) on dairy cattle health outcomes, specifically clinical mastitis, cow culling, IMI cure and new IMI over the dry period .

Methods

A block randomized controlled clinal trial was completed (12/2016-08/2018) on eight California dairy herds. A total of 1,273 cows were block randomized to one of the four treatment groups (AB, TS, AB+TS or control) over summer and winter seasons. Microbiological testing was done on composite milk samples collected at enrollment (dry-off), within 21 days after calving and on quarter milk samples from the first case of clinical mastitis within 150 days in milk (DIM) in the subsequent lactation. Statistical analysis was done using generalized linear mixed models.

Results

There were no significant differences in the odds of clinical mastitis or culling among cows treated with AB, TS, or AB+TS compared to controls. Cows treated with AB+TS had the highest odds of IMI cure (OR 3.05; *P<0.01*), and the lowest odds of developing new IMI (OR=0.45; *P <0.01*). Cows treated with TS had higher odds (OR 2.0; *P<0.01*) of IMI cure and lower odds of new IMI (OR=0.51; *P<0.01*) in comparison to untreated cows. Cows treated with AB had higher odds (OR 2.38, *P<0.01*) of IMI cure and lower odds of new IMI (OR=0.70; *P=0.09*) in comparison to untreated cows. The common bacteria isolated from milk samples collected at dry-off, post-calving and at the first clinical mastitis event were coagulase negative *Staphylococci* spp., *Streptococcus* spp. (excluding *S. agalactiae*), and *coliform* bacteria.

Conclusions

Dry cow treatment with AB+TS, AB, and TS increased the IMI cure, reduced new IMI, and had no significant effect on clinical mastitis and cow culling during the first 150 DIM compared to controls.

Financial Support
University of California Division of Agriculture and Natural Resources

Notes:

408 - Effect of intramammary antibiotics, internal teat sealants, or both at dry-off in dairy cows: II. Milk production

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Session: MASTITIS

Objective

Milk production is the main source of income to the dairy industry with mastitis as the greatest disease challenge for dairy producers. Amongst the approaches implemented to control and prevent mastitis on dairies are vaccination, use of pre- and post-milking teat-dip and treatments at dry-off, including intramammary antibiotics and teat sealants. The objective of our study was to evaluate the effect of different treatments at dry-off on the subsequent lactation's milk production and somatic cell count (SCC).

Methods

A single blinded randomized controlled block design trial was conducted between December 2016 and August 2018 on eight herds in California. Eligible cows were randomly allocated to one of four treatment groups at dry-off (intramammary antibiotics (AB), internal teat sealant (TS), AB+TS, or control [no treatment]) and followed through 150 days in milk (DIM) in the subsequent lactation. Milk production and SCC data records were used from the monthly DHIA test records (Dairy Comp305, Valley Ag Software, Tulare, CA). Monthly milk production (kg) and $\log_{10}$ of SCC (cells/ml) were modeled using two-piece spline linear mixed models.

Results

There was a significant increase in milk production (1.84 kg/day) in cows treated with AB+TS at dry-off in comparison to controls; while there was a numerical increase in milk produced by cows that received either AB or TS (0.12 kg/day; P=0.83 and 0.67 kg/day; P=0.24 respectively) in comparison to untreated cows. These dry cow treatments were associated with a significant reduction in $\log_{10}$ SCC during the first 150 days post-calving. The greatest reduction was reported in cows treated with AB+TS (-0.41; P <0.01), followed by AB (-0.30; P <0.01), and finally TS (-0.19; P = 0.03) in comparison to controls.

Conclusions

Dry cow treatments can be used selectively to address specific herd production and milk quality goals. Dairies with high SCC may benefit from treating cows at dry-off with AB, TS, or both.

Financial Support
University of California Division of Agriculture and Natural Resources

Notes:

409 - Investigations of the impact of intramammary infection on the developing bovine mammary gland

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Session: MASTITIS

Objective

Intramammary infections (IMI) are common in both lactating and non-lactating dairy cattle but their impact on heifer mammary glands that are rapidly growing and developing for the onset of lactation is poorly understood. The objective of this study is to characterize the cellular, tissue architecture, and gene expression changes that occur when the bovine mammary gland is subjected to an IMI during concurrent mammary growth and development, and elucidate how the mammary microbiome is changed during these conditions.

Methods

In a first experiment, 16 non-pregnant heifers will receive estradiol and progesterone injections to induce rapid mammary growth and development. Two quarters of each heifer will be randomly selected for intramammary infusions of saline or *Staphylococcus aureus* in separate quarters, so that each heifer receives both treatments and serves as its own control. Tissues will be collected 10 days post-challenge for analysis. In a second experiment, 21 pregnant heifers will be selected at 3 different stages of gestation (6.25, 7, and 7.75 months gravid) and similarly challenged with 2 quarters of each animal being randomly selected and infused with either saline or *Staphylococcus aureus.* Mammary tissues will be collected 3 weeks post-challenge to represent the impacts of a chronic IMI on mammary growth and development at different gestational stages. In both experiments, lacteal secretions will be collected throughout the trial to measure the somatic cell count and assess changes in the mammary microbiome. Collected mammary tissues will be subject to immunohistological, gene expression, and microbiome analyses.

Results

Experiments are projected to commence in 2021 and 2022.

Conclusions

Data from these experiments will provide information on how IMI during the foundational rapid mammary growth and development that occurs during a heifer's first gestation is altered and define at what stage of gestation these alterations are most pronounced. An understanding of how the mammary microbiome adapts to lactogenesis in the presence or absence of an IMI will also be gained.

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

Notes:

410 - Impact of subclinical mastitis detected in the first month of lactation on performance of organic dairy cows

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Session: MASTITIS

Objective

It is known that subclinical mastitis (SCM), characterized by somatic cell count (SCC) > 200,000 cell/mL, has a negative impact on productivity, reproductive performance, and survivability of dairy cows in conventional herds. However, information about the impacts of SCM in dairy cows from organic herds is scarce. Therefore, our objective was to evaluate how SCM diagnosed in the first month of lactation impacts milk production, fertility and culling of organic dairy cows.

Methods

Data were collected from two organic herds in New Mexico and Texas. A total of 2,124 cows that calved between June 2018 and May 2019 were included. Cows with SCC > 200,000 cells/mL in the first month of lactation were considered as having SCM. Mixed linear regression models accounting for repeated measures were used to assess the effect of SCM on monthly milk production and SCC linear scores. Cox proportional hazards models were used to evaluate the effect of SCM on the risk of pregnancy and culling. Parity, farm, previous gestation length, stillbirth, twin, dystocia, and two and three-way interactions were offered to all models.

Results

The average milk yield for cows with and without SCM was 30.9 kg/d and 32.2 kg/d, respectively ($P < 0.01$). Cows with SCM had higher SCC linear score during the last 3 months of previous lactation than non-SCM cows ($P < 0.01$), and the same was observed throughout the current ($P < 0.01$). The impact of SCM on linear scores was more pronounced in multiparous than primiparous cows ($P < 0.01$). Subclinical mastitis in the first month of lactation did not influenced the likelihood of pregnancy in the first 300 DIM ($P = 0.92$) but increased the likelihood of culling/death ($P < 0.01$).

Conclusions

Elevated SCC in the first month of lactation impairs milk production, increases the risk of culling, but does not impact fertility of organic dairy cows. Cows with SCM had elevated SCC linear scores throughout the entire lactation, and elevated SCC was carried over from the previous lactation.

Financial Support
USDA National Institute of Food and Agriculture

Notes:

411 - Soluble Epoxide Hydrolase enzyme activity during severe bovine coliform mastitis

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Session: MASTITIS

Objective

Systemic clinical mastitis in dairy cattle caused by gram-negative bacteria, such as *Escherichia coli*, is challenging to treat in part because of concurrent oxidative stress that is commonly not targeted during treatment. Soluble epoxide hydrolase (sEH) enzyme is a promising therapeutic target in acute inflammation models characterized by oxidative stress; however, sEH remains unexplored in cattle. The objective of this study was to describe the activity sEH during severe coliform mastitis.

Methods

Holstein dairy cows with severe coliform mastitis (n=5) and matched healthy controls (n=5) were sampled before (blood, milk, urine) and after (kidney, liver, mammary gland tissues) euthanasia or slaughter. The sEH activity was determined by ex-vivo metabolism of a synthetic substrate, (3-Phenyl-oxiranyl)-acetic acid cyano-(6-methoxy-naphthalen-2-yl)-methyl ester (PHOME). Oxygenated lipid metabolites (oxylipids) in plasma, milk, and urine were also used to predict sEH activity. Oxidant status was assessed by quantifying reactive oxygen species (ROS) and antioxidant potential (AOP) in milk and serum. Data were analyzed by Wilcoxon rank-sum tests ($P \leq 0.05$).

Results

We found greater metabolism of PHOME by sEH in the mammary gland of mastitic cows but lower liver activity than control cows. Increased ROS, lower AOP, and higher oxidant status index in milk suggested an increased prooxidant status in the mammary gland during mastitis. In serum, lower ROS, AOP, and overall oxidant index suggested a lower systemic prooxidant status during disease. Oxylipids in milk suggested a decrease in sEH activity in mammary tissue. In plasma, oxylipids from linoleic acid predicted a greater sEH activity, but those from arachidonic acid indicated lower activity during mastitis.

Conclusions

The activity of sEH differed among tissues, and different oxylipids predicted conflicting activity during coliform mastitis. Increased mammary sEH activity was detected when milk oxidant status was increased. The results highlight the need to assess the potential link between sEH activity and oxidant status changes during disease.

Financial Support
USDA National Institute for Food and Agriculture

Notes:

412 - Prospective cohort study of post-calving udder health in cows with intramammary infections in late lactation

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Session: MASTITIS

Objective

Evaluate if late lactation intramammary infection (IMI) with non-aureus *Staphylococcus* spp. (NAS) and *Streptococcus* and Strep-like organism (SSLO) groups would be associated with udder health and productivity in the subsequent lactation.

Methods

A prospective cohort study was conducted using a convenience sample of 74 herds from 10 states in the U.S. Aseptic quarter milk samples from 40 late lactation cows (>180 days pregnant) were collected from each farm and were cultured using standard laboratory procedures and MALDI-TOF. Cows (n=2763) were classified as having been exposed to all pathogens, major pathogens, minor pathogens and for individual pathogen groups or species. Outcomes of interest included clinical mastitis, removal from the herd, subclinical mastitis (somatic cell count [SCC] > 200,000 cells/ml) and milk yield during 1-120 days in milk (DIM) in the subsequent lactation. Cox regression was used to estimate hazard ratios (HR) for clinical mastitis and culling events and generalized estimating equations were used to estimate risk ratios (RR) for subclinical mastitis and differences in test-day milk yield.

Results

The presence of late lactation IMI caused by major pathogens was positively associated with post-calving clinical mastitis (HR = 1.5) and subclinical mastitis (RR = 1.6). Species within the NAS group varied in their associations with post-calving udder health. Late lactation IMI caused by SSLO were associated with increased risk of post calving clinical mastitis (HR = 1.5) and subclinical mastitis (RR = 1.5). DHIA test-day milk yield during 1-120 DIM were 0.9kg lower in late lactation cows with any IMI. No associations were detected between IMI in late lactation and risk for post-calving removal from the herd.

Conclusions

Findings from this observational study indicate that late lactation IMI may have an impact on post-calving health. However, the strength of associations were generally low, indicating that their overall impact is likely to be limited.

Financial Support
Zoetis

Notes:

413 - Retinoic acid affects barrier integrity in bovine mammary endothelial cells

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Session: MASTITIS

Objective

Oxidative stress can cause tissue damage during coliform mastitis when the production of reactive oxygen species (ROS) is excessive due to reduced antioxidant resources. Thus it is imperative to investigate methods to reduce tissue damage from ROS in order to improve clinical outcomes. All-trans retinoic acid (ATRA), the active form of vitamin A, has many important effects on inflammation such as repressing the damaging effects of oxidative stress in human and murine cell models. Previous studies showed that ATRA increased dramatically in the systemic circulation in dairy cows experimentally infected with coliform mastitis. However, it is unknown whether ATRA is cytoprotective or contributes to the pathogenesis of coliform mastitis. The objective of this research was to identify the effects of ATRA on bovine mammary endothelial cells (BMEC) viability and oxidative stress in-vitro.

Methods

An in-vitro BMEC model of oxidative stress was used to evaluate cell viability. Cells were treated with 10 μM all-trans retinoic acid or the reactive oxygen producing compound, 2,2'-azobis-2-methyl-propanimidamide, dihydrochloride (AAPH). Cell viability and reactive oxygen species were evaluated using fluorometric assays. Statistical analysis was performed with one- or two-way ANOVA with Graphpad Prism.

Results

The BMEC viability was unaffected by treatment with ATRA at 24 hours ($p>0.05$). ATRA reduced ROS production 75% when treated concurrently with AAPH (4.4 to 1.1 fold change compared to control, $p <0.0001$).

Conclusions

Based on this data, ATRA has cytoprotective properties for BMEC in the face of oxidative stress. The precise method by which ATRA decreases oxidative stress in BMEC will need to be further investigated. This research indicates that ATRA may be protective against the pathologic effects of coliform mastitis.

Financial Support
USDA National Institute for Food and Agriculture

Notes:

414 - Mastitis vaccine antigen discovery using proteomics and transcriptomics of *Staphylococcus aureus* grown in milk

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Session: MASTITIS

Objective

Bacterial infections of the udder, or mastitis, represent one of the most economically significant challenges facing the dairy industry. *Staphylococcus aureus* is a main cause of mastitis and distinctive for its ability to establish treatment-resistant chronic disease. The use of current *S. aureus* vaccines is limited, and the rational incorporation of conserved antigens will promote efficacy. The goal of these studies was to identify vaccine candidates from proteomic and transcriptomic assay of *S. aureus* grown in milk. Prioritized antigens will be incorporated into a *S. aureus* bovine vaccine platform.

Methods

Surface proteins were isolated after trypsin incubation of bovine *S. aureus* grown in 5% skim milk or Luria broth for 24 hours. Proteins were cleaned and prepared for LC/MS/MS using an ion trap mass spectrometer. Surfaceome analysis was performed using the Thermo Proteome Discoverer 1.3, Sequest and Mascot search engines. Next Gen RNA Sequencing was performed on bovine *S. aureus* grown as above. Total RNA was extracted and shipped for prokaryotic library construction and paired-end 150bp RNA sequencing (Novogene, Chula Vista, CA). Differential gene expression analysis was performed using HISAT2, FeatureCounts, and DESeq2. GO analysis was completed using the ClueGO Cytoscape application.

Results

We have previously reported that *S. aureus* adhesins are upregulated during *in vitro* growth in milk. Results from the current studies support these findings, and show that multiple adhesins and virulence factors are upregulated and surface-exposed after growth in milk. Vaccine candidates were further prioritized *in silico* and include the *S. aureus* adhesins: Fib/Efb, IsaA and IsdF, as well as the MetN2 transporter and SsaA secretory antigen.

Conclusions

Surfaceome and transcriptomic approaches are powerful tools to support vaccine design. These studies describe the identification and prioritization of novel *S. aureus* mastitis vaccine candidates. An effective *S. aureus* vaccine for bovines will reduce animal morbidity and mortality, and will have multiple positive impacts on the dairy industry.

Financial Support
USDA National Institute of Food and Agriculture; U.S. National Institutes of Health

Notes:

415 - Systemic and local responses of cytokines and tissue histology in intramammary endotoxemia in dairy cows

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Session: MASTITIS

Objective

This study aims to delineate systemic and local responses of cytokines and quantify mammary cytostructural changes in lactating cows with intramammary lipopolysaccharide (LPS) challenge.

Methods

Ten multiparous dairy cows, blocked by days in milk, parity and milk yield, were divided into treatment (T) and control (C) groups. In T cows, both the front and rear quarters on one side of the mammary gland were alternatively assigned to receive LPS (50 µg in 10 ml saline, TL); the contralateral quarters received saline (10 ml, TS). Udder-halves of C cows were similarly assigned to receive either saline (10 ml, CS) or no infusion (untreated; CU). Cytokine changes in blood at 3, 6, 12 and 24 h relative to the LPS infusion and in mammary tissue at 3 and 12 h as well as mammary histology changes were evaluated.

Results

The cytokines IFNG, IL6, IL10, IP10, MCP1, MIP1B, and TNFA showed a systemic response in the blood, whereas the cytokines INFG, IL4, IL10, IP10, MCP1, MIP1B, and VEGFA showed a systemic response in TS gland. In addition, the cytokines IFNG, IL1A, IL1B, IL4, IL6, IL8, IL10, MIP1A, IL36RA, MCP1, MIP1B, and TNFA showed a local response in TL glands. Histological changes of mammary tissue due to endotoxin challenge included 5.2- and 7.2- fold increases in number of neutrophils in alveolar lumen at 3 h and 12 h, respectively ($p < 0.01$), whereas neutrophils were rarely observed in the saline-infused control glands (TS and CS). LPS injection did not induce any detectable structural changes in mammary alveoli and mammary epithelial cell numbers, the average numbers of epithelial cells per alveoli and alveolar area did not change by LPS treatment. However, immunolocalization of E-cadherin (expressed as mean % area of positive staining) in TL gland tended to decrease at 12 h ($p < 0.1$). Furthermore, LPS increased mammary apoptosis after 3 h of LPS challenge ($p < 0.01$).

Conclusions

In summary, LPS challenge induced specific local as well as systemic responses in cytokine production and incurs neutrophilic infiltration and apoptosis in the infused mammary gland.

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

Notes:

416 - Metagenomic approach for identification of microbial co-infections in zoonotic infectious diseases

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Session: MICROBIOME

Objective

The importance of detecting co-infections is becoming more recognized, but it remains challenging to get such information. A better understanding of the prevalence of co-infections is required, partly because co-infecting pathogens can interact with each other directly or indirectly via the host's resources or immune system. These interactions within co-infected hosts can alter the transmission, clinical progression, and control of multiple infectious diseases as compared to single pathogen species infection. Furthermore, co-infection can potentially affect the performance of laboratory testing for zoonotic infectious diseases.

Methods

In this study, we used metagenomic data (RNA-seq) from COVID-19 patients. Public next-generation sequencing data (N=68) from SARS-CoV-2 infected patients were retrieved from the NCBI Sequence Read Archive database using SRA-Toolkit. Data screening was performed using an alignment-free method based on *k*-mer mapping and extension, fastv. Taxonomic classification was performed using Kraken 2 on all reads containing one or more virus sequences other than SARS-CoV-2. The results of the Kraken 2 analysis were visualized with pavian and Krona tool which displays hierarchical data (like taxonomic assignation) in multi-layered pie charts.

Results

Influenza type A (H7N9) virus, human immunodeficiency virus, rhabdovirus, human metapneumovirus, Human adenovirus, Human herpesvirus 1, coronavirus NL63, parvovirus, simian virus 40, and hepatitis virus genomes sequences were detected in SARS-CoV-2 infected patients. Multidrug-resistant bacteria associated with hospital-acquired infections worldwide, such as *Enterobacter hormaechei*, *Staphylococcus aureus*, *Pasteurella multocida,* and *Acinetobacter baumannii* were detected in COVID-19 infected patients.

Conclusions

In this study, multiple viral and bacterial genome sequences were detected in COVID-19 patients. Further large-sample studies are warranted to investigate the prevalence of COVID-19 co-infection, the impact of co-infection on the host immune system of COVID-19 patients, and their role in disease progression.

Financial Support
University of Tennessee

Notes:

417 - A single dose of enrofloxacin alters gut microbial diversities irrespective of its dose in beef calves

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Session: MICROBIOME

Objective

Side effects of antibiotics on animal health have been well studied since their discoveries; however, perturbation of gut microbiota has not been recognized as a significant side effect for much of that period. Enrofloxacin is widely used for the prevention and control of bovine respiratory disease complex (BRD), the most economically significant disease of beef cattle in North America. Here, we examined the effects of lower and upper dose limits of enrofloxacin administered subcutaneously to beef calves on their gut microbial diversities.

Methods

Thirty-five calves sourced for this study were divided into three groups: Control (n=7), single Low therapeutic dose (n=14, 7.5mg/kg), and single High therapeutic dose (n =14, 12.5mg/kg). Fecal samples were collected from the calves respectively four and three times pre- and post-enrofloxacin injection to determine microbial diversities and compositions using 16S rRNA extract. We merged Low and High dose groups to assess the effect of the treatment on the microbiota.

Results

Alpha diversities increased after the treatment (mean observed OTUS: pre=279, post=325, q=0.033 and mean Shannon index: pre=5.8, post=6.2; q=0.071), but the two doses did not affect microbial richness and evenness differently (q>0.05). Besides, beta diversities were significantly different between pre-and post-treatment communities (Bray Curtis, q=0.001).), and the microbial diversities significantly shifted in both Low and High doses compared to the control ($q < 0.05$). Besides, 53 of 152 identified families (35%) were altered significantly due to enrofloxacin; at the genus level, the high dose caused a shift in a larger number of genera than the low dose.

Conclusions

In conclusion, this study confirms that subcutaneously administered a single therapeutic dose of enrofloxacin can affect gut microbiota in beef calves irrespective of its dose; however, it appears that the high dose causes a more prominent effect than the low dose. To this end, further studies are warranted to assess the relative impacts of different doses on resistome profiles of gut microbiota .

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

Notes:

418 - Growth performance and gut microbiota in cultured burbot (*Lota lota maculosa*) fed dietary plant-based proteins

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Session: MICROBIOME

Objective

As with many cultured fish species, utilizing plant-based proteins in aquafeed formulations is desirable to ultimately reduce the amount of required fishmeal. The current study investigated the potential for incorporating soybean meal (SBM) and dried distillers grains with solubles (DDGS) as fishmeal replacements in diets for juvenile burbot (*Lota lota maculosa*), and also investigated the influence of these plant-based protein diets on the gut microbiota.

Methods

Young-of the year burbot (8.3±0.2g) were cultured in aerated flow-through, triplicate tanks at 13°C. An Atlantic cod marine-type base diet was formulated to approximately 48% crude protein and 16% lipid and fishmeal protein content was replaced at 25% with inclusions of SBM and DDGS. Fecal material (trial start, 36d, and 72d) was collected for an assessment of both the intestinal microbiota via 16S sequencing (V3 and V4 regions), along with measurements for growth performance calculations. Results were analyzed using both one-way (growth performance) and two-way (microbiota) ANOVAs.

Results

Growth results from the trial endpoint indicate comparable performance (relative growth and specific growth rate) for all diets, but the DDGS diet did not show a favorable tank biomass gain when compared to the SBM diet. For feed conversion ratio (FCR; P=0.019), the burbot fed the REF diet showed the best conversion (0.80±0.01), which was better than the SBM (0.98±0.03) and DDGS (0.96±0.05) diets. With respect to alpha diversity analyses, the REF diet displayed an increase in the Shannon index over time (P=0.031) and was found to be greater than the SBM group at 72d (P=0.034).

Conclusions

Overall, the burbot performed well on the plant-based protein sources at this juvenile life stage. Further, changes to the gut microbiota were noted both over time and among different treatment diets, a consideration that may be used to develop diets that favor specific microbiota profiles catering to burbot health.

Financial Support
USDA National Institute of Food and Agriculture

Notes:

419 - Liver abscess syndrome in cattle: new insights from metagenomic investigations

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Session: MICROBIOME

Objective

Feedlot cattle are susceptible to developing liver abscesses, one of the most important and costly health problems of finishing cattle. Liver abscesses cause decreased growth efficiency and condemnation of meat products at slaughter. Fusobacterium and Trueperella are often considered causative agents, however, recent research shows that liver abscesses contain higher microbial diversity than previously reported, with unknown effects on disease severity. The objective of this study was to characterize the microbial community (microbiome) in liver abscess samples across three different studies of feedlot cattle.

Methods

Post-mortem liver abscess samples were collected from feedlot cattle previously enrolled in studies characterizing the effects of exposure to in-feed antimicrobials (i.e. tylosin) and a Saccharomyces cerevisiae fermentation product (SCFP). Composite fecal and soil surface samples were collected from enrolled cattle and pens prior to slaughter, respectively. Each sample was processed using 16S rRNA amplicon sequencing of the V4 region using an Illumina HiSeq platform.

Results

Samples were sourced from >5 feedlots and two states, yet the liver abscess microbiome was largely homogenous and polymicrobial, with Gram-negative anaerobic bacteria predominating. Microbiome composition was not significantly influenced by exposure to in-feed antimicrobials or SCFP compared to cattle not receiving any treatment.

Conclusions

Preliminary results from network analyses identify phyla found in low abundances such as Verrucomicrobia, Nitrospirae, and Chlorobi are significantly associated with influencing liver abscess microbiome composition and that only a limited number of phyla, such as Actinobacteria appear to be influenced by the environmental microbiome. Future work is needed to understand the role of liver abscess microbiomes in disease severity and must include factors associated with the host, environment, and management decisions at the feedlot.

Financial Support
National Cattlemen's Beef Association - Beef Checkoff

Notes:

420 - Understanding the fescue toxicosis plant-animal integrome in grazing beef through integrative interactomics

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Session: MICROBIOME

Objective

The *Epichloë coenophiala* endophyte is ubiquitous in tall fescue, the predominant grazing forage in the Southeastern US, and it increases plant vigor. However, *E. coenophiala*-infected tall fescue (E+) is referred to as toxic because the endophyte produces detrimental to grazing livestock ergot alkaloids. Grazing of E+ tall fescue leads to fescue toxicosis, economically costly disease with complex pathophysiology. We recently reported that E+ grazing perturbs metabolic homeostasis, induces hindgut dysbiosis, and perturbs the microbiome-metabolome interaction in beef cattle. Notably, no study has investigated these effects globally, i.e., including the rumen and in conjunction with *in planta* effects. Therefore, the goal of this study was to investigate global perturbations in the plant and animal using multi-compartment microbiota-metabolome integrative interactomics.

Methods

Angus steers grazed non-toxic (Max-Q) or E+ tall fescue over a 28-day grazing trial. Plant, rumen, plasma, urine and fecal samples were collected at multiple time points for 16S and ITS2 rRNA sequencing and untargeted high-resolution metabolomics analyses followed by multi-compartment/multi-component integrative interactomics.

Results

E. coenophiala/E+ grazing altered plant and animal microbiota, notably decreasing most ruminal fungi, while having mixed effects on the rumen bacteria and fecal microbiota. Effects on the plant, rumen, plasma, and urine metabolome were also prominent, with some plant-animal overlap (i.e., tryptophan and vitamin B6 metabolism). Integrative interactomics revealed similar overall network structures for Max-Q and E+, but many constituents of the networks were unique. For example, only the E+ had ruminal solid OTUs (bacterial) within the network; fecal fungal OTUs, which were essential to both networks, consisted of unique taxa, e.g., *Anaeromyces* (E+) and *Erthrobasidium* (Max-Q).

Conclusions

This study provides a global view of the plant and animal response to *E. coenophiala* infection and E+ grazing, respectively, while deepening our understanding of FT etiology and possible treatments for it.

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

Notes:

M421 - etagenomic surveillance of foodborne pathogens at the rodent-agricultural interface using Nanopore sequencing

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Session: MICROBIOME

T

Objective

he effective control of rodent populations on farms is a critical component of food-safety, as rodents are reservoirs and vectors for many foodborne pathogens (e.g., Salmonella spp., E. coli O157, etc.). The functional role of rodents in the amplification and transmission of foodborne pathogens is likely underappreciated in the United States. Clear links have been identified between commensal rodents and outbreaks of foodborne pathogens throughout Europe and Asia, however, comparatively little research has been devoted to studying this rodent-agricultural interface in the USA, particularly across the Midwest. Here, we address this existing knowledge gap by characterizing the metagenomic communities of rodent pests collected from Minnesota dairy farms. W

Methods

e leveraged the Oxford Nanopore MinION sequencer to provide a rapid real-time metagenomic survey to identify zoonotic foodborne pathogens. Rodents (mice and rat) were live trapped from two dairy farms and humanely euthanized. DNA extraction was performed using 24 rodent cecal content. Full length 16S amplicon sequencing was performed. O

Results

ur data indicate the presence of pathogenic strains of Salmonella spp., Listeria monocytogenes, Campylobacter spp., Clostridium spp., and Escherichia coli O157, along with many important mastitis pathogens. A critically important observation is that we discovered these pathogens within five species of rodents (Microtus pennsylvanicus, Mus musculus, Peromyscus leucopus, Peromyscus maniculatus, and Rattus norvegicus). D

Conclusions

ata generated from our study will likely result in the identification of new reservoirs for food-borne pathogens and species-specific traits. In light of our results, we recommend a multidisciplinary ‘One Health’ approach to discover, prevent, and control the spread of rodent-borne zoonotic pathogens in our food supply. Furthermore, knowledge gained from our research efforts will directly inform and improve upon farm-level biosecurity efforts and public health interventions to reduce future outbreaks of foodborne disease.

Notes:

422 - Role of nasal microbiome in respiratory immunity in pigs

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Session: MICROBIOME

Objective

The main goal of this study is to investigate the role of nasal microbiome in respiratory immunity in pigs.

Methods

Nasal microbiome from 2-3-week-old piglets was collected and homogenous pool of inoculum was prepared. In first trial, 3-day old colostrum-deprived (CD) gnotobiotic piglets received intranasal inoculation of nasal microbiome. In second trial, CD gnotobiotic piglets received intraperitoneal (I/P) injection of sow serum for 3 days before microbiome inoculation. In third animal trial, we used 10X lower dose of nasal microbiome, gave I/P injection of sow serum 3 days before and 3 days after microbiome inoculation and gave bovine colostrum preparation for first 4 days. In 4th trial, we adapted exact same protocol from third experiment.

Results

In our first experiment, surprisingly all the microbiome inoculated piglets died within 3-4 days following nasal microbiome inoculation. In second trial, nasal microbiome successfully colonized in the nasal cavity and gut; however, within 7 days after colonization, all piglets died or were euthanized due to poor health condition. In 3rd experiment all piglets survived through the entire study period (3 weeks) and were healthy. In microbiome inoculated animals, 16s rRNA sequencing showed change in abundance and diversity between inoculum and post colonization in nasal and fecal samples. TLRs (1,2,3,4,7,8,9), RIG-I, MDA-5, IL-1a, IL-1b, IL-6, IFN-b, IL-8, MCP-1 expressions in the nasal mucosa were significantly higher in nasal microbiome inoculated group than the control gnotobiotic group. Similarly, serum IgA level was higher in nasal microbiome inoculated group than the control group. In 4th experiment, piglets started to die after 8 days of nasal microbiome inoculation and all piglets either died or were euthanized by day 23. Sow serum IgG was around 50% less than that of 3rd experiment and serum concentration of IgG in piglets was low compared to third experiment.

Conclusions

From these studies, we conclude that strong passive immunity is required for the survival of nasal microbiome inoculated CD gnotobiotic piglets.

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

Notes:

423 - Impact of a modified live porcine reproductive and respiratory syndrome virus vaccine on the gut microbiome of pigs

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Session: MICROBIOME

Objective

Porcine reproductive and respiratory syndrome virus (PRRSV) continues to cause the most costly disease of swine production in the United States despite advances made against PRRS control. Although PRRS modified live virus (MLV) vaccines are widely utilized to reduce PRRS-associated loses, currently available vaccines are generally considered inadequate for disease control. Recently, the gut microbiome has been associated with vaccine efficacy and health outcomes following PRRSV infection in pigs. The objective of the current study was to investigate the effects of PRRS MLV vaccination on the gut microbiome composition and diversity of nursery pigs.

Methods

Weaned pigs (average age 23.4 ± 2.1 days) were obtained from a single commercial source and divided into vaccine (n = 12) and nonvaccine (n = 12) groups. The vaccine group was immunized with a commercial PRRS MLV vaccine (Ingelvac PRRS MLV; Boehringer Ingelheim Animal Health) according to the manufacturer's instructions. Approximately four weeks post-vaccination and prior to challenge with wildtype PRRSV and porcine circovirus type 2, fecal samples were collected from both groups. Gut microbiomes were compared between the two groups using the Lawrence Livermore Microbial Detection Array (LLMDA).

Results

Immunization with PRRS MLV vaccine significantly reduced the level of wildtype PRRSV replication and viremia post-challenge. At the level of the gut microbiome, vaccination was associated with an increase in fecal microbial species diversity and increased Firmicutes to Bacteroidetes ratio. Significant differences were also noted in microbiome composition; specifically, the families Carnobacteriaceae and Enterobacteriaceae were detected at significantly higher rates in vaccinated pigs when compared to nonvaccinated pigs ($p = 0.003$ and $p=0.03$, respectively; Fisher's exact test).

Conclusions

Altogether, this study provides data on how the gut microbiome may shift in association with PRRS MLV vaccination and suggests that microbiome characteristics may contribute to vaccine efficacy.

Financial Support
USDA National Institute for Food and Agriculture; State of Kansas

Notes:

424 - Comparison of DNA stabilization methods for preservation of metagenomic samples collected from cattle

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Session: MICROBIOME

Objective

Metagenomic sequencing has become an indispensable tool for investigation of the microbiome found in a variety of ecological niches. Samples that are obtained in highly controlled settings such as a clinic or laboratory can be immediately processed so that growth and metabolism of microbial populations is halted essentially at the time of collection. However, when studies are conducted in remote settings, the conditions under which samples are stored and transported can allow alterations of the microbiome composition before they reach the laboratory. The purpose of this study was to evaluate the use a commercial sample stabilization kit (OMNIgene•GUT, DNA Genotek Inc) and 100% ethanol (EtOH) for stabilization of microbiome in bovine feces and respiratory secretions under a variety of temperatures and conditions that mimicked situations related to collection and transportation from field settings.

Methods

A fresh fecal sample was obtained from a mature Holstein cow and used to prepare multiple replicated samples. Three preservation methods (OMNIgene kit, EtOH, no preservative), four temperatures (20°C, 4°C, -20°C, -80°C), and three holding times (immediate processing, 1 wk, 2wks) were evaluated in a factorial design. Additionally, respiratory secretion samples were collected from the nasopharynx of yearling beef steers, and preservation with EtOH was compared to storage without preservative. DNA was extracted from these samples using DNeasy PowerFecal Microbial Kit (Qiagen) and 16S rRNA gene amplicon sequencing was performed to characterize the microbiome, with a target sequencing depth of 100,000 PE reads per sample. The Qiime 2 software platform was used to classify sequencing reads, and R software package was used to compare differences between preservation conditions.

Results

Results of this experiment demonstrated preservation in commercial stabilization kit and in EtOH at warmer temperatures were equivalent to immediate freezing at -80°C.

Conclusions

DNA stabilization to preserve microbiome composition will greatly facilitate field research and remove important potential biases.

Financial Support
Texas A&M University

Notes:

425 - Differential impact of OTC regimen on growth and gut microbiota of pigs co-infected with respiratory pathogens

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Session: MICROBIOME

Objective

Along with judicious antibiotic use, there is great interest in how the dose regimen of an antibiotic affects the animal gut microbiota. We examined the effectiveness of two dosing regimens of oxytetracycline (OTC) against respiratory pathogen challenge in pigs and what impact this had on the pig gut microbiota.

Methods

Eighty 3-week-old pigs were divided into 4 groups. One group was not challenged and given non-medicated feed (NONINFnm). The other three groups were infected with *Bordetella bronchiseptica* and *Pasteurella multocida* seven days and three days prior to OTC treatment (day 0), respectively, and divided by OTC dosing regimen: no OTC treatment/non-medicated feed (INFnm), OTC injection/non-medicated feed (INFinject), and non-injected/OTC feed (INFfeed) until necropsy (days 4 or 7). Animal weights were recorded to assess average daily gain. OTC levels in nasal wash, plasma, and lung were measured using LC-MS. Lungs were examined and scored for lung lesion severity. *B. bronchiseptica* and *P. multocida* colonization in nasal cavity, lung, trachea, and tonsil were assessed. Fecal microbial population shifts were characterized by 16S rRNA gene sequence analysis.

Results

OTC administration had minimal effect on *B. bronchiseptica* and *P. multocida* colonization. There was some significant impact on lung lesion development detected in INFnm group on day 7. Average daily gain was higher for INFinject and NONINFnm compared to INFnm on days 4 and 7. OTC was detected in plasma, nasal cavity, and lungs at varying concentrations depending on the dosing regimen. Gut microbial shifts of INFnm, INFinject, and INFfeed were significant on day 7. Relative abundances of microbial populations were detected among the three groups at the order level.

Conclusions

While OTC was detected in animals after administration, there was minimal impact on colonization of *B. bronchiseptica* and *P. multocida* and disease pathology. Interestingly, injected OTC limited negative impacts of infection on weight gain. OTC impacted gut microbiota.

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

Notes:

426 - Assessing the microbiome as a tool for the mitigation of viral disease in nursery pigs

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Session: MICROBIOME

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 associated with PRRSV, and reduce the risk of antimicrobial resistance in swine.

Financial Support
USDA National Institute of Food and Agriculture

Notes:

427 - Environmental influences of high-density agricultural animal operation on human forearm skin microflora

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Session: MICROBIOME

Objective

Human forearm skin microbiome ecosystem contains rich and diverse microbes, which are influenced by environmental exposures. The microbial representatives can be exchanged between human and environment specifically animals, by which they share certain or similar epidermal microbes. Livestock and poultry are the microbial sources associated with the transmission of community-based pathogenic infections. Here in this study, we proposed to investigate the environmental influences introduced by livestock/poultry operations on forearm skin microflora of on-site farm workers.

Methods

A total of 30 human skin swab samples were collected from 20 animal workers in dairy or integrated farms and 10 healthy volunteer controls. The skin microbiome was 16S metagenomic sequenced with Illumina MiSeq system. For skin microbial community analysis, the abundance of major phyla and genera as well as alpha and beta diversities were compared across groups using ANCOM and *vegan* package in R.

Results

We identified distinctive microbial compositional patterns on skin of workers in farm with different animal commodities. Workers in integrated farms containing various animals were associated with higher abundances of epidermal Proteobacteria, especially *Pseudomonas* and *Acinetobacter*, but lower Actinobacteria, especially *Corynebacterium* and *Propionibacterium*. For those workers with frequent dairy cattle operations, their Firmicutes in the forearm skin microbiota were enriched. Furthermore, farm animal operations also reduced *Staphylococcus* and *Streptococcus* as well as modulated the microbial biodiversity in farm workers' skin microbiome.

Conclusions

The alterations of forearm skin microflora in farm workers, influenced by their frequent farm animal operations, may increase their risk in skin infections with unusual pathogens and epidermal diseases.

Financial Support
USDA National Institute of Food and Agriculture

Notes:

428 - The microbiome of common bedding materials, before and after use on commercial dairy farms

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Session: MICROBIOME

Objective

Bovine mastitis is considered to be one of the most economically important diseases effecting dairy cows. The choice of bedding material has been identified as an important risk factor contributing to the development of mastitis. However, few reports examine the culturable and unculturable microbial composition of different bedding materials, and how this composition may shift during use of the bedding by dairy cows. Given the prevalence of unculturable microbes in most environments, this information could be an important step to understanding whether and how the bedding microbiome acts as a risk factor for mastitis. Therefore, our objective was to characterize the microbiome composition and diversity of bedding materials, before and after use, through amplicon sequencing.

Methods

We collected bedding samples from 44 dairy farms in the U.S. Unused (from storage pile) and used (out of stalls) bedding materials were collected from four bedding types: new sand (NSA), recycled manure solids (RMS), organic non-manure (ON) and recycled sand (RSA). Samples were analyzed using 16S rRNA sequencing of the V3-V4 region.

Results

We found that microbial diversity was highest in RMS samples, followed by RSA, NSA and ON bedding materials. Used beddings were more diverse compared to unused beddings. A shift in microbiome composition and abundance was observed between used and unused beddings. Proteobacteria, Actinobacteria and Firmicutes dominated all bedding types, but the abundance of Firmicutes increased in used beddings.

Conclusions

Our results support previous findings that suggest differential microbial profiles for different bedding materials. The consistent shift in the microbiome of all bedding types that occurred during use by dairy cows may represent an interventional opportunity in management of mastitis on dairy farms. Future plans for these data will integrate intramammary infection and other udder health parameters from the cows and herds that used these bedding samples to analyze potential associations between the microbiome and mastitis epidemiology.

Financial Support
USDA National Institute of Food and Agriculture; Zoetis

Notes:

429 - Characterization of the bulk tank milk microbiome from Prince Edward Island commercial dairy farms

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Session: MICROBIOME

Objective

The bulk tank milk (BTM) microbiome is thought to be reflective of the health of the cows and management practices of the herd. This study investigated the feasibility of using metagenomics to characterize the microbiome of BTM from commercial dairy farms. Additionally, differences in the microbiome were described across herd characteristics.

Methods

This pilot study included a convenience sample of 12 dairy herds on Prince Edward Island, Canada. BTM samples were shipped to Colorado State University for metagenomic analysis. Shannon diversity index was used to evaluate alpha-diversity, which was compared across stall type using a Wilcoxon rank-sum test. Beta-diversity was assessed with Bray-Curtis dissimilarity.

Results

Firmicutes was the most abundant phylum at 47% of reads across all herds. Proteobacteria (19%), Bacteroidetes (17%), and Actinobacteria (10%) were the next most common phyla. Of the 500 observed genera, the most abundant were *Corynebacterium* (3.9%), *Acinetobacter* (3.9%), and *Lactobacillus* (3.7%). *Streptococcus*, *Staphylococcus*, and *Escherichia*, mastitis related pathogens, made up 2.6%, 1.8%, and 0.98% of reads across all samples, respectively. Genus-level Shannon diversity was higher (P = 0.027) in tie stalls (n = 3) than free stalls (n = 9) (4.52 vs 4.27). The average Bray-Curtis dissimilarity was 0.439 (Range: 0.263 – 0.709). However, the Bray-Curtis dissimilarity between free stall and tie-stall farms was 0.441 (95% CI: 0.425, 0.458), which is not significantly different from the baseline.

Conclusions

In this pilot study, Firmicutes was by far the most abundant phylum, followed by Proteobacteria, Bacteroidetes, and Actinobacteria. The BTM microbiome has higher alpha-diversity in tie stalls than free stalls. However, beta-diversity was not significantly higher between stall types than baseline. This study shows the promise of metagenomics for determining the constituency of the BTM microbiome. However, as differences in alpha-diversity were not substantiated by beta-diversity, it is necessary to build on this pilot study to determine the accuracy of these methods.

Financial Support
Zoetis

Notes:

430 - Development of climactic oral and rectal microbiomes corresponds to peak immunoglobin titers in lambs

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Session: MICROBIOME

Objective

The largest mammalian lymphatic tissue resides in the gastrointestinal tract (GIT) where it interacts with the densest population of resident microbes, the GIT microbiota. Concordantly, the GIT microbiota in several mammalian systems have been shown to affect the developing function of the immune system. However, there remains a paucity of research on this relationship in ruminant animals. Therefore, we sought to assess the relationship between the GIT microbiota and the maturation of the adaptive immune system.

Methods

Blood serum samples were collected via jugular venipuncture, along with rumen, oral, and rectal samples from lambs at 0 (birth), 7, 14, 30, 60, 120, 180, and 365 days of age. Lambs' sera were profiled for circulatory concentrations of IgM, IgA and IgG using antibody-specific ELISAs. Spearman's ranks correlations were used to measure significant relationships between gut microbial diversity of the oral, fecal, and ruminal locations to the specific antibody titers.

Results

The GIT microbiota formed stable adult climatic communities (similar to that of the dams) around 120-180 days of age. This corresponded to the peak in serum titers for each immunoglobin, which, aside from a peak in IgA and IgG at birth (likely maternal transfer), had gradually increased prior to this time point. Immunoglobins peaked and then returned to a sub-peak level between 120 and 365 days. Overall 56 significant ($P \leq 0.05$) correlative relationships between antibody titers and particular GI taxa were determined that included described mutualists, commensals and opportunistic pathogens. Microbial taxa from the fecal and oral GIT locations accounted for 83% (46/56) of all interactions.

Conclusions

Overall our results indicate the lambs immune function peaks between 120-180 days as the GIT microbiota reach a more stable adult-like climactic state and suggest a similarly important role of GIT microbes in shaping the lambs adaptive immune system. Correlations to fecal and oral microbiota are consistant with these regions being the primary locations of GALT in the mucosal/nasopharynx epithelium (MALT/NALT).

Financial Support
USDA National Institute for Food and Agriculture

Notes:

431 - Dietary oligosaccharides modulate microbiome, enteric glia, and epithelial barrier function in a neonatal pig model

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Session: MICROBIOME

Objective

In our neonatal pig model of intestinal ischemia, an age-dependent defect in epithelial barrier restitution can be rescued by ischemic mucosal homogenate from weaned pigs. This is associated with an immature subepithelial enteric glial cell (EGC) network, a known driver of epithelial restitution, which matures postnatally in response to microbial colonization. We have shown that the microbiome can be modulated by dietary prebiotics in our model. Therefore, we hypothesized that dietary oligosaccharide supplementation would accelerate postnatal microbial colonization and EGC network maturation, thus enhancing neonatal restitution responses after intestinal ischemic injury.

Methods

After 24-hours colostrum, pigs were fed control or oligosaccharide-supplemented formula for 14-days and fecal swabs were 16S rDNA sequenced. Jejunal and colonic ischemia were induced surgically for 30-minutes. Injured mucosa was recovered *ex vivo* while monitoring epithelial barrier function by transepithelial electrical resistance (TEER) and restitution was evaluated histologically. EGC were isolated to assess calcium response to ATP and co-culture effects on scratch-wounded IPEC-J2 cells.

Results

Microbial taxa changed in a time- and diet-dependent manner with diets containing oligosaccharides clustering by day seven and becoming progressively more tightly clustered over time ($P<0.05$). TEER and histological appearance of the non-injured and injured jejunum were unaffected by diet. In the injured colon, prebiotic-fed pigs demonstrated higher initial TEER ($P=.0012$) and increased preliminary histological evidence of restitution after recovery. EGC from prebiotic-fed pigs showed decreased intracellular calcium response to ATP ($P=.0075$) versus control-fed pigs. EGC from prebiotic-fed pigs enhanced IPEC-J2 restitution as compared to IPEC-J2 in monoculture ($P=.032$), while EGC from control-fed pigs did not.

Conclusions

Ongoing work to understand microbiome-EGC-epithelial interactions during postnatal development may lead to novel management and clinical practices to improve intestinal health in vulnerable neonates.

Financial Support
U.S. National Institute of Child Health and Human Development; USDA National Institute for Food and Agriculture; U.S. National Institutes of Health

Notes:

432 - Surveillance of ticks and tick-borne diseases in Canada: A framework for a One Heath approach

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Session: PARASITOLOGY

Objective

Global climate, environment, and human socio-economic change, is causing (re)emergence of ticks and tick-borne diseases. Blacklegged ticks, which transmit *Borrelia burgdorferi*, the agent of Lyme disease (LD) are of growing concern in many areas of Canada. Several methods of passive and active surveillance have been employed to monitor the distribution of ticks and tick-borne diseases in Canada, but no single method is ideal. Each surveillance method has benefits and challenges, making it difficult to accurately assess the risk of exposure to ticks and tick-borne diseases in humans and animals. This review was done to assess the drivers of emergence, methods, and challenges of tick and tick-borne disease surveillance, and provide a framework for a One Health surveillance approach

Methods

A Google search was performed to identify priority studies from where key research terms were obtained and grouped into three themes (emergence, methods and challenges of surveillance, and One Health). Four combinations of search strings were used to capture studies from two databases (Web of Science and PubMed)

Results

Of the 3604 studies screened, 140 were retained. These included (i) North American studies addressing tick and tick-borne disease emergence (n= 35), and methods and challenges of surveillance (n=50), (ii) worldwide studies addressing One Health approaches to vector-borne disease surveillance and management (n=38), and (iii) studies relevant to all three research themes (n=17)

Conclusions

The findings of this review will contribute foundational knowledge for the development and evaluation of a One Health approach to tick and tick-borne disease surveillance in Canada. A One Health (integrated) approach to surveillance of ticks and tick-borne diseases is needed to gather data, perform risk assessments and design, implement, and evaluate risk reduction interventions more efficiently and effectively. We believe that this surveillance approach which integrates animal, human, and environmental data will greatly enhance our ability to monitor, prevent, and treat tick-borne disease in humans and animals

Financial Support
University of Guelph

Notes:

433 - A paraprobiotic cure for gastrointestinal nematode parasites of livestock

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Session: PARASITOLOGY

Objective

Gastrointestinal nematode (GIN) parasites are the most common parasites of livestock, causing significant morbidity in ruminants (sheep, cattle, goats) and monogastrics (horses, pigs, poultry). Multidrug resistant GIN parasites are fcommon, and there is an urgent need for new, cost-effective, broadly active anthelmintics. We have shown that Bacillus thuringiensis (Bt) Cry5B protein may safely address this urgent need. Bt Cry proteins are the number one biologically produced insecticide in the world today with a superb safety record. We have shown that Bt Cry5B targets human GINs. Here, we study the efficacy of Cry5B against livestock GINs.

Methods

We engineered Bt to asporagenously express and form Cry5B crystals in the bacterial cytosol. This bacterium can be inactivated (killed) with food-grade essetial oils. We call this engineered bacterium IBaCC5 for Inactivated Bacterium with Cytosolic Crystal (Cry5B). Studies involve oral dosing of IBaCC5 into livestock infected with GINs. Outcomes measured include parasite fecal egg counts and GI burdens relative to placebo. in vitro studies of parasites resistant to common anthelmintics are conducted to ensure that Cry5B can overcome current anthelmintic resistances. A non-GLP repeat/high-dosing tox studied was carried out.

Results

IBaCC5 is broadly effective against GIN parasites in vivo. Single dose in vivo curative studies against target GIN in sheep showed strong efficacy. Preliminary studies of a novel enteric formulation strategy in pigs infected with Ascaris showed promising results, with repeat studies on-going. IBaCC5 efficacy in horses against target GIN show superb efficacy based on fecal egg counts. Tox studied found no evidence of any toxicity, even at high repeat dosing.

Conclusions

IBaCC5 is safe and effective and can provide significant benefit against monogastric and ruminant livestock GINs in situations where anthelmintic resistance is an issue and in general.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture; U.S. National Institute of Allergy and Infectious Diseases

Notes:

434 - Case Report: Fading elk syndrome in a herd of captive elk (*Cervus elaphus*) in the North American Midwest

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Session: PARASITOLOGY

Objective

Fading elk syndrome, or chronic ill-thrift of elk, is a disease associated with abomasal parasitism with *Ostertagia* species, of which elk appear to be particularly susceptible. While this syndrome has been extensively reported to affect wapiti-type red deer hybrids farmed in New Zealand since the mid 1980's, there is only a single report of this disease in North America. Here we report a case of fading elk syndrome in a herd of 34 elk (*Cervus elaphus*) in Ames, Iowa, at the National Animal Disease Center.

Methods

Following the identification of clinical signs, physical examinations were performed, blood samples collected for complete blood counts (CBC) and serum chemistries, and fecal samples were taken for fecal floats. Blood samples were also taken and submitted to the National Veterinary Service Laboratories, for infectious disease testing.

Results

Analysis of complete blood counts were unremarkable, but blood chemistry demonstrated a severe hypoalbuminemia. Fecal floatations were also unremarkable, and non-diagnostic. Histological examination of tissues collected at necropsy revealed proliferative abomasitis and nematodes consistent with *Ostertagia* spp. Anthelmintic treatment consisting of a combination of pour-on Cydectin® and injectable Noromectin Plus®, at double the recommended dose for cattle, showed positive results, as all remaining animals in the herd recovered.

Conclusions

The work presented here is the first report of naturally-acquired disease in a herd of captive elk used for research and sheds light on this seldomly-reported disease in North America.

Notes:

435 - A high level of cryptic cattle fever tick movement occurs in southern Texas via both wildlife and infested cattle

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Session: PARASITOLOGY

Objective

Bovine babesiosis is a lethal cattle disease caused by the parasites *Babesia bovis* and *B. bigemina* and transmitted by cattle fever ticks (*Rhipicephalus microplus* and *R. annulatus*). This disease system is endemic in Mexico and a threat to the US cattle industry. Disease management in the US is focused on preventing spread from Mexico by aggressively eradicating tick infestations in Texas with acaricides. An anti-tick vaccine for cattle is also used inside a tick quarantine zone along the Rio Grande border. Our objectives are to better understand the source of new tick infestations in Texas and examine diversity in vaccine targets, as the current vaccine is not always effective against *R. microplus* in North America.

Methods

We developed genetic tools to support eradication efforts, including DNA fingerprinting markers and qPCR assays for mutations that convey acaricide resistance, and used them to genotype >5,000 ticks collected by APHIS and USDA-ARS. We also evaluated genetic variation in two anti-tick vaccine targets (Bm86 and MP4) using amplicon sequencing.

Results

Our results have led to key insights for tick management, including: 1) ticks frequently use wildlife (white-tailed deer and nilgai antelope) as hosts, which then leads to cattle infestation; 2) ticks are transported long distances within Texas on infested cattle, and 3) acaricide resistance mutations from Mexico are spilling over into the US. In addition, we found a large number of mutations in Bm86 and MP4 which could decrease the effectiveness of these vaccines.

Conclusions

We conclude that a high level of cryptic tick movement occurs in southern Texas. The spread of acaricide resistance mutations over the past decade poses a serious threat to the continued use of acaricides to manage infestations. As acaricides see reduced effectiveness, anti-tick vaccines for cattle will likely become an important management tool for disrupting the lifecycle of ticks. However, given the high number of mutations we identified in two vaccine targets, it will be essential to account for genetic diversity in future vaccine development.

Financial Support
USDA National Institute for Food and Agriculture

Notes:

436 - Host-pathogen coevolution in a changing environment: the fall armyworm (*Spodoptera frugiperda*) and its baculovirus

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Session: PARASITOLOGY

Objective

Rapid ecological changes are being brought about by a warming climate. We are seeing shifts in species ranges, changes in population demography, and altered species interactions. The pressing problems due to changes in temperature affect both ecological dynamics and evolutionary processes. Our research will ask - "How do abiotic factors such as changing temperatures affect eco-evolutionary processes in host-pathogen systems?"

Methods

This research will be carried out using an insect host-pathogen system -- the fall armyworm (*Spodoptera frugiperda*), an agricultural pest, and its species-specific virus. Fall armyworm populations are widespread and, like other outbreaking insects, its population dynamics can be pathogen regulated. To quantify the effects of changing temperatures on host-pathogen coevolution, we will establish separate populations of hosts and pathogens derived from a geographical gradient covering their distributions. We will use these populations to determine how changing temperatures affect host resistance to the virus, pathogen virulence, and disease transmission. We will also track changes in the host and pathogen transcriptome.

Results

Our preliminary data show that temperature affects transmission, that virus origin influences infection, and that there are distinct changes in the host's transcriptome when they are exposed to the virus. Using the established population lines, we will quantify the effects of abiotic factors on disease transmission and whether these effects vary due to ecological processes alone, host evolution, pathogen evolution, or host-pathogen coevolution.

Conclusions

While this research focuses on a particularly devastating pest, the results will be applicable to numerous silvicultural and agricultural pest species that are readily infected by pathogens and whose dynamics will be affected by changing temperatures. This research will also improve our ability to determine how best to use these pathogens as bioinsecticides from an eco-evolutionary perspective for Integrated Pest Management.

Financial Support
U.S. Department of Agriculture

Notes:

437 - A U.S. isolate of *Theileria orientalis* Ikeda is transmitted to cattle by invasive Asian longhorned ticks

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Session: PARASITOLOGY

Objective

Theileria orientalis is a tick-borne hemoparasite that causes significant losses to the global cattle industry. The Ikeda strain is more virulent than others, leading to death in a subset of affected animals. In 2017, *T. orientalis* Ikeda was detected in cattle in VA, U.S. Months earlier, the U.S. was alerted to the invasion of the Asian longhorned tick, *Haemaphysalis longicornis,* in the eastern U.S. *H. longicornis* ticks were identified on cattle in the *T. orientalis*-affected herd in VA, and a subset were PCR-positive for *T. orientalis* Ikeda. In previous U.S. outbreaks of *T. orientalis*, parasites were not transmissible by *H. longicornis*; however, *H. longicornis* is the main vector of *T. orientalis* world-wide. Thus, the objective of this study was to determine whether invasive *H. longicornis* ticks in the U.S. are competent vectors of *T. orientalis* Ikeda.

Methods

A splenectomized Holstein calf was intravenously inoculated with cryopreserved U.S.-VA *T. orientalis* Ikeda blood stabilate. Following innoculation, infection status was monitored via peripheral blood cytology and PCR for the *T. orientalis* MPSP gene. Once patent *T. orientalis* parasitemia was confirmed, *H. longicornis* nymphs were fed on the calf. Replete nymphs were collected and allowed to moult to adults. Adult ticks were fed on three Holstein calves, which were monitored for infection as described above. After four days of feeding, a subset of ticks were removed for salivary gland dissection and *T. orientalis* PCR.

Results

A subset of adult tick salivary glands tested positive for *T. orientalis* Ikeda. *T. orientalis* Ikeda was transmitted to 3/3 calves, each of which developed parasitemia reaching 0.4-0.9%. Infected calves exhibited modest decline in peripheral blood packed cell volume; however, no significant clinical signs of piroplasmosis were observed.

Conclusions

Our findings demonstrate that U.S. *H. longicornis* ticks are a competent vector of the USA-VA *T. orientalis* Ikeda strain. This data provides information for the U.S. cattle industry regarding the necessity of enhanced control measures to curb parasite spread.

Financial Support
U.S. Department of Agriculture

Notes:

438 - Improved blood parameters and reduced parasite eggs detected in pastured goats fed pelleted stinging nettle

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Session: PARASITOLOGY

Objective

Gastrointestinal (GIN) parasitism remains the biggest health challenge in small ruminant production in the US and worldwide. While use of anthelmintic has been the mainstay of treatment and control for decades of years, development of resistance to many of the currently used drugs and demand for organically raised animal products has called for research on alternatives strategies to reduce parasite burden and their economic effect in these animals. Use of plants with nutritional benefits and potentially bioactive compounds that can control parasites or reduce their negative health impact on animals has gained attention lately. Stinging nettle, a nutritionally dense plant is widely consumed as a vegetable by humans in many parts of the world for its many health benefits that include anti-inflammatory and hematological boosting properties.

Methods

In this study, we evaluated the hematological and gut health benefits of supplementing pelleted stinging nettle to naturally parasite infected pastured Spanish and Myotonic yearling goats for three months. Whole blood, serum and fecal samples were collected monthly. Hematocrit was determined from whole blood using a hematocrit centrifuge, total serum protein was determined using a refractometer while fecal egg count was determined using McMaster egg counting technique.

Results

Stinging nettle significantly increased the hematocrit in supplemented animals by the end of the first month, decreased the fecal egg count in Spanish goats and also significantly increased the total serum protein after two months of feeding

Conclusions

These health benefits indicate that this forage may help reduce the negative health impacts of *Hemonchus contortus* and other GIN parasites that affect small ruminants. Thus stinging nettle may be a good candidate for further research on its potential use as a bioactive forage for these species.

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

Notes:

439 - Towards novel acaricide development against cattle fever tick: GPCR target validation by RNAi and chemical leads

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Session: PARASITOLOGY

Objective

The project advances the discovery of novel chemistries to control the tick vector *R. microplus.* Innovation is in the validation of G protein-coupled receptors as acaricide targets, and in advancing tick neurobiology and endocrinology. 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. We focused on the tick kinin signaling system that is hypothesized to regulate water balance, metamorphosis, and feeding. We silenced the pyrokinin receptor.

Methods

1. To determine the identity and function of the tick kinin neuropeptides, their cDNA was cloned from *R. microplus* synganglia. We utilized primers designed based on our curation of the kinin gene from the tick *Ixodes scapularis,* and from NCBI *R. microplus* transcriptomes. We also sequenced the *R. microplus* kinin gene. The kinin precursor cDNA sequences and the kinin peptide sequences were predicted for other seven tick species. Predicted *R. microplus* kinins were tested on the receptor expressed in CHO-K1 cells. 2. RNAi in tick females was performed using pyrokinin receptor dsRNA constructs validated in a luciferase cell assay system. 3. A kinin receptor high-throughput screening was performed with 20,000 small molecules. Hits were validated in dose-response assays with cells.

Results

The kinin gene encodes seventeen kinins. Fourteen kinins tested on the kinin receptor were highly active (nM level). Tick kinins feature a conserved proline in the variable position two (X_2) of the kinin pentapeptide core FX_1X_2WGamide that is needed for activity. Thirty-six kinin receptor antagonists were identified; the most potent had an IC_{50} = 600 nM. Structure-activity relationships of the potent antagonists identified a pharmacophore needed for antagonism. Pyrokinin receptor RNAi resulted in significant negative effects on reproduction (P <0.0001).

Conclusions

Tick neuropeptide GPCRs can be interfered with dsRNA and small molecules.

Financial Support
USDA National Institute for Food and Agriculture

Notes:

440 - Comparison of multiple chlortetracycline regimens to control diverse *Anaplasma marginale* strain infection

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Session: PARASITOLOGY

Objective

Anaplasmosis the most prevalent tick-transmitted disease of cattle worldwide and a major obstacle to profitable beef production in the U.S. Understanding the ecology of antimicrobial resistance and control of hemoparasitic diseases, including anaplasmosis, are national priorities for the USDA and vital to protecting food security. Tetracycline antimicrobials are the only FDA-approved drug to control active anaplasmosis in cattle and may be administered with no limit on duration of use. Continuous exposure to a single drug class for prolonged periods introduces strong selective pressure for the development of resistance in the pathogen species. The objective of this study is to compare the efficacy of different CTC dosage treatment regimens on anaplasmosis infection status in cattle infected with historic or contemporary isolates of *Anaplasma marginale*.

Methods

To compare CTC susceptibility phenotype, cattle were infected with one of four *A. marginale* isolates. Upon entering chronic anaplasmosis, the most common anaplasmosis infection state, cattle were divided into one of four CTC treatment groups and treated for 120 days. Anaplasmosis infection status was evaluated by cELISA, quantitative PCR, and observation of recurrent clinical signs.

Results

Ninety-six calves were successfully infected with one of four *A. marginale* isolates. CTC-treatment was more effective at reducing *A. marginale* infection levels in calves infected with historic versus contemporary *A. marginale* isolates. Infection levels of contemporary *A. marginale* isolates remained high despite high dose CTC treatment suggesting *A. marginale* may be evolving a tolerance to tetracycline antimicrobials.

Conclusions

As tetracycline antimicrobials are the only FDA-approved antimicrobials to control and treat anaplasmosis, it is critical that their efficacy be preserved. The results of this study will aid in developing a judicious and broadly effective CTC anaplasmosis treatment strategy that mitigates development of antimicrobial resistance in cattle systems.

Financial Support
USDA National Institute of Food and Agriculture

Notes:

441 - Assessing the spread of the blacklegged tick, *Ixodes scapularis*, and the agent of Lyme disease in Ontario, Canada

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Session: PARASITOLOGY

Objective

The blacklegged tick, *Ixodes scapularis*, is a vector for *Borrelia burgdorferi*, the causative agent of Lyme disease. Lyme disease is a serious health issue for humans, dogs and horses, and the risk of Lyme disease has significantly increased due to ongoing range expansion of the blacklegged tick. Ongoing monitoring of blacklegged tick populations and its pathogens is needed to assess risk. The purpose of this research is to examine spatial and temporal patterns of *I. scapularis* and its pathogens from 2017 to 2019 in southern, eastern and central Ontario. It is hypothesized that ongoing range expansion will be detected in Ontario over the three-year period.

Methods

Tick dragging was conducted at 38 to 46 sites in each of the last three years. All ticks were identified to species. Blacklegged ticks were tested for *B. burgdorferi*, *B. miyamotoi*, *Babesia microti*, *Anaplasma phagocytophilum* and Powassan virus at the National Microbiology Laboratory. Spatial data will be projected using ArcGIS and assessed using space, time and space-time analyses in SaTScan. Regression models will be built to determine the factors influencing tick and pathogen invasion.

Results

I. scapularis was detected at 11 new sites in 2017, 15 in 2018 and 3 in 2019. *B. burgdorferi* was detected at 6 new sites in 2017, 0 in 2018 and 1 in 2019.

Conclusions

Spatial and temporal patterns indicating the ongoing establishment and expansion of blacklegged tick populations and their associated pathogens are expected to be identified in the upcoming months. A potential 3-5 year gap between the time that blacklegged tick populations establish and the invasion of *B. burgdorferi* will also be considered. Assessing the spatial and temporal patterns of blacklegged ticks can allow for further evaluation of how *I. scapularis* and its pathogens spread, which will provide invaluable information to public and animal health. Additionally, these findings may enhance primary methods of prevention to reduce possible blacklegged tick exposure to help minimize the transmission of tick-borne pathogens.

Notes:

442 - Adaptation of a tick performance model system to cattle infested with *Dermacentor variabilis*

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Session: PARASITOLOGY

Objective

Dermacentor variabilis ticks are indigenous to the eastern US, where it is an important ectoparasite of cattle and a biologic vector of *Anaplasma marginale*. Immunization of cattle with tick midgut (MG) or salivary gland (SG) was shown to reduce different performance parameters of *D. andersoni* ticks. The objective of this study was to test the working hypothesis that immunization of cattle with similarly prepared tissue homogenates will reduce feeding and fecundity performances of *D. variabilis* ticks.

Methods

Female-male pairs of *D. variabilis* were fed on calves (3 calves per group, 9 calves total) before and after immunization with adjuvant alone or with tick MG or SG homogenates, and tick feeding and fecundity performance parameters were measured for each tick group.

Results

Feeding and fecundity performance parameters were reduced in MG and SG groups compared to cohorts fed prior to immunization or on calves injected with adjuvant alone.

Conclusions

This model system used to study experimental reduction in feeding and fecundity performances of various rhipicephaline species fed on dogs and cattle is also applicable to *D. variabilis* ticks fed on cattle. Work is underway to further adapt this system to include acquisition, maintenance and transmission of a bovine tick-borne pathogen, *Anaplasma marginale*.

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

Notes:

443 - Protein antigens associated with seroreactivity of cattle producing reduced tick performance

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Session: PARASITOLOGY

Objective

Immunization of dogs and cattle with tick salivary gland (SG) or midgut (MG) homogenates resulted in distinct impacts on tick feeding or fecundity performance parameters, respectively. We adapted this model system to *Dermacentor andersoni* experimentally fed on cattle, which represents common tick parasites of cattle that are indigenous to the United States. The objective of this study was to identify tick protein candidate antigens uniquely recognized by sera collected from cattle used to feed ticks with significant reductions in performance.

Methods

D. andersoni were fed on steers before/after immunization with MG or SG homogenates with Freund's complete/incomplete adjuvant. ELISAs confirmed Immunoglobulin levels and 2-D Westerns isolated proteins uniquely recognized by protected host immune sera. A Bruker timsTOFPRO generated tryptic peptide m/z ratios submitted to PEAKS X search engine against NCBI-tick protein databases. Predicted and apparent molecular sizes (Mr) and isoelectric points (pI) were compared.

Results

ELISA titers indicated antibody responses to immunization with either antigen. 2-D Western blots confirmed immunization with different homogenates induced both cross-reactive and specific immunoglobulins to both tick tissues. Immune sera from different hosts immunized with the same homogenates were reactive to similar proteins, with some differences in the two trials. Approximately 22 and 25 SG protein spots, in trials 1 and 2, respectively, were uniquely recognized by anti-SG immune sera. MALDI-TOF analysis resulted in 271 tick protein candidates, 195 of which had predicted mass and pI within range of Mr and pI of 41 of the 47 SG proteins uniquely reactive to anti-SG sera.

Conclusions

Multiple MALDI-TOF sequences matched Mr and pI of all but six uniquely seroreactive 2-D Western spots associated with reduced performance of *D. andersoni* female ticks. Work is underway to express and screen uniquely seroreactive tick antigen candidates and to apply this model system to acquisition, maintenance and transmission of *Anaplasma marginale* by *D. andersoni* ticks.

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

Notes:

444 - A Pioneering Approach to Tick Control: Anti-tick Toxins Delivered via Transfected *Babesia bovis*

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Session: PARASITOLOGY

Objective

Ticks are obligate hematophagous ectoparasitic arthropods that transmit a variety of animal pathogens, including *Babesia bovis*. It is estimated that bovine babesiosis cause annual losses of >US $20 billion worldwide. There are no effective vaccines to control *Rhipicephalus* ticks or *Babesia* spp. To minimize tick burden, the use of acaricides is the only effective method available. However, widespread acaricide use has selected acaricide-resistant *Rhipicephalus* tick populations. The discovery of tick populations with acaricide-resistance in Mexico raises concerns regarding geographic and numerical tick expansion into *Rhipicephalus*-free areas including the United States and a corresponding increase in the risk of transmitting *Babesia* spp. to U.S. livestock. Bio-insecticides such as protein toxins derived from spiders have the potential to control tick vectors if an appropriate delivery system were available. We proposed to test if transfected attenuated *B. bovis* expressing an anti-tick protein toxin will reduce infestation by *R. microplus*.

Methods

To test if transfected *B. bovis* expresses eGFP during infection of mammalian and tick hosts, we used whole gene replacement of an alternative locus, BBOV_II005480, to append a promoter and a gene for GFP-BSD.

Results

Using this novel approach, we successfully demonstrated the entire life cycle of the transfected *B. bovis* in the vertebrate and invertebrate hosts by taking advantage of the expression of the fluorescent marker eGFP by the transfected parasites.

Conclusions

The transfected parasites infected the tick vector and subsequently transovarially transmitted to a naïve calf via infected larvae. Through these discoveries, we now have acquired the ability to transfect *B. bovis* with a plasmid cassette that may allow specific expression of an anti-tick toxin exclusively in the hemolymph milieu of the tick vector, with the expectation that it will effectively interrupt the life cycle of *Rhipicephalus* ticks.

Financial Support
USDA National Institute for Food and Agriculture

Notes:

445 - 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 the global livestock industry billions of dollars annually. Use of anthelmintic drugs is the primary means of controlling nematodes in livestock, but there is now high prevalence of anthelmintic-resistant nematodes. Thus, there is urgent need to identify novel strategies for developing new efficacious anthelmintics. Our long-term goal is to identify molecular targets for developing drugs with novel modes of action to kill nematodes and circumvent resistance. Our objective in this project is to identify inhibitors for essential phospholipid biosynthetic enzymes in nematodes as lead compounds for developing novel, broad-spectrum anthelmintics.

Methods

i). Clone and characterize genes encoding putative phosphoethanolamine methyltransferases (PMT) enzymes from different families of livestock nematodes and identity their broad-spectrum inhibitors.
ii). Test the anthelmintic efficacy of optimized PMT inhibitors against a variety of important nematode parasite species of livestock, including multi-drug-resistant strains, using both in vitro and in vivo assays.

Results

We have found that the putative PMTs from different nematode species possess bona fide PMT catalytic activities. Using the PMT assay, we have screened compound libraries several specific PMTs inhibitors that are nontoxic to mammalian cells at their effective concentrations.

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

Notes:

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

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Session: PHYSIOLOGY

Objective

Our objectives are to: (1) Characterize parameters recorded by sensors during health and disease in dairy cows; (2) Demonstrate machine-learning methodology for synthesizing multiple parameters to create Health Status Indexes that identify cows with health disorders (HD); (3) Demonstrate that automated health monitoring can promptly and accurately identify cows with HD.

Methods

Holstein cows (n=1,209) were enrolled from -21 to 30 DIM. Health status was monitored daily. Wearable sensors monitored physical activity (PA), resting time (RT), body temperature (BT), rumination, and eating time. Non-wearable sensors monitored milk volume, milk fat to protein ratio (FPR), milk conductivity, body weight, and environmental conditions. Previous health, production, and reproductive events were retrieved. All data were used for development and testing of machine learning algorithms [XGBoost (XGB), Multi-Layer Perceptron (MLP), Recurrent Neural Networks(RNN)] to predict cow health status.

Results

The pattern of sensor parameters around HD varied depending on the parameter and the type of HD. As examples, cows with displaced abomasum had reduced eating time (-22%), rumination (-34%), PA (-15%), whereas milk FPR increased (+10%) for day -5 vs the day of diagnosis (all $P<0.05$). Cows with metritis had reduced eating time (-14%), PA (-17%), RT (-13%), whereas milk FPR (+11%) and BT (+2%) increased for day -5 vs the day of diagnosis (all $P<0.05$). The sensitivity and specificity of Health Status Indexes on a testing dataset were 88% and 88%, 43% and 96%, and 70% and 67% for XGB, MLP, and RNN, respectively.

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. Combination 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.

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

Notes:

448 - Physiological changes in nursery piglets following transport of different durations in the Canadian summertime

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Session: PHYSIOLOGY

Objective

To assess and compare the physiological response of weaned piglets to different road transport durations by using clinical and hematological indicators of hydration status, injury and stress.

Methods

A cohort study was completed in the summer of 2019 observing multiple shipments of recently weaned piglets from two swine flows representative of Canadian commercial practices. Piglets exposed to long duration (LD) transport (>30 hrs, n=9,400) were compared to piglets exposed to short duration (SD) transport (<3 hrs, n=2,034). Incidence of in-transit mortality was recorded for all piglets. Body weight, the presence of lesions at specified body sites, and lameness were documented the day prior to (t0), immediately following (t1), and 72-hours following (t2) transport for a subset of piglets (SD: n=200, LD: n=240). Complete blood count panels, biochemistry profiles, serum cortisol assays and pen-side glucose and lactate tests were completed on 80 piglets per duration group at t0 and t1.

Results

No difference in mortality risk was observed between groups. Piglets undergoing SD travel had higher odds of lameness observed at t1 compared to LD piglets. Differences in lesion scores observed likely correspond to changing group social dynamics with weaning as opposed to transport duration. At t1, only piglets in the LD group had biologically significant negative weight change from weight at t0. In this group, relatively heavy piglets at t0 lost more weight over the transport period than light piglets at t0. No effect of duration was observed on net weight change from t0 to t3. No indicators of hydration status or injury were outside of normal reference ranges for piglets of this age group, though differences between groups were observed.

Conclusions

These results represent an essential component in understanding the effects of transport duration on piglet welfare and suggest that in these duration cohorts, piglets were physiologically resilient to transport length. However, behavioural data collected during and following transit must be interpreted before a final conclusion on welfare can be made.

Notes:

449 - Periparturient immune depression and disease is due to dysregulation of calcium homeostasis and metabolic syndrome

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Session: PHYSIOLOGY

Objective

This project aims to determine the physiological mechanims that govern hypocalcemia and negative energy balance due to excessive loss of body condition during the tranistion period of dairy cows in order to maintain homeostasis during this time period. Delineation of the physiology underlying the etiology of hypocalcemia and BCS loss that leads to depressed immune function and poor reproductive success is critical for the determination of novel methods to manage hypocalcemia and its associated negative health outcomes.

Methods

Our proposal aims to:
1) Establish the role of inhibition of calcium prepartum on postpartum immune function and disease.
2) Determine the contribution of body condition loss during the peripartum period on hypocalcemia, postpartum immune function, and health disorders.
3) Assess the use of colostrum quality and markers of immune function related to hypocalcemia and NEB as measures of metritis, mastitis, and other health disorders during early lactation.

Results

Our experiments are in progress.

Conclusions

This grant aims to determine the physiological mechanisms underpinning hypocalcemia and NEB that result in depressed immune function and poor reproductive health.

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

Notes:

450 - Early life stress in pigs induces a developmental shift in gut epithelial glucose transporter systems

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Session: PHYSIOLOGY

Objective

Early life adversity (ELA) is a significant risk factor for chronic inflammatory diseases into adulthood. Dysregulation of glucose homeostasis is recognized as a driver of inflammatory response and has been linked with ELA in humans and in animal models. The mechanisms by which ELA impacts glucose homeostasis and its implications for humans and animal disease risk remains poorly understood. Utilizing early weaning (EW) in piglets as a model of ELA, we tested that hypothesis that EW alters the normal developmental trajectory of intestinal epithelial glucose transporters.

Methods

Yorkshire breed female piglets were weaned from their sows at 16 d of age (EW), or 28 d of age (LW, control LW). Weaned piglets were co-housed and received the same diets. At 70 d of age, jejunum and ileum were collected and mounted on Ussing chambers to evaluate Na^+-linked glucose transporter 1 (SGLT1) and glucose transporter 2 (GLUT2) function and expression.

Results

Jejunal and ileal SGLT1 transport, measured as the change in transepithelial short-circuit current (ΔI_{sc}) after luminal glucose addition, was reduced ($P<0.05$) in EW pigs, compared with LW pigs. In contrast, intestinal GLUT2-mediated transport was increased ($P<0.05$) in EW pigs. Opposing effects of EW on SGLT1 and GLUT2 transport coincided with differential localization of SGLT1 and GLUT2 to the brush border membrane (BBM), suggesting altered intracellular trafficking in EW pigs. Further, SGLT1 and GLUT2 function and expression were divergently regulated by β-andrenergic receptor (BAR) pathways in LW but not EW pigs. EW pigs exhibited elevated serum glucose and expression of GLUT in lymph node and spleen.

Conclusions

Together, these data indicate that EW, a common early life stressor in agricultural animal production, causes a developmental shift in intestinal glucose transport from SGLT1 towards GLUT2-mediated uptake. Given the increased transport efficiency of GLUT2, this shift may be a mechanism driving elevated serum glucose and increased tissue GLUT expression which in turn may contribute to increased lifetime risk for chronic inflammatory disease.

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

Notes:

451 - Early life stressors affect health of dairy calves

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Session: PHYSIOLOGY

Objective

Our specific aims are to 1) determine role of VFA and pH in inflammatory state and permeability in intestinal epithelial cells, 2) determine role of weaning method (abrupt vs. gradual) on local and systemic inflammation status, oxylipids profile, and animal health, and 3) determine role of excessive transportation on GIT permeability, oxidative stress, and inflammation. Our hypothesis is that early life insults such as excessive transportation, lack of colostrum feeding within the first 36 h of life, or abrupt weaning at early ages, compromise health and promote inflammatory status.

Methods

For experiment I, The human colon carcinoma Caco-2 cell line will be obtained from ATCC. The cells will be maintained in culture medium composed of Dulbecco's Modified Eagle Medium (DMEM) and will be maintained. Treatments include exposure to low and high pH (via modifying butyric acid relative proportion) with LPS at low (0.5 ng/mL) or high (10 ng/mL) dose for 1-5 days, Inflammatory status of cells and cell-mediated innate response will be evaluated when epithelial cells are dosed with either low or high LPS stimulation. To evaluate the intestinal barrier function, the paracellular permeability of Lucifer Yellow (LY) will be analyzed. For experiment II, 36 day old Holstein dairy calves (18 males, 18 females) will be blocked by gender and BW at birth, and randomly assigned to be on either 1) abrupt weaning on d 42 of age, or 2) gradual weaning on d 56 of age. Daily milk replacer and solid feed intake will be recorded and weekly BW to assess productive responses. Weekly blood sampling and health scores will be performed. Various measures of inflammatory markers and acute phase proteins will be analyzed.

Results

By investigating early life stressors, we aim to identify and develop strategies that will reduce incidence of diseases, the need for antibiotic treatments, and ultimately morbidity and mortality pre-weaning.

Conclusions

Our long-term goal is to develop nutritional and management intervention strategies that reduce gut inflammation and incidence of diarrhea in dairy calves.

Financial Support
USDA National Institute of Food and Agriculture

Notes:

452 - Does early lactation milk yield modify the impact of hyperketonemia in dairy cows?

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Session: PHYSIOLOGY

Objective

High β-hydroxybutyrate (BHB) concentration in the bloodstream might be not always detrimental for dairy cows. Identifying and treating animals with a higher risk of having negative outcomes is fundamental to control hyperketonemia (HYK) more efficiently and to reduce the burden of the disease. The objective of this study is to evaluate whether early lactation milk yield modifies the association of hyperketonemia with health events, early herd removal, and reproductive performance.

Methods

Blood BHB was measured twice between 3 and 10 d of calving in 2,041 Holstein dairy cows from five dairy herds in Minnesota for the diagnosis of HYK (BHB > 1.2 mmol/L). Cows were divided into two groups based on milk yield in the first week post-partum (i.e., high or low). Reproductive performance, health events, and herd removal information were obtained from the farm management software. We created Cox proportional hazard models to evaluate the association of HYK with pregnancy up to 150 days in milk (DIM), and disease events and culling by 60 DIM across milk production groups. Parity was offered to the models as a potential confounder variable and herd was include as a random intercept.

Results

The prevalence of HYK was 15.3% across the study population. High producing cows had similar pregnancy rate by 150 DIM (HR= 1.04, 95%CI: 0.82, 1.32) independently of HYK status. Additionally, high producing cows had a higher hazard of disease events (HR= 1.69, 95%CI: 0.76, 3.80) and herd removal by 60 DIM (HR= 1.28, 95%CI: 0.60, 2.71) if hyperketonemic (HYK+). On the contrary, HYK+ low producing cows had 28% less pregnancy rate by 150 DIM (HR= 0.72, 95%CI: 0.52, 0.99), 3.72 times higher hazard of diseases to 60 DIM (95%CI: 2.22, 6.22), and 1.62 times higher hazard of culling by 60 DIM (95%CI: 0.90, 2.90) when compared to low producing HYK– cows.

Conclusions

These results suggest that HYK+ cows with low first-week milk yield are at a higher risk of impaired reproductive performance, and health events and herd removal, unlike cows with high early milk yield that performed well independently of the blood BHB concentration.

Financial Support
Minnesota Rapid Agricultural Fund

Notes:

454 - Dynamic protemics of PEDV and PRRSV infections

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Session: PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME

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 profilling 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 viral 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
National Research Council Canada; Fonds de recherche du Québec

Notes:

455 - Host transcriptome reveals putative genes involved in persistent infection with a live-attenuated PRRSV strain

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Session: PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME

Objective

Porcine reproductive and respiratory syndrome virus (PRRSV) is an arterivirus that causes reproductive failure and respiratory disease in pigs. It has been well characterized that both virulent and live-attenuated PRRSV strains can persist in lymphoid tissues of infected pigs for a long period of time (up to 5 months), suggesting that the host immune system does not effectively clear the virus. To investigate the mechanisms of PRRSV persistence, we performed a transcriptional analysis of inguinal lymphoid tissue of pigs experimentally infected with a live-attenuated PRRSV strain days 46 post-infection.

Methods

Ten four-week-old PRRSV naïve pigs were divided into two groups: group 1 was injected intramuscularly (IM) with PBS to serve as the negative control, while group 2 was inoculated IM with $10^{5.0}$ $TCID_{50}$ of a live attenuated PRRSV vaccine candidate CON90. Blood samples were collected weekly to determine antibody (Ab) and T cell responses. Lymph node biopsy was collected for RNA sequencing to study host transcriptome signatures associated with vaccination.

Results

A total of 6,404 differentially expressed genes (DEGs) were detected of which 3,960 DEGs were upregulated and 2,444 DEGs were downregulated. Specifically, genes involved in innate immune responses (IRF1, TRAF3, CFD, C1QABC, and C1R) and chemokine and receptors (CCL22, CCL24, CCL19, and CCR6) associated with T cell homing to lymphoid tissues were downregulated. As a result, homing of virus-specific T cells to lymphoid tissues seems to be ineffective, evidenced by the lower frequencies of virus-specific T cells in lymphoid tissue than in peripheral blood. Genes associated with T cell exhaustion (CD274, IDO1, BTLA, FAS, TIGIT, and HAVCR2) were upregulated. Likewise, genes involved in the anti-apoptotic pathway (XIAP, MCL1, BCL2A1, and API5) were upregulated

Conclusions

Collectively, the data suggested that PRRSV establishes a pro-survival microenvironment in lymphoid tissue by suppressing innate immune responses, T cell homing and preventing cell apoptosis

Financial Support
USDA National Institute for Food and Agriculture

Notes:

456 - PRRSV infection and oxidative stress modulation by selenium

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Session: PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME

Objective

Porcine Reproductive and Respiratory Syndrome virus (PRRSV) is one of the most impacting pathogens for swine industry worldwide. Type 2 PRRSV is the main species circulating in North America and PRRSV 1-7-4 strain has been the most common wild type detected since 2015. The lung is the primary site of infection of PRRSV, 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 innate immunity. The phagocytic performance of MNP relies on the respiratory burst and mitochondrial respiration (MR). Compounds containing Selenium (Se) have effects on cellular oxidative stress and immunomodulatory capacities, and are used in pig production as they increase the adaptive ability of animals to stress. The function of Se is mediated through specific proteins called selenoproteins, known to be regulators of the cellular oxidative stress.

Methods

Using a combination of an in vitro and ex-vivo systems, we assessed the differences in MR triggered by PRRSV2 infection, the decrease in the level of infection in cells treated with Se and the variation in cytokine expression of isolated MNP.

Results

Our preliminary data shows that two PPRSV2 strains (NC-1-7-4 (highly pathogenic), NC-1-3-4 (lowly pathogenic)) alter cellular MR during infection. We observed that the use of Se decreases PRRSV2 infection after 24h. We also assessed the interactions between NC PRRSV-2 strains and different MNP: PAM, PIM and dendritic cells (DC): We measured the modulation of cytokine expression in MNP exposed to PRRSV2 NC-1-7-, NC-1-3-4, and the modified live vaccine (MLV) prototype strain. Preliminary data showed different inflammatory cytokine expression between DC and macrophages subsets.

Conclusions

These immune responses will the baseline to analyze how Se modulates them and the oxidative stress. This study will be a stepping-stone on the identification of a specific Se supplementation regime to apply along with vaccination to increase the efficacy of PRRSV control measures and decrease its impact.

Financial Support
North Carolina State University

Notes:

457 - Drivers of PRRS virus dispersal among pig production systems in the United States

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Session: PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME

Objective

Despite improvements in porcine reproductive and respiratory syndrome virus (PRRSV) characterization, remains unclear whether PRRSV infections are a product of viral circulation within a farm, within production systems (local) or across production systems (external). Here we examined the dissemination dynamics of PRRSV and the processes facilitating its spread within and among pig farms in three production systems.

Methods

ORF5 PRRSV sequences were obtained from three commercially unrelated systems of sow-to-finish swine farms collected during 2014-2019 in a defined dense pig populated region of the United States. To determine the spatiotemporal patterns of PRRSV spread, the impact of different potential predictors on PRRSV spread, as well as to infer the migration rates between farms (internal) and external introductions (between production systems) we used a Bayesian phylodynamic approach.

Results

Farms at areas with pig density from 500 to 1000 pig/km^2 and farms located at a range within 0.5 km and 0.7 km from major roads were more likely to infect by PRRSV, whereas farms at an elevation between 41 and 61 meters and denser vegetation acted as dissemination barriers. Results evidenced that dissemination among commercially unrelated pig production systems was intense, reinforcing the importance of farm proximity on PRRSV dissemination.

Conclusions

The understanding of how PRRSV disseminates among production systems and farm types provide valuable information that can guide strategies for disease prevention. The approach and the types of analyses used in this study can be replicated at each relevant region or production system.

Financial Support
North Carolina State University; USDA National Institute of Food and Agriculture

Notes:

458 - Role of the zinc metalloprotease-ZMPSTE24 in Porcine Reproductive and Respiratory Syndrome (PRRS) virus replication

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Session: PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME

Objective

The zinc metalloprotease ZMPSTE24 is a host's natural restriction factor that has been shown to broadly inhibit the replication of a number of viruses and acts as a downstream effector of the IFITM3 protein. Little is known as to how IFITM3 and ZMPSTE24 affect PRRSV replication. The main aim of this study was to investigate the role of host restriction factor- ZMPSTE24 in PRRSV replication. Then we sought to elucidate the mechanism by which this restriction factor inhibits PRRSV.

Methods

MARC-145 cells were transfected with Flag-tagged ZMPSTE24 and then infected with PRRSV SD-23983 at an MOI of 1 for 24 h. Western blot and TCID50 assays were performed to confirm the expression of the desired proteins and to determine the virus titer. Next, siRNA induced knockdown of ZMPSTE24 was performed to study the roles of the endogenous restriction factor on PRRSV replication. Silencing of ZMPSTE24 gene was confirmed by RT-PCR. The role of ZMPSTE24 in restricting PRRSV entry was studied using confocal microscopy.

Results

Exogenous expression of ZMPSTE24 reduced PRRSV replication, confirming the antiviral role of this protein. In addition, silencing of endogenous ZMPSTE24 slightly affected PRRSV replication. Furthermore, over-expression of ZMPSTE24 did not significantly inhibit PRRSV entry at 3 hour post infection. Cells over-expressing ZMPSTE24 showed little or no co-localization with PRRSV at 24 h post infection. Amphotericin B did not restore the replication of PRRSV in cells over-expressing ZMPSTE24. Cytotoxicity assay confirmed that cell viability plays no role in the ZMPSTE24 mediated PRRSV restriction.

Conclusions

In conclusion, ZMPSTE24 restricts PRRSV infection, inhibiting PRRSV at the post-entry step. In future studies, the mechanism of PRRSV inhibition will be explored. These findings may improve our understanding of PRRSV infectivity and host-pathogen interaction.

Notes:

459 - Inhibition of PRRSV and swine influenza virus by knockdown of host factors

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Session: PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME

Objective

Porcine Respiratory and Reproductive Syndrome Virus (PRRSV) and swine influenza A virus (SIAV) are important viral infections in pigs worldwide. PRRSV is an enveloped, single-stranded, positive sense RNA virus and causes reproductive failure in pregnant sows and causes respiratory disease in young pigs. SIAV belongs to the family *Orthomyxoviridae* and causes influenza outbreaks in pigs. Elucidating host factors involved in virus infection improves our understanding of virus-host interactions and provides the basis for new intervention strategies. Protein disulfide isomerases (PDIs), oxidoreductases of the thioredoxin superfamily, play key roles as promoting native protein folding and have been involved in the replication of some viruses. The aim of this study is to elucidate the effects of PDIs on the replication of PRRSV and SIAV.

Methods

MARC145 cells or LLC-PK cells were transfected with siRNAs for PDI genes and infected with PRRSV or SIAV, respectively. Viral titers were determined by real-time quantitative RT-PCR or the TCID50 method and western blotting was performed to confirm inhibition of virus protein synthesis. In addition, porcine macrophage cell line 3D4/21 cells expressing porcine-derived CD163, a receptor for PRRSV, were generated. To investigate if a PDI enzyme is involved in virus entry step, cellular trafficking of PRRSV was examined using the confocal microscopy.

Results

Knockdown of PDIA genes with specific siRNA significantly reduced the replication of PRRSV or SIAV in MARC145 cells or LLC-PK cells. It was confirmed that 3D4/21 cells expressing porcine-derived CD163 can support the replication of PRRSV. Studies with confocal microscopy suggest that the inhibitory effects of the PDI knockdown is not related to the entry events.

Conclusions

These findings suggest that the PDI genes are critical for PRRSV replication in cells and may serve as a potential target for new intervention strategies for PRRSV infections.

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

Notes:

460 - Host factors involved in porcine reproductive and respiratory syndrome virus entry

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Session: PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME

Objective

Porcine reproductive and respiratory syndrome virus (PRRSV) is the etiologic agent of PRRS, an economically devastating disease of swine that is characterized by reproductive failure in breeding herds and respiratory problems and growth retardation in growing pigs. PRRSV is found in most pig-producing countries, causing enormous economic losses each year. The aim of this study is to understand how PRRSV gains access to the interior of susceptible cells, the initial step in an infection process that involves a cascade of multiple, highly coordinated interactions between the virus and its target cells.

Methods

This work involves 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 ZMAC, 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 RNAi screen strategy with a lentiviral porcine shRNA library to identify one or more cellular genes that play an important role in PRRSV entry into the PRRSV-susceptible porcine macrophage cell line ZMAC.

Results

This is an ongoing project currently screening both the iterative cDNA library and the multiplexed RNAi library.

Conclusions

The outcomes of this study will provide a unique opportunity to gain a complete understanding of how PRRSV-host cell interactions occur at the level of PRRSV entry, shed new light on the cell/tissue tropism and pathogenesis of PRRSV, and provide new targets for the development of novel antiviral interventions capable of inhibiting the early steps of PRRSV infection.

Financial Support
USDA National Institute of Food and Agriculture

Notes:

461 - Validation of a PRRSV live-virus potentiated by replication-competent expression of porcine interferons

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Session: PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME

Objective

Interferon (IFN) genes were cloned into a PRRS DNA-launched reverse genetics system to generate vaccine candidates with specific changes. This recombinant virus was evaluated *in vitro* and *in vivo* to determine the effects of transgene expression on the virus, the cell, and the host animal.

Methods

The pCMV-P129 infectious cDNA clone was constructed from the virulent PRRSV-2 (Linage 8) field virus P129, isolated in Indiana in 1995. The infectious clone pKermit (Zoetis) contains the GFP gene within an additional dedicated sgRNA expression cassette. In this study, the GFP gene was replaced with genes encoding a cohort of optimized antiviral IFNs including each of IFN-α, IFN-β and IFN-ω subtypes. The IFN cohort-expressing virus (PRRSV-P129-IFNmix) was tested with or without an adjuvant and compared with a commercial MLV vaccine, Ingelvac PRRS® ATP (MLV-ATP). The vaccine challenge study used outbred pigs (5-wk-old, n=10/group) challenged by intramuscular inoculation of strain NADC-34 ($1x10^4$ $TCID_{50}$/ml).

Results

The PRRSV-P129-IFNmix virus generated 0.5-2 logs more vaccine virus than the MLV-ATP vaccine during the 4-wk vaccination period. The febrile responses in pigs vaccinated with the PRRSV-P129-IFNmix were similar to the MLV-ATP group, particularly post challenge. Lung lesion scores were not significantly different for the PRRSV-P129-IFNmix groups from the MLV-ATP group. At challenge (0 DPI), the control and non-vaccinated groups were PRRSV antibody negative, while each vaccinated group was antibody positive. At 14 DPI, all challenged groups had antibody titers that were significantly higher than the non-vaccinated, non-challenged sham group; and the vaccinated groups were significantly different from the non-vaccinated groups.

Conclusions

This vaccine platform is designed to directly reverse PRRSV suppression on the host IFN signaling and associated immune response, which will enhance vaccine efficacy against both homologous and heterologous PRRSV strains. Initial studies have shown the vaccine prototype(s) efficacy is comparable to a commercially available PRRSV MLV vaccine

Financial Support
USDA National Institute for Food and Agriculture

Notes:

462 - Quantifying sequential dominance of PRRSV strains through classification of sub-lineages

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Session: PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME

Objective

Porcine reproductive and respiratory syndrome virus (PRRSv) is comprised by genetic variants that evolve in parallel and complicate efforts to control the disease. We hypothesize that, at the population level, partial cross-immunity against differing strains might lead to sequential turnover in the dominant phylogenetic lineage through time. In the U.S., most field viruses belong to lineage 1, which is highly diverse, suggesting that sub-lineages exist. Our objective is describe sub-lineages within lineage 1 and quantify their occurrence through time as a first-step to understanding multi-strain dynamics.

Methods

We performed a discriminant principal component analysis on 7,252 lineage 1 ORF5 sequences. Clusters were identified through a k-means analysis of the principle components that accounted for 80% of the genetic data variance. The optimal number of clusters was considered the smallest BIC that preserved the known sub-lineage assignment of 75 reference sequences. We also investigated if ORF5-based lineages are preserved in phylogenies based on whole genome sequences.

Results

A total of 8 (L1A to L1H) sub-lineages were identified, including three previously undescribed. In addition, the emergence of new sub-lineages occurred every 2 to 4 years. In 2018, approximately 40% of all sequences belonged to sub-lineage L1H (mostly RFLP type 1-8-4) and 33% to L1A (mostly types 1-7-4 and 1-4-4). However, 13 years ago, the dominant sub-lineages were L1B (mostly type 1-18-2) and L1F (mostly type 1-8-4). The fact that RFLP type 1-8-4 appears in different sub-lineages highlights the ambiguity and inconsistency of typing viruses based on RFLP rather than phylogenetic ancestry. ORF5-based lineages remained largely consistent within whole-genome phylogenies.

Conclusions

By classifying PRRSV lineage 1 sequences into sub-lineages, we were able to document the periodic emergence of new sub-lineages through time, which is consistent with our hypothesis of lineage turnover. This research paves the way to answer evolutionary questions related to the emergence of new and often more virulent sub-lineages.

Financial Support
U.S. National Science Foundation; USDA National Institute of Food and Agriculture

Notes:

463 - Modified live virus vaccine induces heterologous immunogenicity and partially protects against type-2 PRRSV strains

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Session: PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME

Objective

Cross-protection against heterologous strains is a major hurdle of vaccines against porcine reproductive and respiratory syndrome virus (PRRSV). Their efficacy relies on the induction of a strong immune response leading to heterologous immunological memory. Both the humoral and cellular immune responses play a pivotal role in vaccine efficacy. Thus, this study evaluated the induced antibody and cellular immune responses as well as the efficacy of a modified live virus (MLV) vaccine against four strains from three type-2 PRRSV lineages.

Methods

Sixty PRRSV negative four-week-old pigs were divided into ten groups. Five groups were Mock inoculated and five MLV intramuscularly immunized. Each of the Mock and MLV vaccinated groups were intranasally challenged 28 days post-vaccination (dpv) with either Mock, NC174 or NADC30 (both lineage 1), VR2332 (lineage 5), or NADC20 (lineage 9). Pigs were clinically monitored daily. At 0, 28, and 42dpv, serum and PBMCs were isolated to study the heterologous neutralizing antibody (nAb) response, viremia via RT-qPCR, and the heterologous cellular immune response by flow cytometry after in vitro restimulation of PBMC with their analogous challenge strains. Nasal swabs were collected weekly to study viral shedding and local nAbs. At 42 dpv, pigs were sacrificed to assess lung pathology.

Results

All vaccinated animals developed viremia by 28 dpv. While the post-challenge viral load data are still outstanding, intranasal challenge led to a range of PRRSV-associated clinical signs and lung pathology with variable severity depending on the challenge strain. The MLV vaccine protected to variable degrees against disease and lung pathology. Analysis of the nAb and detailed evaluation of the cellular immune response are still in progress, but our current results demonstrate that MLV vaccinated pigs showing protection at necropsy also had a higher T cell cytokine production and proliferation.

Conclusions

These data demonstrate that the MLV vaccine was able to elicit various degrees of both immunogenicity and protection against heterologous PRRSV strains from different lineages.

Financial Support
North Carolina State University

Notes:

464 - Preventing PRRS through modifications in the virus receptor CD163

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Session: PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME

Objective

CD163 on macrophages is the receptor for porcine reproductive and respiratory syndrome virus (PRRSV). The goal of this research is to construct a pig possessing the smallest modification in CD163 sufficient to prevent PRRSV infection while retaining normal CD163 functions. The first objective is the use of an in vitro system to map the CD163 peptide sequences important for PRRSV infection. For the second objective, the results were applied to the construction of CD163-modified pigs. The final objective is to understand the participation of CD163 in inflammation and immunity.

Methods

For Objective 1, HEK293T (HEK) cells were transfected with CD163 cDNAs possess different mutations fused to EGFP. Transfected cells were infected with a PRRSV isolate expressing a red fluorescent protein (RFP) and viewed under a fluorescence microscope. Insertion of proline-arginine (PR) dipeptides along the SRCR5 peptide sequence was used to probe peptide sequences and secondary structures within SRCR5 involved in virus infection. For Objective 2, CRISPR quides were designed and used to create domain deletions in CD163. Edited embryos were placed in preganant guilts. For Objective 3, CD163 KO and WT pigs were infected with a systemic virus PCV2d. Clinical signs, viremia, and pathology were evaluated.

Results

PRRSV-resistant cells expressing domain deletions, such as the removal of SRCR5, were identified. The location of PR insertions in SRCR5 possessing the greatest effect on infection were identified for both PRRSV-1 and PRRSV-2 isolates. Pigs with transplanted embryos possessing several CD163 modifications did not become pregnant. Following PCV2d infection, CD163 KO pigs showed decreased viremia and fewer PCV2d tissue lesions compared to WT pigs.

Conclusions

The genetic modification of CD163 presents the opportunity to construct pigs, which are resistant to infection with PRRSV and other porcine viruses. The partial resistance of CD163 KO pigs to PCV2d infection creates the opportunity to explore new roles for CD163 in viral pathogenesis

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

Notes:

465 - Immunological responses are dampened during co-infection of NF-κB activation negative PRRSV and Streptoccus suis

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Session: PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME

Objective

Porcine reproductive and respiratory syndrome virus (PRRSV) suppresses type I interferons (IFNs-α/β) response and also activates NF-κB signaling during infection. In swine farms, pigs are commonly co-infected with PRRSV and other secondary bacterial pathogens, which will trigger enhanced expression of proinflammatory cytokines. As a consequence, the disease becomes more severe to cause the porcine respiratory disease complex (PRDC). PRRSV non-structure protein 1β (nsp1β) has been demonstrated as an IFN antagonist, and we show that leucine at position 135 is the active residue for IFN suppression. Furthermore, the nuclear localization signal (NLS) of the PRRSV nucleocapsid (N) protein was identified as the NF-κB activation domain. In the present study, double-mutant PRRSV was generated by reverse genetics to eliminate both IFN suppression and NF-κB activation functions. The immunological phenotype was examined in cells during co-infection of PRRSV and a bacteria pathogen.

Methods

A series of mutant PRRSVs were then rescued by reverse genetics. For co-infection, MARC-145 were first infected with wild-type PRRSV or mutant PRRSV for 1 hour followed by infection with Streptococcus suis (S. suis) at 48 hpi. NF-κB response was examined by luciferase assay, and pro-inflammatory cytokines were determined by RT-qPCR. Statistical analyses were performed using Student t-tests, and statistical significance was expressed as $P < 0.05$.

Results

First, double-mutant PRRSV produced a lower level expression of pro-inflammatory cytokines, IL-1b, IL-6, IL-8, and TNF-α, which were driven by the NF-κB. Compared to the co-infection of wild type PRRSV and S. suis, NF-κB activation and expression of IL-1b, IL-6, and TNF-α were decreased in co-infected cells.

Conclusions

Our study demonstrates that the double-mutant PRRSV attenuates the expression of proinflammatory cytokines compared to wild-type PRRSV. Co-infection of double-mutant PRRSV and other swine pathogens supports the hypothesis that the mutant PRRSV may relieve the clinical severity of PRDC in pigs caused by the co-infection.

Financial Support
USDA National Institute of Food and Agriculture

Notes:

466 - Inhibition of type 1 IFN signaling by PRRSV nsp5 through blocking STAT2 nuclear translocation

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Session: PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME

Objective

Type 1 interferons (IFNs-α/β) play a critical role in the innate immune response to viral infection. Porcine reproductive and respiratory syndrome virus (PRRSV) has been shown to inhibit the expression of type 1 IFNs and interfere with the IFN signaling pathway. Since signal transducer and activator of transcriptions (STATs) are crucial in the IFN signaling, the STATs are hypothesized to be the target of the virus to hamper the antiviral response induced by the IFN signaling. PRRSV has been shown to interfere with the STAT signaling pathway, but the relationship between the suppression of IFN responses and PRRSV-mediated STAT2 signaling remains unclear. In the present study, the mechanisms for IFN inhibition by PRRSV nonstructural protein (nsp) 5 and STAT2 signaling were investigated.

Methods

HeLa cells were co-transfected with the nsp5-expressing and IFN-reporter plasmids and stimulated with IFN for 30 min. Induction of IFN response by nsp5 was examined by luciferase assay, and interferon-induced gene expressions were determined by RT-qPCR. Subcellular localization of proteins was determined by immunofluorescent staining and confocal microscopy. For proteasomal pathway experiments, MG132 treatments, cell fractionations, and Western blot assay were conducted.

Results

While the control showed an increase of the interferon-sensitive responsive element (ISRE) activity by IFN, nsp5 exhibited strong suppression of ISRE activity. The nsp5 protein also downregulated the IFN-induced gene transcriptions, including interferon-stimulated gene (ISG) 15 and promyelocytic leukaemia (PML). After IFN stimulation, PRRSV nsp5 blocked the STAT2 nuclear translocation, but STAT1 and STAT3 were no affected by nsp5. Treatment with MG132 restored the nuclear translocation of STAT2, suggesting the proteasomal degradation of STATs by nsp5.

Conclusions

Our results demonstrate that PRRSV nsp5 interferes with the type I IFNs signaling pathway by blocking the STAT2 nuclear translocation through the proteasomal pathway.

Financial Support
USDA National Institute of Food and Agriculture

Notes:

467 - Small Molecules Block the Interaction of PRRSV with CD163 Receptor and the Infection of Pig Cells

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Session: PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME

Objective

Porcine reproductive and respiratory syndrome (PRRS) is one of the most economically devastating diseases affecting the pork industry globally. PRRS is caused by PRRS virus (PRRSV). Currently there are no effective treatments against this swine disease. We hypothesized that small molecules could be used to block the infection of the PRRSV to pig cells.

Methods

Through artificial intelligence molecular screening, we obtained a set of small molecule compounds predicted to target the scavenger receptor cysteine-rich domain 5 (SRCR5) of CD163, which is a cell surface receptor specific for PRRSV infection. These compounds were screened using a cell-based bimolecular fluorescence complementation (BiFC) assay, and the function of the positive hit was further evaluated and validated by PRRSV-infection assay using porcine alveolar macrophages (PAMs).

Results

Using the BiFC assay, we identified one compound with previously unverified function, 4-Fluoro-2-methyl-N-[3-(3-morpholin-4-ylsulfonylanilino)quinoxalin-2-yl]benzenesulfonamide (designated here as B7), that significantly inhibits the interaction between the PRRSV glycoprotein (GP2a or GP4) and the CD163-SRCR5 domain. We further demonstrated that compound B7 inhibits PRRSV infection of PAMs, the primary target of PRRSV in a dose-dependent manner. B7 significantly inhibited the infection caused by both type I and type II PRRSV strains. Further comparison and functional evaluation of chemical compounds structurally related to B7 revealed that the 3-(morpholinosulfonyl)aniline moiety of B7 or the 3-(piperidinylsulfonyl)aniline moiety in a B7 analogue is important for the inhibitory function against PRRSV infection.

Conclusions

Our study identified a novel strategy to potentially prevent PRRSV infection in pigs by blocking the PRRSV-CD163 interaction with small molecules.

Financial Support
USDA National Institute of Food and Agriculture

Notes:

468 - Reproductive PRRS: Gene expression differences elucidate mechanisms of fetal viral level and demise

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Session: PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME

Objective

The mechanisms leading to variation in fetal outcome in response to reproductive PRRS virus (PRRSV) infection are not fully understood. Our objective was to assess targeted immune-related gene expression patterns and pathways in the placenta and fetal thymus to elucidate the molecular mechanisms involved in the resistance/tolerance and susceptibility of fetuses to PRRSV infection.

Methods

Fetuses were collected at 12 days post infection from pregnant gilts challenged with PRRSV2 in late gestation. Fetuses were grouped by preservation status and PRRS viral level (VL): mock infected control, no virus detected, virus in the placenta only with either viable or meconium-stained fetus, low VL with either viable or meconium-stained fetus, and high VL with either viable or meconium-stained fetus. Expression of 286 immune-related genes were quantified using NanoString; differentially expressed genes (DEG) were calculated for each fetal group, contrasted to control with adjusted $P \leq 0.05$ as significant, and pathway analysis was completed using Ingenuity Pathway Analysis software.

Results

The host immune response (measured by detection of immune DEG) was initiated only after PRRSV reached detectable levels in the fetus. Upon fetal infection, a set of core responsive interferon inducible genes (CXCL10, IFIH1, IFIT1, IFIT3, ISG15, and MX1) were strongly upregulated in both tissues. Gene expression in the thymus differentiated fetal VL; we observed high VL in fetuses undergoing strong thymic downregulation of several immune pathways (e.g., B-Cell development). Gene expression in the placenta differentiated fetal demise. Potential placental biomarkers of susceptibility (e.g., dysregulation of the Apoptosis and Ubiquitination pathways) may be contributing to fetal demise.

Conclusions

Our data supports the concept that fetal outcome in response to PRRSV infection is determined by fetal and more significantly placental response, which is initiated only after fetal infection. This conceptual model represents a significant step forward in understanding the mechanisms underpinning fetal susceptibility to the virus.

Financial Support
U.S. Department of Agriculture, Agriculture and Research Services; Genome Canada; Genome Prairie; Genome Alberta; PigGen Canada

Notes:

469 - Simultaneous measurement of antibody reactivities against PRRSV structural proteins

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Session: PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME

Objective

The primary objective of this study was to comparatively evaluate immunogenicity of the PRRSV structural proteins.

Methods

Luciferase-immunoprecipitation system (LIPS), a liquid phase immunoassay, was used to measure antibody reactivities against the structural proteins of PRRSV. This assay utilizes a luciferase-fusion antigen as the bait to capture antigen-specific antibodies. Specifically, the target antigen is cloned in-frame with a luciferase reporter gene and expressed in mammalian cells. A crude cell extract containing the luciferase-tagged antigen is mixed with a test serum sample in the presence of protein A Sepharose beads. If the test serum samples contain antibodies specific to the luciferase-tagged antigen, the antigen will be immobilized on the beads. The amount of antigen-specific antibody present in the test serum will be quantified by adding a luciferase substrate, followed by measuring light production

Results

Using PRRSV N protein as the model antigen to validate the LIPS, we demonstrated that the LIPS with N protein gave results that were highly comparable to the results produced by the commercial IDEXX PRRS X3 ELISA. We then applied the LIPS to simultaneously measure antibody reactivity against all viral structural proteins. Antibody reactivities were highest against GP3, M and N proteins and intermediate against GP2, E, GP4 and GP5 and lowest against ORF5a protein. Although GP5 is a major viral envelop glycoprotein, antibody reactivity against GP5 was not significantly different from the antibody reactivities against the minor proteins GP2, E and GP4.

Conclusions

The results of this study indicate that the levels of immunogenicity are highest with GP3, M and N, intermediate with GP2, E, GP4 and GP5 and lowest with ORF5a-protein. This study expands our knowledge on the humoral immune response against PRRSV infection.

Financial Support
USDA National Institute for Food and Agriculture

Notes:

470 - Assessment of primary and secondary bile acids against chicken necrotic enteritis

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Session: POULTRY

Objective

Necrotic enteritis (NE), mainly induced by pathogens of Clostridium perfringens and coccidia, causes huge economic losses in the poultry industry. This study was to investigate the impact of various bile acids on prevention of chicken NE.

Methods

Day-old broiler chicks were randomly assigned to 7 groups of diets supplemented with 0 (basal diet), 1.5% commercial bile (CMB), 1.5% deoxycholic acid (DCA), 1.5% lithocholic acid (LCA), and 4000 ppm of chicken bile (CB). The birds were challenged with *E. maxima* (15,000 oocysts/bird) at d 18 and C. perfringens (109 CFU/bird) at d 23 and d 24 to induce NE. 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 analysis. The date was analyzed by one-way ANOVA and followed by Fisher LSD comparison test with statistical significance when $p \leq 0.05$.

Results

Notably, birds infected with E. maxima and C. perfringens developed acute NE and suffered severe growth performance reduction of daily body weight gain (BWG) during the NE phase compared to noninfected birds (-14 vs. 56 g/day/bird, $P < 0.001$). Both CMB and CB failed to attenuate body weight gain loss (-6 and -15 vs -14 g/day/bird) compared to NE, respectively. Interestingly, DCA at 1.5 g/kg in feed alleviated the NE-induced BWG loss (16 vs -14 g/day/bird, $P < 0.006$) compared to NE birds. *C. perfringens* colonization was decreased significantly in the DCA group (5.31 vs 6.36 logs, $P = 0.04$) compared to NE infected group. NE infection modulated the major bile acids (CA and CDCA) composition in ileum content. Dietary DCA, LCA, CMB, and CB significantly increased the T/DCA (4410 vs 60 nmol/gram, $P = 0.007$), total LCA (885 vs 2.5 nmol/gram, $P = 0.015$), total T/G/CA (606 vs 3036 nmol/gram, $P < 0.001$), and total T/G/CDCA (1267 vs 2805 nmol/gram, $P = 0.09$) in ileum content, respectively.

Conclusions

The results showed that dietary secondary bile acids DCA, but not other bile acids, reduces *C. perfringens* colonization and improve NE infected birds health.

Financial Support
USDA National Institute for Food and Agriculture

Notes:

471 - Inhibition of mTOR Signaling attenuates chicken necrotic enteritis induced intestinal inflammation

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Session: POULTRY

Objective

Necrotic enteritis (NE) has reemerged as a prevalent chicken disease with huge losses to the poultry industry every year. The aim of this study is to investigate whether mTOR signaling mediated *C. perfringens*-induced chicken small intestinal inflammation of necrotic enteritis (NE).

Methods

Day-old broiler chicks were randomly allotted to one basal diet and six diets supplemented with four groups of rapamycin (0.075, 0.15, 0.3 0.45 mg/kg), 1.5 g/kg of deoxycholic acid (DCA), and 1.5 g/kg DCA plus 0.3 mg/kg rapamycin (rap). Feed supplementation was started from d 17. Additional two groups of chickens were subcutaneously injected with the rap of 8 and 64 µg/kg BW starting from d 17. At d 18, birds were orally infected with *Eimeria maxima* (15,000 sporulated oocysts/ bird) to induce coccidiosis. The birds were subsequently infected with 10^9 CFU/bird of *C. perfringens* at d 23 and 24. Growth performance of body weight gain (BWG) was measured at d 18, 23, and 25. The birds were euthanized at d 25. Ileum tissue and content were collected for *E. maxima* and *C. perfringens* colonization and histopathology. The date was analyzed by one-way ANOVA and followed by Fisher LSD comparison test with statistical significance when $p \leq 0.05$.

Results

Notably, birds infected with *E. maxima* and *C. perfringens* developed acute NE and suffered severe growth performance reduction. Interestingly, rap treatments alone did not significantly inhibit the BWG loss. During the NE phase (d23-25), DCA and DCA plus rap attenuated the BWG loss (16, 29 vs -14 g/day/bird) compared to the NE group. Notably, DCA plus rap improved histopathology score compared to DCA alone ($P < 0.05$). Only DCA treatment alone reduced both *C. perfringens* and *E. maxima* colonization compared to NE infected group ($P < 0.05$). Invitro assay showed rap inhibited immune cell migration ($P < 0.05$).

Conclusions

Together, these results suggest that mTOR signaling in immune cells exacerbates *C. perfringens*-induced intestinal inflammation by promoting immune cell infiltration, while the signaling in epithelial cells is indispensable for wound healing.

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

Notes:

472 - Predictors of death on arrival (DOA) among chickens slaughtered at a poultry abattoir, South Africa

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Session: POULTRY

Objective

Although death-on-arrival (DOA) among broilers contributes significantly to losses by the South African poultry industry, the phenomenon of DOAs has not been extensively investigated. We investigated the prevalence and predictors of DOA at a South African poultry abattoir.

Methods

Secondary data collected by a poultry abattoir in Limpopo province between January 2014 and December 2016 was used in this study. The following variables were extracted from the data: farms of origin, the number of farm rejects, distance travelled, mass of birds delivered, total number of birds delivered, the number and total mass of DOA, number and total mass of birds slaughtered, the number of rejected carcasses and biosecurity measures on the farms.

Results

The DOA constituted 0.48% of the total number of birds delivered over the study period. The highest proportion of DOA (0.56%) was recorded in 2014 and the lowest (0.36%) in 2016. There was an upward trend in the proportion of DOA observed from November to January, followed by a decrease from January to April in 2014, 2015 and 2016. The live mass for the broilers (RR=5.706; 95CI: 3,696-8,738), the number of birds rejected at farm level (RR= 3.66; 85% CI: 2,437- 5,596), Summer season (RR= 2.071; 95% CI: 1,715-2,491) and the quantity or number of birds delivered to the abattoir (RR=1.194; 95% CI: 1,135- 1,257) were positively associated with DOA. However, winter was negatively associated with DOA (RR=0.572; 95% CI= 0.472-0.689).

Conclusions

Transporting of birds to the abattoir early in the morning or at night in summer months, maintaining the birds live mass as much as possible close to the prescribed harvesting mass to avoid over crowing in crates, and more training of catchers on the welfare of birds could be employed as control measures to reduce the number of DOA. Although, the proportion of DOA observed in this study was low, there is room for improvement and hence measures are needed to further improve the production environment and reduce the incidences.

Financial Support
Carnegie African Diaspora Progamme; University of South Africa

Notes:

473 - Protection against *Campylobacter* by fecal microbiota transplantation in newly hatched broiler chickens

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Session: POULTRY

Objective

Campylobacter jejuni is a leading cause of foodborne illness worldwide. In a recent longitudinal study, we found some commercial farms consistently produced *Campylobacter*-negative flocks, while others had *Campylobacter*-positive flocks despite the fact the farms operated under similar production and management practices. We hypothesize that this colonization difference may be associated with the composition of chicken gut microbiota between farms.

Methods

A fecal microbiota transplantation (FMT) experiment was performed to evaluate the effect of adult cecal microbiota from a *Campylobacter*-negative broiler farm on the colonization of young broiler birds by *C. jejuni*. Two weeks after the FMT, birds were orally challenged with *C. jejuni* and cecal contents were collected periodically to determine *Campylobacter* colonization levels via culture and for microbiota (16S rRNA gene-based) analysis.

Results

Campylobacter colonization levels in the FMT group were significantly reduced compared with the control group. Microbiota analysis indicated that the overall alpha diversity of the FMT group was much higher than the control group, and significant temporal shifts in the bacterial community structure were observed throughout the experiment. The composition of the chicken microbiome was significantly different between the FMT group and the control group. Taxonomic analyses at the phyla level showed that *Firmicutes* (90.6% and 60.7%), *Bacteroidetes* (4.8% and 18.8%), *Epsilonbacteraeota* (2.1% and 9.8%), and *Proteobacteria* (1.8% and 6.6%) were the top four most abundant taxa in the control group and FMT group, respectively.

Conclusions

FMT using the cecal contents of *Campylobacter*-free adult commercial broilers significantly affects the subsequent temporal development of the gut microbiota and has a measurable inhibitory effect on *Campylobacter* colonization in young broilers.

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

Notes:

474 - Pathogenicity and transmission of Canadian wild type and vaccine revertant Infectious Laryngotracheitis Virus

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Session: POULTRY

Objective

Infectious laryngotracheitis (ILT) is an upper respiratory disease in chickens caused by a member of the Herpesviridae family, infectious laryngotracheitis virus (ILTV). ILT control relies on biosecurity and vaccination with live attenuated vaccines. In common with global trend, in Canada, ILT is caused dominantly by vaccine revertant ILTV strains and, to a lesser extent, wild type ILTV. In this study we studied the pathogenicity and transmission potential of three Canadian ILTV strains, two wildtypes and one vaccine revertant strain.

Methods

Three weeks old specific pathogen free chickens were infected experimentally with two wild type and one revertant ILTV strains along with mock infected controls (n=8 per group). When the ILT clinical signs were at peak, three contact chickens were introduced to each group for 3 days. The experimentally infected and contact chickens and their controls were observed for morbidity and mortality for 14 days, then the chickens were euthanized for tissue collection. In addition, feather follicles, oropharyngeal and cloacal swabs were taken at predetermined timepoints for viral load quantification.

Results

Infection with vaccine revertant ILTV resulted in significantly higher morbidity (clinical signs and bodyweight loss) and mortality when compared to the wild type ILTV strains (P<0,05) with contact chickens showing more severe morbidity and mortality when. Compared to experimentally infected chickens.

Conclusions

The results of this study indicate that vaccine revertant ILTV can possess a greater pathogenicity potential in comparison to the wildtype ILT virus.

Financial Support
Egg Farmers of Canada; Alberta Agriculture and Forestry

Notes:

475 - Establishing experimental transmission models for H9N2 avian influenza virus (AIV) in chickens

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Session: POULTRY

Objective

Avian influenza viruses continue to be a major threat to the poultry industry. Transmission of low pathogenicity avian influenza virus (LPAIV) is speculated to occur through aerosol, large droplets, direct contact or fomites. There is, however, paucity of experimental evidence for routes of transmission of AIV viruses and the relative importance of various routes. The present study focussed on the establishment of respiratory and direct contact (combination of oral, intranasal, intra-tracheal and intra-ocular) transmission models simulating natural infection using 2-week-old SPF chickens.

Methods

Chickens in each group (n=16) were inoculated with a dose of H9N2 AIV and were kept along with uninfected contact birds (n=8) in Horsfall units in each transmission model. Chickens infected via the aerosol route were placed in an aerosol chamber and held for 20 minutes for maximum uptake of the aerosolized virus. Titers of the virus in oropharyngeal and cloacal swabs were determined by Tissue culture Infectious dose 50 (TCID50/ml) in both infected and exposed groups.

Results

Results indicated viral shedding at -1, -3, -5, -7 days post-infection (DPI) in inoculated chickens and earliest at day 3 post-exposure (DPE) in exposed groups. The ratio of infected birds in exposed group was 7/8, 6/8, 6/8 at -3, -5, -7 DPE respectively. Cloacal shedding in the aerosol exposed birds was 1.7 (1.2×10^5 TCID50/ml) and 1.5 (9.2×10^4TCID/ml) fold higher compared to the direct contact group at 3 and 5 DPE, respectively. Viral shedding in the oropharyngeal swabs (1.2×10^5 TCID50/ml) in the infected aerosol group was also significantly higher compared to the direct contact group (7.1×10^4 TCID50/ml). In support of these observations, antibody-mediated immune response in the serum of exposed birds was five fold high on 7 and 14 DPE in the aerosol group than the direct contact group via Haemagglutination inhibition (HI) assay.

Conclusions

These findings suggest that transmission of the virus was established in both models, wherein the transmission of H9N2 AIV appeared to occur more readily in the aerosol route.

Notes:

476 - *Campylobacter* dynamics in broilers raised to five and eight weeks of age

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Session: POULTRY

Objective

Campylobacter colonizes the chicken gut after the decline of the passive humoral immune response, and broilers are mostly unable to clear the infection during the typical production lifespan (~five weeks of age). However, some broilers are raised to eight weeks of age or longer; data regarding *Campylobacter* colonization during this extended time frame are limited. The objective of this study was to compare the *Campylobacter* dynamics in broilers raised to five and eight weeks of age in commercial settings.

Methods

Ten and 16 flocks of broilers were followed throughout the production cycle until five or eight weeks of age, respectively. Weekly sample collections included two litter samples (from front and back sections of each house) and ceca from five broilers. Most probable number (MPN) was estimated from the litter and the presence of *Campylobacter* in litter wash and cecal content DNA was assessed via PCR. A zero-inflated negative binomial regression was built to model the temporal dynamics of *Campylobacter* MPN in litter. A logistic regression was used to model the longitudinal *Campylobacter* presence in ceca.

Results

Preliminary results showed the temporal changes in *Campylobacter* prevalence followed a concave shape with a peak around the fifth week of age and reached 100% in all flocks in at least one sampling. The rise of *Campylobacter* prevalence in ceca preceded, by at least a week, the detection of *Campylobacter* in litter using the MPN method. There was no difference in which section of the house *Campylobacter* was first detected via MPN.

Conclusions

In conclusion, viable *Campylobacter* was detected in litter, one or more weeks after the *Campylobacter* prevalence in ceca was very high. The age effect observed on *Campylobacter* colonization of broiler ceca can be used in combination with preharvest and postharvest measures to reduce *Campylobacter* loads in chicken carcasses.

Financial Support
USDA National Institute of Food and Agriculture

Notes:

481 - Evolution and spread of rabies virus in Connecticut and its neighboring states

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Session: PUBLIC HEALTH AND ZOONOSIS

Objective

Rabies virus is a zoonotic pathogen that is prevalent throughout the United States. Rabies is endemic in the US Northeast. Since the year 2000, the Connecticut Department of Public Health has reported 3408 confirmed cases of rabies. According to this data, the rabies virus has been detected, via the direct fluorescence assay (DFA), in 17 animal species including livestock and wildlife. Most of the rabies virus infections have been observed in raccoons (*Procyon lotor*) (1900 cases) and skunks (*Mephitis mephitis*) (822 cases). Interestingly, the population dynamics of circulating rabies viruses and potential drivers of virus changes in the State has not been fully established.

Methods

In this study, we conducted full-length genome sequencing followed by a phylogenetic analysis to trace the origin of rabies viruses currently circulating in Connecticut. Maximum likelihood (ML) and Bayesian phylogenies of the assembled full-length genome sequences with representative sequences retrieved from the NCBI Genbank were generated.

Results

Based on our phylogenetic analysis, multiple subgroups (genetic group 1, 2, 3, and 4) of rabies virus are present in CT which could have independently evolved. The CT genetic group 1 was genetically close to viruses originated from NY state. The CT genetic groups 2 – 4 are genetically close to viruses circulating in the Northeast US, including Vermont, Maine, Massachusetts, New Hampshire and New Brunswick, Canada.

Conclusions

We have established a systemic workflow for the characterization of rabies virus detected at the Connecticut Veterinary Medical Diagnostic Laboratory. Phylogenetic analysis showed that multiple viral lineages are circulating in various animal species in Connecticut. Understanding how the rabies virus spread in wild animals and which animal species act as a major reservoir in this region will allow us to predict transmission dynamics of the virus. Such findings can directly influence us to implement prudent measures to cease rabies circulation in the U.S.

Notes:

482 - Assessing rodents as carriers of pathogenic leptospires in the U.S. Virgin Islands.

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Session: PUBLIC HEALTH AND ZOONOSIS

Objective

Leptospirosis is a global zoonotic disease. Rodents are a primary reservoir host of disease transmission; leptospires colonize the proximal renal tubules and are excreted via urine to contaminate water and soil where they may survive for weeks. The aim of this study was to determine if rodents in the U.S. Virgin Islands are reservoir hosts of leptospires.

Methods

Rodents were trapped in each of the three main US Virgin Islands: Saint Croix (STX), Saint Thomas (STT) and Saint John (STJ). Kidney samples were collected aseptically at necropsy into liquid HAN media for investigation for the presence of leptospires by dark-field microscopy (DFM), culture, fluorescent antibody testing (FAT) and *lipL32* rtPCR. Cultured isolates were serotyped by the MAT, and molecular typing performed by analysis of *secY*.

Results

A total 140 rodents were captured comprising *Mus musculus* (N=112) and *Rattus rattus* (N=28): 73 in STX, 28 in STT and 39 in STJ. Forty-nine (35%) of samples were positive by DFM; 32/73 (44.4%) in STX, 5/28 (17.8%) in STT and 12/39 (30.8%) in STJ. By FAT, 63/140 (45%) were positive; 39/73 (53.4%), 6/28 (21.4%) and 18/39 (46.1%) on STX, STT and STJ respectively. Sixty (42.8%) samples were culture positive; 37/73 (50.7%) in STX, 6/28 (21.4%) in STT and 17/39 (43.6%) in STJ. By rtPCR 61/140 (43.6%) were positive, 38/73 (52%) in STX, 6/28 (21.4%) in STT and 17/39 (43.6%) in STJ. All samples positive by DFM were positive by FAT, culture and rtPCR. Molecular typing identified 48 isolates as *L. borgpetersenii,* and serological characterization by MAT identified them as belonging to serogroup Ballum; the remaining 12 cultures appear to be mixed species.

Conclusions

This study demonstrates that rodents are important reservoir hosts of pathogenic leptospires in the US Virgin Islands and highlights their potential role in the transmission of infection to humans and domestic animals.

Financial Support
U.S. Department of Agriculture

Notes:

483 - Detection of influenza A virus in pig farm workers

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Session: PUBLIC HEALTH AND ZOONOSIS

Objective

Influenza A virus (IAV) affects many hosts, including pigs and people, which represents a public health concern. Bidirectional transmission of IAV leads to both disease morbidity and the potential to generate novel IAV strains. US swine herds currently experience frequent outbreaks of acute influenza that can impact both swine and human health. Despite the recognition that bi-directional transmission of influenza virus occurs between people and pigs, little is known about how frequently this transmission or exposure takes place. Therefore, the objectives of this study were to implement a surveillance system at the farm worker-swine interface, quantify how frequently farm workers tested positive for IAV and whether workers have identifiable risk factors that can be predictive of virus introductions into farms.

Methods

Three sow farms located in the US-Midwest were selected for the study. The farms were representative of US commercial farms with an average of 4,000 sows. The farms had a history of IAV infections and 2 of the farms tested IAV positive at the time to initiate the study. We aimed to recruit swine farm workers that worked with pigs at least 2 days a week. After collecting baseline information, we asked each participant to self-collect a nasal swab before entering the farm and at the end of the working day, after completing the daily chores, twice a week for 8 weeks during the human influenza season as determined by the Centers of Disease and Prevention (CDC). At each sampling points, participants also collected their body temperature using disposable thermometers and answered a short survey regarding the activities performed during the day and whether they had influenza-like illness (ILI). Pigs were also sampled at three time points during the study. Each time 30 nasal swabs were collected from 20 day old pigs.
Farm worker samples were tested individually with an influenza A specific rRT-PCR test that detects both human and swine IAV's. Samples with a cycle threshold <38 were considered positive and selected for virus isolation on Madin-Darby canine kidney cells.
Pig samples were tested in pools of 3 using an rRT-PCR that targets the conserved matrix gene of IAV.
The study took place during the 2019 peak of human seasonal influenza (January-March).

Results

There were 34 workers enrolled from the three participating farms and a total of 1027 farm worker samples were obtained. Out of the samples collected, 35 samples (3.4%) tested IAV rRT-PCR positive and out of 34 workers, 20 of them tested IAV positive at least once during the study. However, we did not detect significant risk factor associated with testing IAV positive in the workers.

Conclusions

We were able to establish an IAV surveillance system where workers complied with self-sample collection protocols which indicates our ability to perform studies at the pig-human interface in pig farms.
Our results provide evidence that a number of farm workers can test influenza positive when they report to work at swine farms and at the end of the working day and can be indicative of IAV exposure while working with pigs at the farms.
Further characterization of the samples is needed to understand the implications for virus introduction into farms and risk of bi-directional transmission between pigs and people.

Notes:

486 - What was in that food?! A scoping review of risk factors for infection with antimicrobial-resistant *Campylobacter*

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Session: PUBLIC HEALTH AND ZOONOSIS

Objective

Campylobacter spp., a leading cause of acute diarrheic illness around the world, have developed resistance to antimicrobials important for human medicine. Infection with antimicrobial-resistant (AMR) *Campylobacter* spp. (Campy*)* is an important public health concern as it may be linked with increased severity of illness and risk of death. The objective of this study was to perform a scoping review of factors associated with human infection with AMR Campy.

Methods

The scoping review followed the methods in the Joanna Briggs Institute Reviewer's Manual & PRISMA-SR guidelines. Criteria for inclusion were English publications investigating humans with an AMR-Campy infection (resistant to macrolides, tetracyclines &/or quinolones) that reported factors potentially linked with the infection (e.g., food sources, prior antimicrobial use). Databases were: ProQuest® AGRICOLA, CAB Abstracts® & Global Health®, Ovid EMBASE® & MEDLINE®, & Scopus®. Grey literature sources were WHO's Global Index Medicus, the first 250 Google Scholar results & Bielefield Academic Search Engine. Primary and secondary screening will be completed by two reviewers using Distiller SR®.

Results

The search returned 7910 de-depulicated articles. 532 articles made it to secondary screening of which 380 had no abstract. Current documented risk factors for infection with Campy include: poultry consumption, travel, unpasteurized milk, & contaminated water. However, these risk factors don't differentiate between susceptible and resistant Campy infections. While screening is ongoing, article themes include: HIV, travel, proximity to animals, and age.

Conclusions

This scoping review will identify gaps in the literature, help to focus future research, provide data for future quantitative modeling & most importantly start the process of organizing the AMR in human Campy infection puzzle. The results will provide insight into if there are risk factors specific to a resistant Campy infection. These data will be incorporated into the AMR Integrated Assessment Model for human exposure to resistant Campy.

Financial Support
Government of Alberta

Notes:

487 - Epidemiology of *Campylobacter jejuni* in raccoons on swine farms and conservation areas in southern Ontario, Canada

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Session: PUBLIC HEALTH AND ZOONOSIS

Objective

Campylobacter is a leading cause of foodborne illness in humans worldwide. Sources of infection are often difficult to identify, and are, generally, poorly understood. Recent work suggests that wildlife may represent a source of *Campylobacter* for human infections. The aim of this work was to assess the impact of seasonal, climatic, location, annual and raccoon demographic factors on the occurrence of *Campylobacter jejuni* in raccoons.

Methods

Using a repeated cross-sectional study design, raccoons were trapped on five swine farms and five conservation areas in southern Ontario from May through November, 2011-2013. Multi-level multivariable logistic regression was used to model the odds of isolating *Campylobacter jejuni* from raccoon fecal swabs. The following independent variables were examined: raccoon age and sex, year, location type (swine farm vs. conservation area), season (May-July vs. Aug.-Nov.), and the sum of rainfall and mean temperature over the 14 days prior to sampling. Only biologically plausible two-way interactions were tested. In order to account for potential clustering, site and animal were included as random intercepts.

Results

A total of 1086 samples were obtained from 625 raccoons; 46.2% (95%CI: 43.2-49.2%) were positive for *C. jejuni.* Along with both random effects, the following interactions and their main effects were significant ($p<0.05$) and kept in the final model: seasonXyear, yearXrainfall, yearXtemperature. The carriage of *C. jejuni* by raccoons was consistently greater in Aug.-Nov., however, trends in relation to temperature, rainfall and year were not consistent.

Conclusions

Based on the results from our multivariable model, climatic variables (i.e., rainfall, temperature, and season) were associated with the carriage of *C. jejuni* by raccoons, but the effects were not consistent, and varied by location and year. Although raccoons may pose a zoonotic risk due to their carriage of *Campylobacter,* further work is required to characterize the transmission and movement of this microorganism within the ecosystem.

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:

488 - Main risk factors for perpetuating *Salmonella* spp. serovars in dairies in Mexico

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Session: SALMONELLA

Objective

The main objective of this cross-sectional study was to determine the risk factors for the presence of *Salmonella* spp. in maternity areas in relationship with close-up cows and calves on the main milk production areas of Mexico. The secondary objective was the detection of *Salmonella* spp. serovars from group O.

Methods

The sampling took place from October 2019 to January 2020 in 13 states of Mexico. Based on the sensitivity (85%) and specificity (85%) of the diagnostic test, and less than 40% prevalence with a 95% confidence interval, the least number of samples were six from each dairy, depending on the herd size. Samples were taken directly from the rectum of asymptomatic calves from 0 to 60 days of age, asymptomatic close-up cows, and maternity floors with Q-swabs. A total of 378 samples were collected and analyzed.

Results

From the 55 dairies sampled, 35 had at least one positive result, conforming a 63.6% prevalence with 57 isolates in total. Stratified analysis indicated that 10 calves were found positive, in eight dairies (n= 127); 16 close-up cows were positive, in 14 dairies (n=116); and 31 isolates were positive from maternity areas, accounting for 25 positive dairies (n=134) ($p < 0.05$). Herds were classified by size: less than 500 cows, from 500 to 999, and more than 1000 cows. The largest dairies showed 72.1 % of the *Salmonella* spp. isolates ($p < 0.05$). The most frequent serotype was C (41.03%), followed by D (30.8 %).

Conclusions

This study indicates that the main source of *Salmonella* spp. are the maternity areas, where periparturient shedders are contaminants and perpetuate the pathogen within the dairy. The larger the dairy, the higher the risk of having *Salmonella* as a contaminant with C and D serovars. However, simple biosecurity measures can diminish the pathogen such as cleaning and disinfecting frequently the maternity areas as well as avoiding group maternities. To our knowledge, there is no other study of serotyping and risk assessment of this scale in Mexico.

Financial Support
Zoetis

Notes:

489 - *Salmonella* infection in nursery pigs: a matter of concern?

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Session: SALMONELLA

Objective

To assess the prevalence of *Salmonella* infection at the beginning of the nursery period on a piglet population that had not been previously treated with antibiotics, and to determine whether piglets may be a source of *Salmonella* infection for the growing phase.

Methods

A total of 389 six-week-old pigs from 5 *Salmonella*-seropositive breeding farms and 191 floor fecal samples from gilt units from those farms were included in this study. Mesenteric lymph nodes (MLN) and intestinal content (IC) samples were collected for bacteriology from each piglet at slaughter (EN ISO 6579:2002/A1:2007). Serotyping was performed on all positive samples. PFGE and antibiotic resistance analyses were carried out to characterize *Salmonella* isolates.

Results

The prevalence of infection in piglets was 36.5% (95% CI 31.9-41.4), similar to the proportion of shedding piglets (37.3%, 95% CI 32.6-42.2). A significant association between infection (MLN+) and shedding (IC+) was observed (OR=14.3; CI 8.3-22.7, $P<0.001$). *Salmonella* Rissen was the most frequent serotype, followed by the monophasic variant of *S*. Typhimurium (*S*. 4,[5],12:i:-) and *S*. Derby. The overall proportion of *Salmonella*-positive floor fecal samples was 27.3%. The most frequent serotype was Rissen, which was present in all farms, followed by Derby and *S*. 4,[5],12:i:-.
PFGE clusters matched well with serotypes and AMR profiles. Most (75.5%) of the *Salmonella* isolates from piglets were genetically similar to those found in gilt units.

Conclusions

The prevalence of *Salmonella* infection and shedding among weaning pigs was high. Nursery pigs can become subclinically infected and act as active carriers of *Salmonella* spp. in the farm. Major *Salmonella* serotypes found in piglets were also found in gilts, suggesting a likely transmission from nursery to the growing phase. PFGE and antimicrobial profile analyses confirmed this relationship. Implementing on-farm strategies to reduce *Salmonella* infection during the lactation period may help to prevent further infections at the growing phase and reduce *Salmonella* contamination at slaughter.

Notes:

490 - *Salmonella* spp. prevalence and antimicrobial resistance in broiler chicken and turkey flocks in Canada (2013-2018)

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Session: SALMONELLA

Objective

Salmonella infections are a major health concern that can cause life threatening infections and may require antibiotic therapy, particularly in the elderly or immunocompromised persons. Fluoroquinolones and extended spectrum cephalosporins (ESC) are the antibiotic therapy of choice where treatment is required.

Methods

This study utilised data from the Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) to compare the prevalence of *Salmonella* serovars between chicken and turkey flocks across Canada, and to gain an understanding of the prevalence of AMR to antimicrobials categorized as important to human health. There were 1,596 *Salmonella* isolates obtained from 514 broiler chicken flocks, and 659 *Salmonella* isolates obtained from 217 turkey flocks (2013-2018). All isolates were obtained from pooled faecal samples.

Results

The top three serovars isolated from chicken flocks were Kentucky (n =573, 36%), Enteritidis (n = 314, 20%) and Heidelberg (n = 127, 8%). In serovar Kentucky, resistance to ceftriaxone decreased from 27% in 2013 to 22% in 2018. No resistance among serovar Enteritidis was reported until 2018 when one isolate from British Columbia was resistant to ampicillin, streptomycin, sulfisoxazole, and tetracycline. Resistance to ceftriaxone in serovar Heidelberg decreased from 19% in 2013 to 14% in 2018. The top three serovars among turkey flocks were Uganda (n = 109, 16.5%), Hadar (n = 85, 12%) and Muenchen (n = 66, 10%). No isolates from turkey flocks were resistant to quinolones. No isolates of serovars Uganda or Muenchen were resistant to any β-lactams. Serovar Hadar exhibited resistance to ampicillin (34/81, 42%). Resistance to β-lactams and quinolones was higher in chickens. Resistance to aminoglycosides, folate pathway inhibitors and fluoroquinolones was higher in turkeys.

Conclusions

Emerging resistance among serovar Enteritidis, and resistance to β-lactams and fluoroquinolones among serovar Kentucky from chickens are cause for concern as these classes of antimicrobials are important for treatment of salmonellosis.

Financial Support
Alberta Agriculture and Forestry

Notes:

491 - Insights into antimicrobial resistance and virulence in *Salmonella* spp. from snake samples through genomic approach

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Session: SALMONELLA

Objective

Non-typhoidal *Salmonella spp*. (NTS) are responsible for enteric infections in both humans and animals. Considering the fact that reptiles carry NTS and shed in their feces, the potential for exotic pets to harbor antimicrobial resistant NTS is a one health concern that needs to be addressed. To fight against the rise in antimicrobial resistance (AMR) and predict potential outbreaks using virulence matches in serovars from the environment and clinical isolates, we aim to characterize NTS isolates to determine the presence of indicators that contribute to virulence and confer AMR using genome sequence data.

Methods

We sequenced the whole genomes of 9 NTS isolates from snake fecal samples submitted at Animal Disease Diagnostic Lab (ADDL) Purdue. We compared the genomes of the isolates for AMR and virulence gene detriments. Antimicrobial susceptibility testing was performed for 17 different antibiotics phenotypically and looked for presence of resistance and virulence genes in Resfinder and virulence finder databases, respectively.

Results

Whole genome-based analysis revealed several virulence factors associated with the pathogenicity of NTS which include but are not limited to adhesion, invasion and secretion system. The genetic analysis indicates the presence of adherence genes, outer membrane proteins and secretion systems that are of clinical microbiology importance. The minimum inhibitory concentration report also confirmed that all 9 samples had same resistance profile with resistance to the following antibiotics: amikacin, cephalexin, cefazolin and gentamicin. Genetic analysis revealed the presence of resistance genes conferring resistance to aminoglycoside antibiotics.

Conclusions

This data provides insightful public health implications to mitigate outbreaks; gain better understanding of host-pathogen interaction and benefits microbial source tracking and possible prophylactic planning approaches for clinicians in the future.

Financial Support
Purdue University

Notes:

492 - Occupational exposure of dairy farm workers and working environment to multidrug resistant *Salmonella enterica*

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Session: SALMONELLA

Objective

Dairy cattle operations are a working environment with a high risk for occupational exposure to antimicrobial resistant (AMR) *Salmonella enterica* among workers. Despite the risk, there is a lack of research on their spread in dairy farms. The objectives of our study were to characterize AMR of *S. enterica* serovar Dublin from worker clothing and environment and perform genetic profiling. Our hypothesis was that *S.* Dublin from farm worker clothing and environment would identify diverse AMR patterns and genes revealing potential exposure of workers to health hazards.

Methods

Dairy farm workers were recruited for participation from two Colorado farms, swab samples from clothing (bibs, boots, gloves) and environment (milking parlors, pens) were collected. Identification of *S.* Dublin was confirmed with PCR, antimicrobial susceptibility testing was conducted, and genetic profiling done. To evaluate AMR genes, whole genome sequencing was performed using Illumina Miseq platform. Assembled short-read gene sequences were screened using MEGARes database.

Results

Clothing from 42 workers and environment were sampled. In total, 37 *S.* Dublin isolates were identified, some from the same sample. Clothing accounted for ~92% of isolates, and ~8% were environmental. Whole genome sequencing identified 237 AMR genes. Major gene classes identified across all sample types included beta lactams (48 genes) and multidrug resistance efflux pumps (44 genes). One boot had four fosfomycin resistant genes not previously reported in *S.* Dublin.

Conclusions

This study identified MDR *S.* Dublin on dairy worker clothing and the environment. Fosfomycin resistance has not been reported in *S.* Dublin isolated from dairy cattle and warrants further exploration for its public health implications. Examination of theclonal relatedness of clothing and environmental strains will be conducted.The results from this study can help inform disease control practices on dairy farms to limit the spread of drug-resistant zoonotic pathogens from livestock operations.

Financial Support
U.S. Centers for Disease Control and Prevention

Notes:

493 - High-resolution comparative genomics identifies *Salmonella* Kentucky ST198 and ST152 lineage-specific mutations

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Session: SALMONELLA

Objective

Salmonella Kentucky is a globally emerging foodborne bacterial pathogen. *S.* Kentucky is a polyphyletic serovar comprised of two major sequence types (STs), ST152 and ST198. ST152 is prevalent in U.S. poultry and is less commonly associated with human illness, whereas ST198 is prevalent in international poultry and is more commonly associated with human illness. The objective of this study was to identify *S.* Kentucky lineage-specific mutations and to deduce the likely genetic mechanisms underlying the epidemiologic divergence of these two lineages.

Methods

S. Kentucky strains isolated from poultry (n=140) and humans (n=26) were tested for antimicrobial resistance against 11 antimicrobials, presence of *ColV* plasmid by PCR, and genetic relatedness by PFGE. Representative isolates (n=14) were whole genome sequenced for comparative genomic analysis with 50 ST152 and 400 ST198 sequences from the NCBI Sequence Read Archive to identify lineage-specific SNPs, and phylogenies were constructed.

Results

ST152 and ST198 lineages of *S.* Kentucky were separated by more than 30,000 SNPs/lineage. ST152 isolates harbor fewer antimicrobial resistance genes and formed a closely genetically related cluster. ST198 isolates harbor a variety of antimicrobial resistance genes and form multiple genetic clusters. Highly conserved, lineage-specific SNPs resulting in extension, start codon loss, and truncation in encoded proteins were identified.

Conclusions

S. Kentucky is comprised of two major STs which are distinguished by unique lineage-specific SNPs. In addition to presence or absence of virulence factors and antimicrobial resistance genes, the lineage-specific SNPs result in protein effects which have likely contributed to epidemiologic divergence and differential host adaptation.

Financial Support
Washington State University

Notes:

494 - Antimicrobial resistance and genomic characterization of *Salmonella* Dublin isolates in cattle from United States

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Session: SALMONELLA

Objective

Salmonella enterica subspecies *enterica* serovar Dublin is a host-adapted serotype in cattle, associated with enteritis and systemic disease. While rare in humans, it can cause severe illness, including bacteremia, with hospitalization and death. In the United States, *S.* Dublin has become one of the most multidrug-resistant serotypes. The objective of this study was to characterize *S.* Dublin isolates from sick cattle by analyzing phenotypic and genotypic antimicrobial resistance (AMR) profiles, the presence of plasmids and phylogenetic relationships.

Methods

S. Dublin (n=140) isolates were selected from submissions to the NVSL for *Salmonella* serotyping (2014 – 2017) from 21 states. Isolates were tested for susceptibility against 14 class representative antimicrobial drugs. Resistance profiles were determined using the ABRicate with Resfinder, NCBI databases, AMRFinder and PointFinder. Plasmids were detected using ABRicate with PlasmidFinder. Phylogeny was determined using vSNP.

Results

98% of isolates were resistant to more than 4 antimicrobials. Only 1 isolate was pan-susceptible and had no predicted AMR genes. All *S.* Dublin isolates were susceptible to azithromycin and meropenem. They showed 96% resistance to sulfonamides, 97% to tetracyclines, 95% to aminoglycosides and 85% to betalactams. The most common AMR genes were: sulf2 and tetA (98.6%), aph(3")-Ib and aph(6)-Id (96.4%), floR (94.3%), and blaCMY-2 (85.7%). All quinolone resistant isolates presented mutations in *gyr*A. Ten plasmid types were identified among all isolates with IncA/C2, IncX1, and IncFII(S) being the most frequent. The *S.* Dublin isolates show low genomic genetic diversity.

Conclusions

This study provided antimicrobial susceptibility and genomic insight into *S.* Dublin clinical isolates from cattle in the U.S. Further sequence analysis integrating food and human origin *S.* Dublin isolates may provide valuable insight on increased virulence observed in humans.

Financial Support
U.S. Department of Agriculture

Notes:

495 - Cellular activity of ArtAB toxin from bovine *Salmonella* Typhimurium

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Session: SALMONELLA

Objective

Salmonellosis is one of the most common bacterial foodborne illnesses in the U.S. Non-typhoidal strains of *Salmonella* can infect humans and animals; resulting in acute gastroenteritis that can lead to death. Cows can also harbor subclinical *Salmonella* and are a main reservoir of disease for humans. An effective bovine vaccine is needed to reduce the health and economic impacts of *Salmonella* disease. Bacterial AB5 toxins are virulence factors and antigens in many licensed vaccines. Bovine *S.* Typhimurium DT014 harbors an AB5-type toxin, ArtAB, that is not well-characterized. The objective of this study was to determine the *in vitro* activity of ArtAB. These studies will help to define the virulence potential of this toxin and inform its inclusion into a novel vaccine.

Methods

S. Typhimurium *art*AB was previously cloned into *E.coli* and purified to high efficiency using affinity chromatography. Cytotoxic and metabolic activity assays were performed using purified ArtAB on Vero and CHO epithelial cells. Confocal and light microscopy was used to assess ArtAB trafficking, as well as toxin effects on cellular morphology.

Results

Results revealed that ArtAB induced a slow cytotoxic response after more than 18 hours of incubation. During short incubation times, ArtAB consistently stimulated cell growth and metabolic activity, even at high concentrations. Confocal microscopy revealed binding and internalization of ArtAB-HIS into Vero cells. Analysis of cellular structure indicated that ArtAB induced a clustering phenotype on CHO cells, similar to that of pertussis toxin (PT), but distinct from the elongation phenotype induced by cholera toxin (CT). On Vero cells, ArtAB uniquely stimulated dendrite formation at lower concentrations.

Conclusions

Findings indicate that purified ArtAB can bind to and enter cells *in vitro*, and that this toxin has a reproducible cellular activity and phenotype similar to that of PT. Future studies will focus on the *in vivo* activity of ArtAB. Findings have identified contributions of ArtAB to pathogenicity and represent steps to a potential mucosal *Salmonella* bovine vaccine.

Financial Support
USDA National Institute of Food and Agriculture

Notes:

497 - The Peste des Petites Ruminants vaccine value chain in Karamoja: who has access to the vaccine and who does not?

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Session: VACCINOLOGY

Objective

Mapping the Peste des Petites Ruminants (PPR) vaccine value chain in Karamoja, Uganda to identify the barriers women and other marginalized groups have when accessing PPR vaccines using a gender and intersectional approach.

Methods

A series of Focus Group Discussions (FGDs) and interviews were done in November 2019 and January-February of 2020 in four districts of the Karamoja subregion of Uganda (Abim, Amudat, Kotido, and Moroto). The interviews and FGDs were done with farmers and animal health service providers. Some additional Key Informant Interviews were done at the national level. Interviews and FGDs with farmers and community members were done in the local language with local facilitators. FGDs were disaggregated by sex.

Results

Access to PPR vaccines, and livestock vaccines in general, is very restricted for all farmers in Karamoja. There is no private sector involvement regarding livestock vaccines in Karamoja; access to PPR vaccines is limited to those provided by the government or NGOs. The government's vaccination strategy, which is to respond to outbreaks, occasionally fails to act on time, and vaccines sometimes arrive months after an outbreak is detected. When vaccines do arrive, the quantity is insufficient to vaccinate all livestock. Additionally, there are other barriers for farmers to access vaccines, and among those, certain groups face even more challenges. Women (widows in particular), the elderly population, those who live in remote areas, and those who have physical disabilities are far more affected, routinely left out of campaigns, and are often unable to access vaccines regardless of availability. Ethnic differences and a preference for animals owned by men likewise impact access to vaccines. Finally, lack of knowledge about vaccines and how vaccines work is also prevalent across the region.

Conclusions

An effort to improve the PPR vaccine value chain is needed in order to eradicate the disease from the region; however, this effort must be inclusive of those who have been systematically left out, as failure to reach them will result in disease reservoirs remaining.

Financial Support
International Development Research Centre

Notes:

498 - Peste des petits ruminants and newcastle disease Vaccine Value Chains in Senegal: access and womens engagement

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Session: VACCINOLOGY

Objective

Mapping the Peste des petits ruminants (PPR) and Newcastle disease (ND) vaccine value chain in Kaffrine, Senegal to identify the barriers women face in the VVC. Using a gender and intersectional lens.

Methods

Two phases of data collection occurred between July and October 2019, both of which were overseen by an in-country coordinator. Document review, key informant interviews(KIIs), individual interviews(IIs), and focus group discussions (FDGs) were conducted to document the Vaccine Value Chain (VVC), followed by a gender and intersectional analysis of data from these methods. Fieldwork started in Dakar, where KII identified vaccine wholesalers and importers. The team then advanced to the four departments of Kaffrine Region to conduct KIIs, IIs and FGDs. Data was collected from participants in their local language (Wolof). Data from KIIs, IIs, and FDGs were disaggregated by gender. Thematic analysis was conducted by the research team to map the VVCs and identify themes with regard to women's engagement in and benefit from the PPR and ND VVCs.

Results

Vaccine distribution in Senegal is regulated by the state either through the public or the private system. The public veterinary system has representation at national, regional and district level, utilizing a network of community animal health workers to reach out to rural communities. Private system importers/wholesalers sell directly to private veterinarians, who then serve the public directly (or use the same network of community animal health workers). The ITA-new vaccine for ND comes through the private system. PPR/H vaccine is produced in Senegal by the only vaccine producer ISRA and is transported through the state veterinary system. Knowledge about the VVC also played a role in communities vaccinating their poultry or small ruminants. Lack of incentives, access and geographical restrictions impact the ability for effective vaccine campaigns and women's engagement in administrating the vaccines.

Conclusions

Including women in the VVC for ND and PPR is needed to reduce the mortality rate among poultry and small ruminants.

Financial Support
International Development Research Centre

Notes:

499 - H1N1 influenza virus preimmunity improves antibody response to vaccination through regulation of germinal center

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Session: VACCINOLOGY

Objective

Influenza viruses circulate annually through the human population, accumulating genome mutations that affect antigenicity. Due to constant circulation individuals are exposed to multiple viruses over a lifetime, creating an immune history or preimmunity, which has been shown to have significant effect on vaccination outcomes. Most experimental studies investigating vaccine efficacy utilize naïve hosts, not accurately representing the human condition. In the present study, we sought to determine the mechanism of preimmunity-induced vaccination outcomes and hypothesized the germinal center reaction was central to improved antibody response to vaccines in a preimmune host.

Methods

C57Bl/6J mice were infected with a non-lethal dose of A/FM/1/1947 creating H1N1 preimmunity or A/Hong Kong/1/1968 creating H3N2 preimmunity. Mice recovered, allowing for development of adaptive memory before subsequent vaccination. H1N1 preimmune animals were then challenged with a lethal dose of a 2009 pandemic H1N1 virus. Animals were assessed for weight loss, antibody dynamics, and immune responses in lungs as well as secondary lymphoid organs.

Results

H1N1 preimmune mice had increased antibody response post-vaccination (pv) to the H1 antigen. Titers peaked at 8 HAI units by 14 days pv, compared to 2 HAI units in naïve-vaccinated mice. H3N2 preimmune mice showed no detectable titers to any component of the vaccine on day 7 pv. Preimmune-vaccinated mice had increased IgG class-switched isotypes, demonstrating affinity maturation, and were protected at challenge. The mediastinal and inguinal lymph nodes, proximal to the site of infection and vaccination, respectfully, were assessed via RNASeq. Preimmune-vaccinated mice revealed distinct regulation of lymphocyte and germinal centre specific genes, including *bcl6*, which is essential for germinal-center dependant B-cells.

Conclusions

H1N1 preimmunity, but not H3N2 preimmunity, was shown to lead to a more efficient vaccine response regulated by the germinal centre reaction in the lymph nodes, findings that should be considered for future vaccine design.

Notes:

501 - An inactivated, universal *Salmonella* epitope vaccine protects piglets from *Salmonella choleraesuis* disease

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Session: VACCINOLOGY

Objective

The infection of swine with *Salmonella* presents a continuing problem to human and animal health and food safety. *Salmonella enterica* serovar Choleraesuis (Sc) is the causative agent of swine paratyphoid and can lead to economic losses due to high morbidity and mortality. In this study, our universal *Salmonella* epitope vaccine for poultry was adapted for swine. The vaccine uses a *Bacillus sp.* to produce a protective epitope and is encapsulated for oral delivery to the intestinal mucosa.

Methods

Three-week-old pigs (n=10) were transferred from a farm nursery to weaning boxes. Pigs appeared healthy. It was determined that the pigs had no previous exposure to *Salmonella* as detected by seroconversion. The pigs were individually tagged, weighed, and randomized into 1 of 2 treatment groups. Commercial feed and water were provided *ad libitum*. Pigs were vaccinated on study days 0 and 14 via oral gavage with 2 ml of vaccine (T1) or saline (T2) and challenged via oral gavage 10 days later with 10^{10} CFU of NAL^R Sc. Samples were taken at study days, 0, 14, 24-31, and 35 to determine specific antibody response to the vaccine and Sc enumeration in feces; as well as a series of clinical observations during the 35-day study. All pigs were humanely euthanized.

Results

The T1 group had better ADG and total weight gain with little to no clinical signs observed. The T2 group experienced weight loss and bouts of diarrhea during the study. T1 had peak mean fecal Sc counts 3 days dpi (10^3 CFU/g) and then decreased (10^2 CFU, LOD). T2 maintained elevated mean fecal Sc counts (10^3-10^4 CFU/g) during the study. The ELISA S/P ratio measuring vaccine-specific IgG was 1.2 on study D35.

Conclusions

There were no observable side effects of the *Salmonella* vaccine. Overall, the vaccine prevented the overt clinical disease from a strong Sc challenge and the T1 group gained more weight and had lower mean Sc CFU counts in their feces at the end of the study compared to the T2 group. The results from this study indicate that our *Salmonella* subunit vaccine can control Sc infections in swine with the same efficacy as in poultry.

Financial Support
Vetanco

Notes:

502 - Characterization of *Mycobacterium paratuberculosis* membrane vesicles and *E. coli* outer membrane vesicles

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Session: VACCINOLOGY

Objective

Johne's disease is a chronic granulomatous enteritis occurring worldwide among domestic ruminants. *Mycobacterium avium* subspecies *paratuberculosis* (MAP) not only being a pathogen that causes significant economic losses in the world, but also a potential factor in the pathogenesis of Crohn's disease in humans. Because no current MAP vaccine is available in the U.S., the development of an effective vaccine is urgently needed in the animal industry. Gram-positive bacterial membrane vesicles (MVs), which were released from the bacterial cell wall, were considered as potential vaccine candidates because of their immunogenicity. We also cloned Ag85A-Ag85B-SOD fusion protein gene using vector pBAD18H6ClyA and expressed in *E. coli* strain JH8033. The ultimate goal of this project is to develop the MAP vaccine capable of protecting ruminants against Johne's disease infection using MVs produced from the MAP or outer membrane vesicles (OMVs) produced in an engineered *E. coli* strain.

Methods

MAP K-10, an isolated bovine strain, is cultured in 7H9 broth supplemented with OADC and Mycobactin J at 37℃ for four weeks. During the culture period, MVs were produced by MAP and released into the culture medium. After 4-week incubation, the culture medium was collected and filtered by 0.45μm of PVDF membrane filters. MVs were isolated by ultracentrifugation (100,000 xg, 2h, 4℃) followed by gradient ultracentrifugation (30,000 rpm, 16h, 4℃) and resuspended in PBS forTEM and proteomic analysis. Ag85A-Ag85B-SOD-expressing *E. coli* was induced by L-arabinose when the bacterial density (OD_{600}) reached 0.4 and followed by 16-hour incubation at 37℃. OMVs in the supernatant was collected as described above.

Results

MVs was produced by MAP during growth and isolated via a series of ultracentrifugation. MAP MVs were identified by SDS-PAGE and transmission electron microscopy. Further proteomic analysis of MVs is ongoing and expected to reveal the protein composition of MAP MVs. We also isolated OMVs from engineered *E. coli* and confirmed with SDS-PAGE, Western blotting, and TEM.

Conclusions

According to our results, MAP MVs can be isolated from the culture successfully. The recombinant OMVs are also expressed well in *E. coli*. Future work will proceed to identify the competency of MVs/OMVs being a vaccine candidate on using goats as an animal model for the protection efficiency and host immune responses after challenge.

Financial Support
USDA National Institute for Food and Agriculture

Notes:

503 - Outer membrane vesicles of *Fusobacterium nucleatum*: antigenicity, composition, and virulence

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Session: VACCINOLOGY

Objective

Fusobacterium nucleatum is a Gram-negative bacterium commonly found in the oral cavity and is often involved in periodontal diseases. Recent studies have shown increased *F. nucleatum* prevalence in colorectal cancer (CRC) tissues, and causal data has linked this bacterium to CRC tumorigenesis. Outer membrane vesicles (OMV) are naturally produced by Gram-negative bacteria, consisted with complex components and may elicit protective immune responses if used as vaccines.

Methods

OMVs were isolated and purified from *F. nucleatum* cultures by ultracentrifugation. Antigenicity of OMVs was characterized using dendritic cells (DCs) and evaluated by flow cytometry and Luminex. The proteins contained within the OMV were identified by nano-LC/MS/MS analysis. 20 proteins identified from *F. nucleatum* OMVs were expressed to immunized mice. Anti-adhesion and bactericidal assay were performed to evaluate the antigens.

Results

Our results demonstrated *F.nucleatum* OMV stimulates DCs to express higher costimulatory molecules and T cell polarizing cytokines levels. Of 98 proteins consistently identified from duplicate analyses, 60 were predicted to localize to the outer membrane or periplasm via signal peptide-driven translocation. Of these, six autotransporter proteins, which constitute the majority of the protein mass of OMV, were associated with Type V secretion system. In addition, other putative virulence factor proteins with functional domains, including FadA and MORN2 were identified by *in silico* analysis. Both OMV and glycerophosphodiester phosphodiesterase, one of the 20 expressed recombinant proteins, displayed sufficient anti-adhesion ability.

Conclusions

Understanding the constituents of *F. nucleatum* OMV will provide fundamental information and potential strategies for OMV-based *F. nucleatum* vaccines design. Altogether, the non-replicative OMVs of *F. nucleatum* contain multiple antigenic virulence factors that may play important roles in the design and development of vaccines against *F. nucleatum*. The protectability and efficacy will need further evaluation in challenge and colorectal murine models.

Financial Support
U.S. National Institutes of Health

Notes:

504 - Antibody response against Canine Parvovirus of client-owned dog puppies after vaccination with bivalent vaccines

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Session: VACCINOLOGY

Objective

The objective of the study was to describe the antibody response against Canine Parvovirus (CPV) generated in sera after vaccination of client-owned puppies with bivalent vaccines containing live attenuated strains.

Methods

Healthy 6-8 weeks old puppies were recruited from private owners and assigned to two groups according to age. Group 1 (8 weeks old; n=62) was vaccinated with HIPRADOG® DP and group 2 (6 weeks old; n=58) was vaccinated with an equivalent commercially available bivalent product. The vaccines were administered in accordance with the manufacturer's instructions. This implies the administration of two doses of each vaccine. Blood samples were collected before (1st dose and 2nd dose) and 21 days after completion of the vaccination plan (21 days after the administration of the second dose). The purified sera were tested using commercially available ELISA kits to determine antibody titres against CPV. Results were compared between groups by Mann-Whitney U-test for the variable antibody titre or Chi square for the variable percentage of positive animals.

Results

Results showed that a CPV-specific antibody response was generated in all groups (Group 1 = 93.55%, group 2 = 77.59%) despite the presence of weak maternal derived immunity in some of the recruited puppies (Group 1 = 11.29%, group 2=18.97%) before the vaccine administration (1st dose). However, the characteristics of those responses were different depending on the product that was administered. In particular, group 1 showed a higher coverage and homogeneity of the immune response compared to group 2. Notably, this latter produced slightly higher levels of antibodies despite showing a higher variability.

Conclusions

Bivalent vaccines tested were able to produce a specific antibody immune response against CPV in puppies under field conditions and in face of weak maternal antibody response. Despite this, the immunization performances can differ significantly depending on the product used.
HIPRADOG® DP showed to be able to produce a high immunization coverage and homogeneity.

Financial Support
Universitat Autònoma de Barcelona of Spain

Notes:

505 - Stabilizer selection for a live recombinant Herpesvirus vaccine candidate

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Session: VACCINOLOGY

Objective

Herpesviruses (HV) are enveloped viruses that contain a linear dsDNA genome ranging from 125 to 240kbp. An attenuated HV vaccine strain was experimentally used as a live vector vaccine in animals, but susceptibility to freeze-drying conditions were reported. In this study, a recombinant HV vaccine candidate was formulated using two different stabilizers, and the loss in infectivity assessed after lyophilization.

Methods

Two stabilizers were tested in this study, containing similar concentrations of the same sugar and gelatin components, and a different peptide source. Stabilizer A contained 25% v/v of the excipients, while Stabilizer B contained 26.8% v/v. To determine if the antigen input affected virus stability, different concentrations of the HV candidate were tested within each formulation, ranging from 1% to 50% antigen input (titer range 5.9–8.3 $\log_{10}$ $FAID_{50}$/vial); media was used to achieve the desired virus concentration. A LyoStar 3 freeze dryer was used, with a fixed proprietary lyophilization cycle. A Fluorescence Assay Infectious $Dose_{50}$ ($FAID_{50}$/vial) assay was used to assess the virus infectivity before and after the lyophilization process.

Results

A low loss in infectivity (≤0.6 $\log_{10}$ $FAID_{50}$/vial) after lyophilization was observed when the antigen input was equal or higher than 8% of the formulation, regardless of the stabilizer used. In comparison, higher loss in infectivity (1.1 $\log_{10}$ $FAID_{50}$/vial) was observed when using Stabilizer A with an antigen input equal or lower to 5% of the formulation; a follow-up study demonstrated that this loss occurred during the initial freezing step of the cycle. Formulations containing Stabilizer B consistently showed low loss in infectivity (≤0.6 $\log_{10}$ $FAID_{50}$/vial), regardless of antigen input.

Conclusions

Selection of an optimal stabilizer for a live virus vaccine is an essential step during vaccine development. Due to its cryopreserving properties, Stabilizer B was selected as an excipient for the recombinant HV vaccine candidate. This novel formulation should be considered for other live virus vaccine candidates in the future.

Notes:

506 - Kinetics of ruminant immunity elicited by live attenuated vaccine against Johne's disease.

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Session: VACCINOLOGY

Objective

Johne's disease (JD) caused by *Mycobacterium paratuberculosis* is a chronic infection characterized by the development of granulomatous enteritis in ruminants. It is one of the most significant livestock diseases in the U.S. and worldwide. The currently licensed inactivated vaccine does not help in controlling disease transmission. Recently, our group developed a live-attenuated vaccine (LAV) with the deletion of *LipN* gene from the virulent strain of *M. paratuberculosis*. This LAV showed significant improvement over the inactivated vaccine. The main objective of this project is to characterize safety and immunogenicity of LAV in ruminants.

Methods

The current study evaluated the safety, persistence and the type of generated immune responses of the novel LAV in the caprine model of paratuberculosis. All animal groups (N=4 for 8 animals/group) were vaccinated at 4 weeks of age with different vaccine candidates and followed for 6 months to monitor vaccine shedding, tissue damage and generated immune responses.

Results

Goats vaccinated with the LAV via subcutaneous (SC) or intranasal (IN) routes didn't suffer from any skin induration or granuloma formation at the site of inoculation, unlike the group vaccinated with the inactivated vaccine. By 3 weeks post immunization (WPI), *M. paratuberculosis* were recovered only from SC site of inoculation and the associated pre-scapular lymph nodes while all other lymph nodes and organs had no detectable levels of *M. paratuberculosis* (>30 lymph nodes and organs). By 6 months post immunization (MPI), no detectable levels of *M. paratuberculosis* were found in any collected lymph node or tissue samples. Interestingly, the LAV, regardless given via SC or IN routes, induced a robust T cell mediated immunity by 6 MPI compared to the inactivated vaccine given SC.

Conclusions

Overall, the obtained results indicate the safety and immunogenicity of LAV in ruminants and provide more support to initiate field trials to curb JD that will help in the control of Johne's disease in the USA and worldwide. u

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

Notes:

507 - We've got a live one! Generation of a live-attenuated vaccine to piscine streptococcosis

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Session: VACCINOLOGY - AQUACULTURE

Objective

Streptococcus iniae is an emerging pathogen in freshwater and marine aquaculture worldwide, costing the industry over US$100 million in annual losses. There are few commercial vaccines available for *S. iniae,* which are restricted to killed whole-cell preparations with limited protection against heterogenous strains. Live-attenuated vaccines (LAV) represent an advantageous alternative to these bacterins, as they induce robust cellular and humoral immunity, and may provide longer lasting protection through less stressful routes of administration. We hypothesized that accumulation of mutations in *S. iniae* by serial passage in the presence of rifampin can generate immunogenic LAV that confer protection to vaccinated fish challenged with heterologous wild-type (WT) *S. iniae* strains.

Methods

Three lineages of rifampin-resistant strains were generated from three genetically distinct parent strains (n=9) by multiple passages in increasing increments of Rifamycin SV sodium salt. Growth in liquid media, extent of capsulation, survival in Nile tilapia (*Oreochromis niloticus*) whole blood and cytotoxicity in an *O. mossambicus* endothelial cell line were compared between the passaged and parental strains. Nile tilapia challenges were used to assess strain virulence, generation of anti-*S. iniae* IgM, and the protection conferred by LAV candidates against virulent *S. iniae*.

Results

Multiple passaged strains demonstrated changes in growth rate and cytotoxicity in endothelial cells, as well as significant reductions in whole blood survival ($p < 0.05$). Selected strains showed attenuated virulence in the Nile tilapia challenge model, and anti-*S. iniae* IgM generated against these strains demonstrated considerable cross-reactivity. Immunization by intracoelomic injection conferred protection against a virulent WT strain of *S. iniae,* with relative percent survival ranging from 4.22 to 95.05%.

Conclusions

Passaging of *S. iniae* in increasing concentrations of antibiotic is a viable method for generating LAV candidates with the potential to protect against heterologous WT strains.

Financial Support
University of California at Davis

Notes:

508 - Protective effects of vaccine and killed culture against atypical A*eromonas hydrophila* (aAh) in I*ctalurus punctatus*

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Session: VACCINOLOGY - AQUACULTURE

Objective

An atypical form of the gram-negative pathogen, *Aeromonas hydrophila (aAh),* causes dramatic losses in US catfish aquaculture. Research has identified two predominant aAh haplotypes within the industry, hereafter ML09-119 and S14-452. A live, attenuated vaccine has been derived from the ML09-119 strain (ML09-119 Δ*gfcD*) but the protective effects against the S14-452 haplotype are unknown. This study evaluated oral delivery of the prospective vaccine against both aAh haplotypes compared to formalin-killed preparations.

Methods

Cryopreserved aAh isolates were revived on blood-agar plates and individual colonies expanded overnight in static porcine BHI broth. Channel catfish fingerlings received 0.1 mL (~$1x10^6$ CFU/fish) IP injections of the live vaccine or formalin killed ML09-119 or S14-452 culture. Oral vaccination consisted of 454g of commercial feed mixed with 100 mL of diluted live vaccine or formalin-killed culture (~$4x10^6$ CFU/g of feed). Fish were fed twice daily to apparent satiation over 2-days. After 30 days, fish were challenged with IP injections of 0.1 ml of diluted ML09-119 or S14-452 (~$1x10^6$ CFU/fish). Fish were monitored twice daily for 10 days and mortality recorded.

Results

Injection of live, attenuated or formalin-killed cultures significantly improved survival (relative percent survival [RPS]: >80%). Oral vaccination using the live, attenuated ML09-119 vaccine also improved survival (RPS: 60.5%) but did not infer protection against the S14-452 haplotype at the administered dose. Further, none of the formalin-killed treatments showed protective effects when delivered orally.

Conclusions

These results reveal oral vaccination of channel catfish against aAh provides less protection than IP injection, however, oral administration of the live, attenuated ML09-119 vaccine outperforms orally delivered formalin killed preparations and increases survival against the ML09-119 parent strain. Comparably, oral delivery of the live, attenuated ML09-119 strain did not protect against S14-452. This warrants further study but suggests the need for haplotype-specific or combinatory vaccines.

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

Notes:

509 - Development of a Generalized Recombinant Attenuated *Edwardsiella piscicidan* Vaccine Vector System (RAEVs)

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Session: VACCINOLOGY - AQUACULTURE

Objective

Characterization of *E. piscicida* mutant strains with (i) regulated delayed attenuation, (ii) regulated delayed protective antigen synthesis and delivery by type 2 secretion system and (iii) that exhibit regulated delayed lysis in vivo.

Methods

Insertion of defined deletion mutations with and without insertions was accomplished by conjugational transfer of suicide vectors to *E. piscicida* J118 using the suicide vector donor strain χ7213. The I-antigen gene was inserted into the lysis plasmid pG8R114 and named as pG8R8040, which was electroporated into *E. piscicida* lysis strain χ16027. Synthesis of I-antigen was confirmed by western blotting.

Results

The regulated delayed attenuation and programmed self-destructing features designed into these *E. piscicida* strains enable them to efficiently colonize host lymphoid tissues and allow release of the bacterial cell contents after lysis. None of the bacterial vaccine cells are able to survive and thus exhibit complete biological containment. The system is composed of two parts. The first component is *E. piscicida* strain χ16027 with deletions of *asdA* and arabinose-regulated expression of *murA*, two genes required for peptidoglycan synthesis and additional mutations to enhance attenuation. The second component is plasmid pG8R8040, which encodes arabinose regulated *murA* and *asdA* expression and also encode codon optimized IAG52B ich I-antigen gene. The RAEV-ich strain χ16027(pG8R8040) exhibits arabinose-dependent growth. Upon invasion of host tissues, an arabinose-free environment, transcription of *asdA, murA*, and concentrations of their gene products decrease because of cell division and confer the cell lysis. Zebrafish bath immunized with χ16027(pG8R8040) developed mucosal and systemic antibody responses to ich membrane protein.

Conclusions

Our work highlights the potential for developing *E. piscicida* live vaccines against *I. multifiliis*. This multidisciplinary approach using cutting-edge technologies will address the sustainability challenges of aquaculture, increased food quality and quantity

Financial Support
USDA National Institute for Food and Agriculture

Notes:

510 - Infection kinetics and immune response to vaccination in cultured sablefish (*A. fimbria*) against *A. salmonicida*

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Session: VACCINOLOGY - AQUACULTURE

Objective

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

Methods

Groups of forty fish where intraperitoneally (ip) injected with different doses of atypical *A. salmonicida* J410, where the medium lethal dose (LD_{50}) was estimated to be ~$3x10^5$ CFU/dose. Samples of blood, head-kidney, spleen, brain, and liver were collected at different time points to determine the bacterial colonization. Immune protection for the different vaccine preparations was evaluated in a common garden experiment. Blood samples were obtained biweekly to evaluate IgM titers. After 10 weeks post-immunization the fish were ip challenged with 100 times the LD_{50} dose (10^7 CFU ml^{-1}). Thirty days post-challenge (dpc) the relative percent survival (RPS), compared to the control, was calculated for each vaccine.

Results

The RPS for the bacterin mix was 63.7%, for the Forte vaccine 54.57%, and for Alpha Ject vaccine was 27.28%. *A. salmonicida* tissue colonization 10 dpc negatively correlated with the RPS. Additionally, ELISA assays indicated differences in IgM titers between vaccines at 6 weeks post-immunization. Vaccine immune protection was associated with the IgM titers, where the autogenous vaccinated fish had the highest RPS and IgM titers. *A. salmonicida* A-layer binds to immunoglobulins in a non-specific fashion, questioning the hypothesis that could be used as immune protective antigen.

Conclusions

This study provides novel insights about sablefish vaccinology for the prevention of furunculosis, further research is required to develop an effective cross-protective vaccine for this species.

Financial Support
Canada-First Ocean Frontier Institute; 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:

511 - Efficacy of intranasal vaccination with *Moraxella spp* antigens plus growth medium against bovine pinkeye

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Session: VACCINOLOGY - CATTLE

Objective

Control of infectious bovine keratoconjunctivitis (IBK; 'pinkeye') with currently available parenterally-administered vaccines can be challenging. To determine if an intranasal vaccine containing concentrated extracts of *M. bovis* and *M. bovoculi* culture supernatant plus concentrated growth medium could prevent IBK and reduce disease morbidity, a randomized controlled field trial was conducted during summer 2020 in northern California crossbred beef steers.

Methods

Steers without evidence of active IBK were randomly assigned to receive an intranasal vaccine (and booster on day 21) of either adjuvant plus water (control vaccine; n=89), or adjuvant plus *M. bovis* and *M. bovoculi* concentrated culture supernatant and concentrated uninfected heart infusion broth (experimental vaccine; n=87). The adjuvant was a combination of polyacrylic acid and emulsified oil-in-water containing dimethyldioctadecyl ammonium bromide (PA-DDB). Ocular exams were conducted once a week for 16 weeks following primary vaccination to identify corneal ulcers associated with IBK. Ulcers were photographed and corneal ulcer surface areas were measured. Steers exhibiting ocular pain from IBK were treated with flunixin meglumine and oxytetracycline was administered for IBK-associated ulcers >0.5 cm in widest diameter. Nonparametric statistical methods were used to evaluate differences between groups in IBK occurrence, drug treatments, and ulcer severity.

Results

While significant differences were not found between groups in the proportion of animals with corneal ulcerations associated with IBK, consistently lower metrics associated with ulcer severity were observed in IBK-affected animals that received the experimental vaccine.

Conclusions

The results from this study suggest that intranasal vaccination with *M. bovis* and *M. bovoculi* concentrated culture supernatant plus concentrated growth medium adjuvanted with PA-DDB may reduce requirements for IBK treatment.

Financial Support
USDA National Institute of Food and Agriculture

Notes:

512 - Development and testing of *Mycobacterium avium* subsp. *paratuberculosis* DIVA vaccines in ruminants

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Session: VACCINOLOGY - CATTLE

Objective

Johne's Disease (JD), caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP), is one of the most significant problems in animal health. We developed a unique approach to generate live-attenuated vaccines 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. Antigens MAP_1152 and MAP_1156 have been also shown to differentiate between seropositive and seronegative samples.

Methods

In this study, we measured secreted cytokines in supernatants from peripheral blood mononuclear cells isolated from non-infected and JD-positive cows after infection with wild type (K-10) or mutant strains for 24 hr. Secreted cytokines were measured using multiplex cytokine arrays for IL-1β, IL-6, IFN-γ, TNF-α; and commercial ELISAs for IL-10, IL-13 and IL-17. Data was analyzed by one-way ANOVA.

Results

A significant increase was observed for IL-17 with strain treatments DMAP56 ($P = 0.0230$) and K-10 ($P = 0.0373$), as well as a trend for DMAP52 ($P = 0.1329$). Thus, responses were equally robust for mutant and wild type strains. Future studies in progress are planned to (1) generate attenuated unmarked in-frame deletion mutants and test for invasion and intracellular survival in bovine macrophages, (2) test these antigens for DIVA capabilities in assays of humoral and cellular immunity and (3) assess the immunogenicity and pathogenicity of unmarked mutants in calves.

Conclusions

These results indicate that the mutants can elicit a protective and modulated immune response in the host with secretion of both pro- (IL-1β, IL-17, IFN-γ and TNF-α) and regulatory anti- (IL-6, IL-10 and IL-13) inflammatory cytokines. Achievement of future goals would be of significant importance in ameliorating the negative impact of JD on national and international trade.

Financial Support
USDA National Institute of Food and Agriculture

Notes:

T513 - argeting subdominant rhoptry-associated proteins of Babesia bovis in a subunit vaccine to protect cattle against bovine babesiosis

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Session: VACCINOLOGY - CATTLE

E

Objective

mergence of bovine babesiosis, caused by Babesia bovis, is a significant threat to the US cattle industry. Live attenuated B. bovis vaccines are available but not permitted in the US, and development of efficient vaccines is a priority. B. bovis rhoptry-associated protein 1 (RAP-1) is involved in erythrocyte invasion and has been historically considered as a candidate antigen for a subunit vaccine. RAP-1 consists of an immunodominant C-term and a subdominant N-term, which contains the RAP-1 signature domains. RAP-1 vaccination failed to protect cattle despite induction of IFNγ and IgG. Possible explanations for the failure of RAP-1 as a solo vaccine antigen are that: (a) immunodominant segments in the repetitive C-term region of RAP-1 skew the host immune responses; (b) and alternative expression of RAP-1 related antigen (RRA), a potential and truncated subdominant functional equivalent of RAP-1 lacking the C-term region. H

Methods

ereby we are testing the immunosubdominant segment NT of RAP-1 and full-length RRA fused with the Salmonella flagellin Flic molecular adjuvant (RAP-1NT/RRA/Flic) as a subunit vaccine to control acute bovine babesiosis. W

Results

e predict that subdominant proteins are essential for parasite survival and rational vaccine antigens, considering that the parasite is able to cause persistent infections in the face of strong immune responses of the host, driven mainly against immunodominant proteins. This novel vaccination approach combines two B. bovis subdominant antigens fused with the Flic molecular adjuvant, with the expectation to turn these proteins into immunodominant-like antigens. W

Conclusions

e plan testing the RAP-1NT/RRA/Flic vaccine in cattle, natural host of B. bovis and expect that vaccinated animals will control parasitemia, show mild signs of infection, and survive challenge with a virulent strain of B. bovis. Our premise is that RAP-1NT/RRA/Flic vaccination can prime the immune system of cattle to mount a protective adaptive immune response, allowing the animals to survive acute bovine babesiosis.

Notes:

514 - Novel subunit targeted vaccine against BVDV: development and field results

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Session: VACCINOLOGY - CATTLE

Objective

Bovine viral diarrhea virus (BVDV) has a worldwide distribution and infects ruminants, BVDV infections cause a broad spectrum of clinical signs and economic losses. Vaccination against BVDV is an important component of prevention and control programs. Currently, only modified live vaccines (MLV) and inactivated vaccines are used. Both have disadvantages; MLV in terms of safety and inactivated vaccines in terms of immunogenicity. Subunit vaccines provide the opportunity of developing safe and effective vaccines, however, in the field of veterinary medicine, the challenge is to produce a protective recombinant vaccine at a cost affordable price. Here we report the development and efficacy of the first targeted subunit vaccine against BVDV. The core of the vaccine is a fusion of the BVDV structural protein, E2, to a single-chain antibody, APCH, together termed, APCH-E2. The APCH antibody targets the E2 antigen to the MHC-II present on antigen-presenting cells.

Methods

Experimental Design: 300 Brangus cows were divided randomly into two groups, 150 vaccinated with the targeted vaccine and 150 with a conventional vaccine, which contains inactivated BVDV. All animals were vaccinated twice at day 0 and 30. Blood was drawn from all animals on days 0, 30, 60, 120, 180 and 360. Sera were analyzed by competition ELISA. Results are expressed as a percentage of displacement of a positive serum. Serum samples of all animals at day 0 and 60 were also evaluated by virus neutralization assay. Three field trials of 100 animals were conducted simultaneously.

Results

This new subunit vaccine induces strong BVDV-specific neutralizing antibodies in vaccinated cattle. Importantly, in cattle with low levels of natural BVDV-specific neutralizing antibodies, the vaccine induced strong neutralizing antibody levels to above the protective threshold.

Conclusions

The APCH-E2 vaccine induced a rapid and sustained neutralizing antibody response compared to a conventional vaccine in cattle. The development of this subunit targeted vaccine provides producers with an inexpensive, safe, and efficacious BVDV vaccine.

Notes:

515 - Development of a protective BRSV IgA mucosal response IFOMA with a parenteral adjuvanted vaccine

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Session: VACCINOLOGY - CATTLE

Objective

Bovine respiratory syncytial virus (BRSV) is major viral contributor to bovine respiratory disease (BRD). The purpose of this study was to evaluate the efficacy of an adjuvanted modified live virus (MLV) vaccine in the presence of well-defined maternal passive immunity.

Methods

Calves were vaccinated at approximately 1 month of age and challenged ~90 days later when BRSV systemic antibodies were less than 1:4. Clinical signs, nasal secretions and blood samples for virus measurement [polymerase chain reaction (PCR) and virus isolation (VI)] and n to measure for mucosal BRSV IgA antibodies were collected and the animals were euthanized and necropsied 8 days post infection.

Results

Body temperature and other clinical signs were lower at 6 and 7 days post challenge in the vaccinates. Nasal viral shed was 3–4 times lower in the vaccinated animals as measured by VI and PCR and peaked 5 days post challenge compared to the controls (who peaked at days 6 and 7). On day 8 following challenge, animals were necropsied, and lung lobes were scored and tested for virus by PCR and indirect fluorescent assay (IFA). There was a 25-fold reduction in PCR virus detection in vaccinates and two of the vaccinated calves' lungs were PCR negative. Only 29.4% of vaccinated calves were BRSV positive on IFA testing at necropsy, while 87.5% of control calves were BRSV positive. Vaccinated calves developed a mucosal BRSV IgA response with over 50% of the vaccinated calves having IgA prior to challenge and all vaccinated calves were positive following challenge. Additionally, vaccination stimulated the production of Interferon gamma in mononuclear cells to prime the immune system.

Conclusions

This study established that an adjuvanted MLV vaccine could provide protection against BRSV as measured by clinical, virological, and pathological parameters while also activating both mucosal and systemic immunity

Notes:

516 - Simultaneous epitope recognition by CD4 and CD8 T cells

essential for cytotoxic T-cell development

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Session: VACCINOLOGY - CATTLE

Objective

Demonstrate simultaneous epitope recognition by CD4 and CD8 T cells
essential for cytotoxic T-cell development.

Methods

Bovine PBMC depleted of monocytes (mdPBMC) were stimulated twice with APC primed with a membrane protein (MMP) from *Mycobacterium avium* subsp. *paratuberculosis* (*Map*) encoded by *MAP2121c* ex vivo alone, or in the presence of monoclonal antibodies to MHC I and MHC II together or separately.

Results

The CTL response of mdPBMC to APC pulsed with MMP was completely blocked in the presence of mAbs to both MHC I and II molecules and also blocked in the presence of mAbs to either MHC I or MHC II alone.

Conclusions

The results demonstrate simultaneous cognate recognition of Ag by CD4 and CD8 T cells is essential for delivery of CD4 T cell help to CD8 T cells to elicit development of CTL.
The results explain why it has been so difficult to develop peptide-based vaccines. The vaccine peptides must contain the epitopes for CD4 and CD8 T cells for processing and simultaneous presentation.

Notes:

518 - Passive immunity from colostrum offers clinical protection to young calves against experimental challenge with BRSV

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Session: VACCINOLOGY - CATTLE

Objective

Maternal antibodies against bovine respiratory viruses protect calves against acute viral infection. The objective of this study was to determine if passive immunity transferred from colostrum of vaccinated cows resulted in clinical protection of calves against challenge with BRSV.

Methods

Forty pregnant beef cows were randomly assigned to two different treatment groups. Group VAC (n = 20) was vaccinated with 2 doses of an inactivated BRSV-vaccine 21 days apart between 6-7months of gestation. Group CON (n=20) received saline solution and acted as the control group. After calving, all calves nursed colostrum from their dams without assistance. Serum and nasal secretion samples were collected from all calves at 48 hours and 30 days of age to evaluate initial titer and decay of BRSV antibodies. All calves were early weaned at 90 days of age. After weaning, calves were experimentally challenged with BRSV by intranasal inoculation. Serum and nasal secretion samples were collected from all calves immediately before challenge (day 0) and on days 2, 4, 6, 8, 10, 14, 21 and 28 after challenge for evaluation of BRSV shedding and specific BRSV antibody responses. Clinical evaluation and scoring on respiratory signs were performed in all calves.

Results

A greater proportion of Group CON (60%) calves developed fever (> 39.5 ºC) compared with VAC calves (36.8%). On day 21 after challenge, the proportion of calves with abnormal respiratory scores was greater in CON calves compared with VAC calves (85% vs. 73%, respectively). The mean Log2 BRSV-specific antibody titers in serum at 48h and before challenge was greater VAC calves (6.2 +/- 0.5 and 5.35 +/- 0.6) compared with CON calved (4.7 +/- 0.5 and 4.2 +/- 0.5). A greater number of Group CON shed BRSV in nasal secretions compared with VAC (5 vs. 1). After challenge CON calves had higher levels of BRSV IgA in nasal secretions compared VAC calves.

Conclusions

Vaccination of beef cows during pregnancy with 2 doses of an inactivated-BRSV vaccine provides clinical protection of calves against experimental challenge with BRSV through passive immunity.

Financial Support
Auburn University

Notes:

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

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Session: VACCINOLOGY - CATTLE

Objective

Determine whether immunization of calves with novel BVDV mosaic antigens will confer broad protection against diverse BVDV strains.

Methods

Bovine Viral Diarrhea Virus (BVDV) plays a role in causing Bovine Respiratory Disease Complex (BRDC) in cattle. The study goal was to evaluate cross-protection potential of a prototype vaccine containing recombinant mosaic BVDV antigens. Three mosaic antigens [$E2^{123}$, NS2-3[1], and NS2-3[2]] were expressed in Expi-293F cells and the $E2^{123}$ antigen was also expressed in Hi-5 insect cells. The antigens contain conserved protective epitopes from BVDV-1a, -1b, and -2, as well as unique neutralizing epitopes from disparate strains. Affinity purified proteins were used to formulate two prototype vaccines, using MONTANIDE ISA 201 VG adjuvant, which were tested for immunogenicity and protective efficacy in calves. The positive control was ViraShield commercial BVDV killed vaccine and the negative control was an irrelevant antigen.

Results

Both prototype vaccines induced significantly ($p<0.05$) higher BVDV-1- and -2-specific IFN-γ-secretion compared to the commercial vaccine. The 293F-expressed antigens elicited virus neutralization (VN) titers against BVDV-1 and -2 strains, whereas the Baculovirus-expressed $E2^{123}$ antigen mostly generated VN titers against BVDV-1 strains. Upon challenge, the calves immunized with the 293F-expressed antigens had delayed fever, were protected from leucopenia and had significantly delayed and lower viremia compared to the calves immunized with the commercial vaccine. Similar outcomes were observed in the calves immunized with the Baculovirus-expressed $E2^{123}$ antigen. Overall, the 293F-expressed antigens induced robust BVDV-specific cross-reactive IFN-γ responses, broadly VN antibodies and significantly reduced viremia and BVD disease compared to the commercial vaccine. The Baculovirus-expressed $E2^{123}$ antigen was not as effective as the 293F-expressed antigens, but it protected calves from BVD disease better than the commercial vaccine.

Conclusions

The findings supports development of a broadly protective subunit BVDV vaccine.

Financial Support
USDA National Institute for Food and Agriculture

Notes:

520 - Efficacy of blocking IBR viral circulation using a double-deleted gE-/tk- vaccine on a dairy farm

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Session: VACCINOLOGY - CATTLE

Objective

This study aims to evaluate the efficacy of blocking virus circulation using HIPRABOVIS® IBR MARKER LIVE vaccine under field conditions.

Methods

In November 2017 and February 2020, blood samples were collected from the animals at random, selected by lactation number, to analyse IBR prevalence. BoHV-1 gE antibody response was tested using a commercial ELISA kit. The sample size was considered representative, with blood samples taken from over 5% of the total number of animals. By examining the IBR prevalence by lactation number, it is possible to evaluate changes in viral circulation over time.

Results

The serology obtained in early 2016, at the time of the outbreak, clearly indicated that only the calves were infected, while the adult herd remained negative. In November 2017, most of these infected animals had already calved once. This explains why the results show a prevalence of 41% at first lactation, while younger and older animals were negative. Taking the normal dairy production system into consideration, the animals that were infected in the past should have had three calvings by February 2020. The serology results show that the three-lactations group was the only group with IBR-positive animals. The samples also show that total IBR prevalence decreased from 50% in February 2016 to 11% in November 2017 and 5.2% in February 2020. This improvement could be due to the slaughter of IBR-positive animals with bad production and reproductive performance over this period.

Conclusions

By analysing the serology results from this farm, we can state that the animals infected in 2016 retained their positive status without infecting new animals over the years. On this farm, the IBR virus has been blocked in those animals since its appearance in early 2016. From the results of this field study, we can conclude that the application of the HIPRABOVIS® IBR MARKER LIVE vaccine every 6 months achieved the primary objective of IBR vaccination, blocking viral circulation and preventing new infections.

Notes:

521 - Impact of vaccination with HIPRABOVIS SOMNI/Lkt on ADG, days on feed, mortality and treatments associated with BRD

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Session: VACCINOLOGY - CATTLE

Objective

The aim of this study was to evaluate the impact of vaccination with HIPRABOVIS SOMNI/Lkt on different production parameters in a feedlot.

Methods

440 beef calves from one feedlot were included in the study. Animals of similar weights were grouped into batches of 25-30 per pen. Pens were randomly allocated into “control” or “vaccinated” groups.
233 calves were vaccinated with HIPRABOVIS SOMNI/Lkt and 207 calves were used as a control. Data from 25 animals were excluded. Therefore, data from 226 and 189 calves were included in the vaccinated and control groups, respectively. Upon arrival, all animals in both groups received metaphylaxis (tulathromycin) and were vaccinated with a polyclostridial vaccine and a multiviral vaccine containing BRSV, IBR, BVD and PI3, with a second dose of the vaccines 3 weeks later. The vaccinees also received HIPRABOVIS SOMNI/Lkt on arrival and 21 days later.
Data was collected during the fattening period, including individual weights at entrance and slaughter, days on feed, mortality and its cause (BRD/not BRD associated), and treatments for BRD. Data analysis was carried out with R® Studio.

Results

The ADG was 0.214kg/d higher in the vaccinated group (1.394±0.261kg/d) than in the control group (1.180±0.223kg/d). The difference was significant ($p<0.001$).
The animals in the control group gained 249.86kg (±67.61SD) and the vaccinees gained 249.60kg (±48.13SD). However, in average, vaccinated animals spent 31 days less on feed than animals in the control group (180 days ±18SD vs. 211±43SD, respectively).
There were nine deaths due to BRD in the control group (4.8%) while there were none in the vaccinated group. Therefore, the difference of mortality due to BRD was significantly higher ($p=0.001$) in the control group than in the vaccinated group.
The antibiotic treatments for BRD were significantly ($p<0.001$) reduced in the vaccinees (0.4%) compared to the control group (16.9%).

Conclusions

The results herein demonstrate vaccination with HIPRABOVIS SOMNI/Lkt is cost-effective and can decrease the impact of BRD in fattening units.

Notes:

522 - Efficacy in the reduction of IBR prevalence when Hiprabovis® IBR marker live vaccine was used after an outbreak.

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Session: VACCINOLOGY - CATTLE

Objective

Since 2015, a dairy farm in Portugal has been monitoring IBR prevalence, the young animals being negative and the adult animals positive by bulk milk tank (BMT). Because of the high density, some of the young animals became infected with IBR. For this reason, the farmer decided to rear the post-weaning animals on another farm and switched from a killed IBR marker vaccine to a live one, namely HIPRABOVIS® IBR MARKER LIVE. The objective of this field study was to analyse the efficacy of the virus reduction when the live vaccine was used.

Methods

As part of routine practice in the control of IBR, a bulk milk tank (BMT) was analysed four times a year using a very sensitive commercial ELISA kit testing for BoHV-1 gE antibodies. Young animals over 6 months of age were tested by means of serology twice a year, avoiding interference with maternal antibodies. Since May 2016, the farm has been using the live IBR vaccine HIPRABOVIS® IBR MARKER LIVE.

Results

In 2015, the BMT test was positive in the adult animals and negative in the serologies obtained from the animals between 6 and 12 months. During 2016 and 2017, these young animals became positive, showing a prevalence of 41% and 22.7%, whilst the BMT was negative. It was in 2018, in the first calving period of the animals infected in 2016, when they joined the milking herd, that the BMT showed positive again for a year. On the other hand, during this year, the young animals tested were all negative, and are currently still negative.

Conclusions

Since the infection in 2016 when the young animals tested positive, the virus circulation has been reduced. In 2017, the prevalence was reduced and there were no new infections found in this group of animals in the following 2 years. In conclusion, with these results it can be assumed that the objective of IBR vaccination – which is to prevent virus circulation – was achieved with the vaccine HIPRABOVIS® IBR MARKER LIVE. It is not only vaccination to control IBR which is important, but also monitoring of the herds to determine the performance of the vaccination protocol.

Notes:

523 - Bovine immune response to *Leptospira* infection and vaccination: antigen delivery in novel adjuvants and platforms

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Session: VACCINOLOGY - CATTLE

Objective

Leptospirosis is a zoonotic bacterial infection that affects both cattle and man globally. In cattle, leptospirosis is a leading cause of reproductive failure. The culmination of over 5 years of research, 2 University collaborations and 3 completed studies have led to 1) characterization of the bovine immune response to Leptospirosis bacterin vaccination protocols and experimental infection, and 2) to enhancement of immune response, and probable vaccine efficacy, by modifying vaccine adjuvants.

Methods

Novel adjuvants were chosen for their additional properties such as shelf life and stability, single dose administration or enhancement of cellular responses. Using standard methodology immune responses were measured to vaccination and infectious challenge.

Results

Results demonstrate that effective vaccines elicit the cytokine IL-17, as well as IFN-gamma, in memory T cells not in circulating PBMCs but also in local lymph nodes. An increase in gamma-delta T cells was observed following infection which is hypothesized to clear the bacteria from the bloodstream and resident tissues (kidneys and reproductive organs). Additionally, novel adjuvants performed equal or superior to current vaccines in eliciting the measured immune responses.

Conclusions

This examination of immune response allows for some key characteristics to be elicited by protective vaccines, thus new constructs can be evaluated without the personnel risk of infectious challenge. These novel adjuvants could enhance vaccine design and development around the world in creating better vaccines not just for leptospirosis but other bacterial livestock diseases.

Notes:

524 - Probiotic lactobacilli enhance immunogenicity of an inactivated H9N2 influenza virus vaccine in chickens.

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Session: VACCINOLOGY - POULTRY

Objective

Avian influenza viruses (AIVs) infect a wide range of hosts, including humans and many avian species. Efforts have been made to control this pathogen in chickens using vaccination programs, but that has been met with varying degrees of success. Therefore, identification of more efficacious vaccination strategies is warranted. This study was undertaken to investigate the potential effects of probiotics on the immunogenicity of a beta-propiolactone-whole inactivated virus (WIV) vaccine of H9N2 subtype adjuvanted with the Toll-like receptor-21 ligand, CpG oligodeoxynucleotides 2007 (CpG).

Methods

Eighty-four 1-day-old White Leghorn layers were allocated into six groups. Two out of six groups received a mixture of probiotic *Lactobacillus* spp. (PROB) biweekly from days 1-35 of age. Chickens were intramuscularly vaccinated with WIV either alone or adjuvanted with AddaVax™ (WIV+Add) or CpG (WIV+CpG), one group received saline (phosphate-buffered saline). Primary and secondary vaccinations occurred at days 14 and 28 of age, respectively.

Results

The results revealed that the group that received probiotics and was vaccinated with CpG-adjuvanted WIV H9N2 vaccine had higher hemagglutination inhibition titers than the other treatment groups at days 14 and 21 postprimary vaccination. Probiotics did not induce higher IgM or IgY titers in chickens receiving the WIV vaccine only. Concerning their effect on cell-mediated immune responses, probiotics enhanced interferon-gamma (IFN-γ) gene expression and significantly increased secretion of IFN-γ protein by splenocytes in chickens vaccinated with CpG-adjuvanted WIV H9N2.

Conclusions

Together, these findings suggest the use of probiotics to enhance the immunogenicity of CpG-adjuvanted WIV H9N2 vaccines. Additional studies are required to better understand the specific interactions between probiotics and the gut microbiota and different types of cells of the gastrointestinal tract to decipher the underlying mechanisms of how probiotics modulate immune responses to vaccines.

Financial Support
Agriculture and Agri-Food Canada; Egg Farmers of Canada; Canadian Poultry Research Council; University of Guelph

Notes:

525 - Combination of probiotics and vaccination for enhancing protection against Marek's disease virus infection

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Session: VACCINOLOGY - POULTRY

Objective

Although several Marek's disease (MD) vaccines have been used to control MD in chickens, the emergence of new strains of Marek's disease virus (MDV) causes a threat to vaccine protection. Therefore, the objective of the current study was to investigate whether administration of probiotics improves the efficacy of Herpesvirus of turkeys (HVT) vaccine to provide protective immunity against MDV infection.

Methods

A probiotic cocktail comprised of four Lactobacillus species was administered with HVT vaccine at embryonic day 18 (ED18) and/or from day 1 to day 4 post-hatch chicks. At day 5 post-hatch, spleen, cecal tonsils (CT) and bursa of Fabricius (BF) were collected to evaluate the composition of various cell types by flow cytometry. Subsequently, chicks were infected with MDV. At 4, 10- and 21-days post-infection (dpi), spleen and CT were collected to investigate the cytokine gene expression and feathers were collected to determine the MDV genome copy numbers. At 21 dpi, tumor incidence and lesion score were recorded.

Results

Following administration of probiotics with HVT at ED18 and oral gavage of probiotics from day 1 to day 4, the increased expression of major histocompatibility complex (MHC)-II on macrophages and B cells in spleen was observed. Interestingly, the number of CD4+CD25+ T regulatory cells was significantly reduced in spleen. Following infection with MDV, the expression of interferon (IFN)-β in CT and INF-α in spleen was increased. Moreover, the expression of tumor growth factor (TGF)-β was also reduced in the group that received both vaccine and probiotics at ED18. In addition, concurrent probiotics administration has also reduced tumor incidence by half when compared to HVT vaccine only.

Conclusions

Administration of probiotics modulated immune responses and moderately improved the efficacy of HVT, marked by reducing tumor incidence in MDV infected chickens. The findings of this study suggest the potential use of probiotic lactobacilli as adjuvants with HVT vaccine against MDV infection in chickens.

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:

526 - Evaluation of host responses induced by two infectious bronchitis vaccination strategies in laying hens

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Session: VACCINOLOGY - POULTRY

Objective

Vaccination is practiced to control infectious bronchitis (IB) in both broiler and layer industries. Live attenuated and inactivated IBV vaccines are commonly used at various combinations to control IB. The objective of the study was to compare two vaccination strategies adapted by the Canadian poultry industry in terms their ability to induce immune response in various tissues in laying hens.

Methods

Two-weeks old specific pathogen free White Leghorn layer chickens were divided randomly into three groups. The Group 1 (n=5) was vaccinated with IB live attenuated vaccine, Mass serotype at 3, 8, 12 and 16 weeks of age via eye drop route with Mass and Conn combination vaccine given at 5 weeks of age via eye drop route. The Group 2 (n=7) was given the same vaccines as Group 1 except the 16 weeks age vaccination. For the Group 2, inactivated (Mass) vaccine was given at 16 weeks of age. The Group 3 (n=5) was remained as mock vaccinated controls. Blood was collected at 3 weeks following the last vaccination. At peak of lay, chickens in all 3 groups were euthanized and blood, reproductive tract washes and tissues (lung, trachea, kidney, cecal tonsil and spleen) were collected. Blood and reproductive tract washes were used to quantify anti-IBV antibody response using a commercial enzyme link immunosorbent assay (ELISA) kit. Spleens were used for isolation of mononuclear cells and quantification of CD4+ and CD8+ T cells using flow cytometry technique. Tissues collected to quantify immune genes and immune cell recruitment.

Results

We found, the chickens that received combination of live attenuated and inactivated vaccines (Group 2) induced significantly higher anti-IBV antibody response systemically ($P<0.0001$) whereas differences were not observed in spleen T cell response ($P>0.05$). This is a work in progress and we are in the process of determining the recruitment of T cell subset in reproductive tract mucosa, kidney and lungs.

Conclusions

The generated data will be useful in informing table egg layer industry of the choice of IB vaccination strategies that protect laying hens.

Financial Support
Alberta Agriculture and Forestry; Egg Farmers of Canada

Notes:

527 - MDV vaccination monitoring with feather pulp and spleen in (layer/breeder) by POCKIT™ central PCR system

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Session: VACCINOLOGY - POULTRY

Objective

Marek's Disease (MD) causes serious economic problems especially to the long-living birds and vaccination with CVI988/Rispens is considered the "gold standard" for prevention. MD vaccination is applied subcutaneously at day of age at the hatchery. Inappropriate storage/reconstitution and incorrect vaccination results in sub-optimal vaccinal protection of flocks. The on-site fully automated POCKIT™ Central PCR system is suitable for timely detection of MD vaccination effectiveness/uniformity without skilled personnel and specialized high-cost equipment. In this study, we evaluated the feasibility and POCKIT™ Central provides a good indicator for vaccination effectiveness/uniformity.

Methods

Spleen and Feather pulp were collected from individual chick and labeled in pair. At each check point, 10 chicks were sampled from 25,000 flock size at DOC, Day 3, 7, 10, 14, and 21. Less than 40 mg spleen were homogenized in the tube with 500 µl taco™ Sample Storage Solution and 3 to 5 feather pulps were homogenized in another tube with 500 µl taco™ Sample Storage Solution. For each test, 200 µl sample were used for evaluation and the positive rate were recorded. The virus titer were checked with real-time PCR system in parallel.

Results

MDV post vaccination uniformity check showed the uniformity can be evaluated with POCKIT™ Central as early as 7dpv with positive rate up to 100%. The MDV titer gradually increased in feather pulp while the MDV titer remain constant in spleen. Vaccination uniformity can be varied from batch to batch. In this case, post vaccination uniformity check is necessary every batch to ensure the quality of vaccination.

Conclusions

In summary, POCKIT™ Central allows on-site MD vaccination uniformity check and gets the results in 85 minutes without skilled personnel and specialized high-cost equipment. This application allows the farmer to check the vaccination procedure once the uniformity is poor. Both spleen and feather pulp are ideal sample type, but feather pulp is even better because of less invasiveness.

Notes:

528 - Recombinant attenuated *Salmonella* vaccines reduce *Campylobacter* colonization and induce IgY antibodies in chickens

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Session: VACCINOLOGY - POULTRY

Objective

Campylobacter is the most common bacterial cause of human gastroenteritis worldwide, making it a major food safety and public health concern. Preharvest control is critical because studies show a direct link between the high load of *Campylobacter* in the chicken intestinal tract and the high contamination of poultry carcasses. However, there are no commercially available vaccines or other antibiotic-independent competitive exclusion products for reducing *Campylobacter* under production conditions. In this study, we analyzed the ability of *Salmonella* Typhimurium derived recombinant attenuated *Salmonella* vaccines (RASV) to significantly reduce the colonization of *C. jejuni* and induce an immune response in chickens.

Methods

One-day-old *Salmonella* and *Campylobacter* free chickens were orally immunized with RASV constructs ($2x10^8$/ bird) and a booster was given ten days later. Birds were challenged with a cocktail of 5 *C. jejuni* strains on day 18. Colonization load was determined by enumerating *C. jejuni* in the cecum of the birds. Three trials were conducted analyzing the RASVs effect on *Campylobacter* load; Trail 1 examined the effect at 7 and 14 days post infection ($8x10^5$/ bird), trial 2 at 9 days post infection ($4x10^4$/ bird), and trial 3 analyzed the improved vaccines effect at 9 and 17 days post infection with a low ($1x\ 10^3$ per bird) and a high ($1x\ 10^4$ / bird) *C. jejuni* challenge dose. Blood serum was used to assess the levels of systemic *Campylobacter*-specific IgA and IgY antibodies in an indirect ELISA.

Results

Three vaccine constructs showed significant reduction of *C. jejuni* colonization; RASV-86 yielded the largest reduction (3.7 logs)17 days post infection. RASV-90/201 showed consistent reduction across trials 2 and 3 in low and high dose challenged groups. Additionally, in multiple trials RASV-90 showed a significant induction of IgY antibody titers compared to the untreated and unchallenged control group.

Conclusions

Our vaccine constructs are promising candidates for mitigating *C. jejuni* in birds and can potentially help reduce antibiotic resistant bacteria from entering the food chain.

Financial Support
USDA National Institute of Food and Agriculture

Notes:

529 - Enhancing the production of type I interferon to create rationally-defined Marek's disease vaccines in chickens

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Session: VACCINOLOGY - POULTRY

Objective

Marek's disease (MD) is a lymphoproliferative disease of poultry caused by Marek's disease virus (MDV), an alphaherpesvirus. MD is typically controlled by vaccination with the live attenuated virus strain CVI988 ("Rispens"). New and more virulent strains of MDV will evolve and break the protection provided by the CVI988 vaccine. We propose to identify and then ablate MDV viral gene products which inhibit or ablate the IFN-I response during infection. Our initial task will be to identify MDV gene products which inhibit the IFN-I response in an *in vitro* chicken macrophage model. We will use this new information to rationally design recombinant mutants of MDV which are less able to inhibit the production type I IFNs than the wild type MDV. We postulate that these recombinant strains will result in the production of greater amounts of type I IFNs both *in vitro* and *in vivo*, which will attenuate their pathogenicity and increase their immunogenicity, resulting in improved vaccinal protection. Our collaboration with the Boeke laboratory (NYU-Langone) will give us access to rapidly-assembled MDV viral genomes. This project will result in new and more protective vaccine strains for MDV.

Methods

In the initial stages of this proposal we will use CRISPR/Cas9 to construct an HD-11 cell line which expresses a fluorescent protein under control of the native IFNB/IFNW1 control elements. This recombinant cell line will be used to test expressed MDV genes in isolation for their ability to ablate the IFN-I response. These genes will be subjected to random mutatagenesis by the Boeke laboratory, which will again be tested in our system.

Results

Project is scheduled to initiate on July 1, 2020.

Conclusions

We anticipate that this project will result in several new and highly effective vaccine candidates for MDV, suitable for commercial use.

Financial Support
USDA National Institute of Food and Agriculture

Notes:

530 - Vaccinating chickens with crude *Clostridium perfringens* sporulation product reduces necrotic enteritis

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Session: VACCINOLOGY - POULTRY

Objective

Necrotic enteritis (NE) is a prevalent and costly intestinal disease in chickens, of which the main causative pathogen is *Clostridium perfringens*. Enterotoxin (CPE) is responsible for *C. perfringens*-mediated enteritis/diarrhea, which is produced and released only when *C. perfringens* sporulates in the intestine in response to stressed conditions.

Methods

We hypothesize that *C. perfringens* sporulation and host inflammation mediate pathogen-induced enteritis. Vaccines produced from the sporulation medium of two *C. perfringens* isolates were labeled as CP1 and CP2. CPE presence, epithelial, and immune cellular toxicity were examined. Birds were vaccinated at d 0 and 10 with CP1 and CP2 native vaccine at three doses. Birds were then infected with 15,000 sporulated *E. maxima* M6 oocysts at d 18 and *C. perfringens* with 10^9 CFU/bird at d 23 and 24. Body weight was measured at d 0, 18, 23 and 25. Ileal tissue was collected at d 25 for analysis of histopathology and inflammation. Differences between treatments were analyzed using Prism 7.0 software. Data were also analyzed using the nonparametric Mann–Whitney U test.

Results

In vitro, the vaccine induced expression of p-MLKL in macrophage cells and more than 1000- and 400-fold increase of proinflammatory mRNA Il1β and Cxcl2, respectively. In vivo, birds grew comparably between different groups during the uninfected phase of d 0 to 18. Body weight gain in noninfected birds was significantly heavier compared to *E. maxima*-infected only and CP1-3 vaccinated birds during d 18 to 23. At the NE phase of d 23 to 25, NE birds significantly lost body weight compared to NC birds (-13.71 vs. 56.08 g/bird/day). Remarkably, birds vaccinated with CP2-3 were able to continue to significantly gain body weight compared to NE birds (13.25 vs. -13.71 g/bird/day), while birds vaccinated with other vaccine doses didn't gain significant body weight. Upon histopathology examination, we found that CP1-2 and CP2-3 attenuated acute NE-induced ileal inflammation.

Conclusions

In conclusion, vaccinating chickens with the crude *C. perfringens* sporulation product reduces chicken NE.

Financial Support
Arkansas Biosciences Institute; USDA National Institute of Food and Agriculture

Notes:

531 - Genomic comparison of *Mycoplasma gallisepticum* vaccine and virulent strains

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Session: VACCINOLOGY - POULTRY

Objective

Mycoplasma gallisepticum (MG) infection can lead to chronic respiratory disease in chickens and infectious sinusitis in turkeys. MG infection leads to economic loses for poultry producers due both to morbidity and mortality associated with infection but also due to restrictions imposed on poultry that test positive for the presence of MG. Currently both killed and live attenuated vaccines are available that work to varying degrees to lessen the morbidity and mortality associated with infection. However, neither current vaccines nor treatment with antibiotics prevent life-long colonization with MG, severely limiting the utility of current vaccines and treatments.

Methods

A comparison of MG genomes from 3 commercial vaccine and 2 pathogenic strains was performed to identify potential targets for vaccine development and improved diagnostic methods. Core genes were identified using the Roary Pan Genome Pipeline and pathogenicity genes and islands were identified using IslandViewer4.

Results

An analysis of core genes identified about 140 of the 750 to 800 genes of the MG strains as being present in all strains. This included ribosomal proteins, housekeeping genes, hypothetical proteins, and fibronectin binding protein PlpA. Although the MG VlhA protein family appears to have a role in host infection, no VlhA proteins were found in the list of core genes, likely due to the great diversity of *vlhA* genes between the different strains. Analysis for known virulence factors suggests that all MG strains carry a P80 lipoprotein family homologue. Further, two of the analyzed strains carry the hypothetical virulence factor RmuC inserted in conjunction with a transposase. All strains had at least one putative pathogenicity island identified, although many of the genes associated with the putative islands are hypothetical.

Conclusions

This work has identified several potential virulence genes common to all MG strains analyzed. This work further identified a set of core genes for further study as targets for improved vaccine development and improved differentiating diagnostic methods.

Financial Support
U.S. Department of Agriculture

Notes:

532 - Evaluation of immunity with the application of Newcastle inactivated vaccine in different doses to day-old chicks

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Session: VACCINOLOGY - POULTRY

Objective

Hipraviar® BPL2 is a commercially available Newcastle inactivated vaccine with a titre of $EID_{50} \geq 10^{8.0}$ at the time of inactivation and 0,5 ml dose, the high titre suggest the possibility of the use lower doses. The objective of this trial was to evaluate the immune response using of different doses after vaccination of day-old chicks.

Methods

Forty healthy White Leghorn layer-type Specific-Pathogenic-Free (SPF), mixed sex chickens were used. Ten more animals were also included to test the initial antibody responses and 5% of the animals were added due to losses associated with selection. Selected day-old chickens were stratified according to weight and allocated equally between 4 groups of 10 birds each. Groups A, B and C were vaccinated with doses of 0.1 ml, 0.2 ml and 0.5 ml of Hipraviar® BPL2 a respectively per bird. Group D was vaccinated with 0.2 ml of Placebo. The antibody response against NDV was measured weekly starting from 7dpv to 42dpv (days post-vaccination).
The Immune response was evaluated using the ELISA CIVTEST®AVI NDV and Haemagglutination Inhibition (HI). The interpretation for the ELISA was done using the Kit standard and for the HI an antibody titre of 16 and above (≥ 24) was considered positive.

Results

The positivity at 21dpv and 42dvp in Groups A and B was 80% and 70%, respectively, using CIVTEST®AVI NDV and was 20% for Group A and 10% for Group B using HI at 21dpv (Group A had 80% and Group B 70% at 42dpv).
Group C, using the CIVTEST®AVI NDV and HI, did not have negative seroconversion at 42 dpv.
The trial shows, using the CIVTEST®AVI NDV that the titre at 21 dpv for group A was 2406, group B was 1670 and group C was 4947. The HI titre for group A was 3.76, group B it was 2.95 and 5.55 for group C.

Conclusions

All the vaccinated groups showed significant seroconversion. The titres detected in groups A and B indicate the ability of the vaccine to generate a dose-dependent seroconversion. The negative seroconversion on HI in samples from Groups A and B indicates a challenge to vaccine application in lower doses as some of the birds did not seroconvert later on.

Notes:

533 - The role of exosomes in Marek's disease virus lymphomagenesis and immunity

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Session: VACCINOLOGY - POULTRY

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 this 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

To address these hypotheses, we have purified exosomes from the serum of tumor-bearing, vaccinated/challenged, and vaccinated/not challenged chickens using size-exclusion chromatography. These were characterized by TEM, nanotracking analysis (NTA) and protein expression, as well as whole transcriptome sequencing. Transcripts were mapped to the chicken, MDV and other viral genomes. In addition, proteomes were also mapped to the chicken and viral genomes. MicroRNA target pathways and proteomic data were mapped to KEGG pathways. Purified exosomes were labeled via CFSE for examining uptake by macrophages and dendritic cells.

Results

Our data show that vaccine-associated exosomes (VEX) contain microRNAs that target proliferation-associated pathways and mRNAs expressed from the whole MDV genome. Tumor-associated exosomes (TEX) however show miRNAs that target phosphatidylinositol signaling and mRNAs expressed from oncogene-associated regions of the virus.

Conclusions

We found that VEX may be key to systemic anti-viral and anti-tumor immunity through providing viral mRNAs in the absence of vaccine virus replication. Additionally, we are gaining insight into TEX-affected pathways likely to be important to MDV-mediated immune suppression and lymphoma progression.

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

Notes:

534 - Recombination signals present in Infectious Laryngotracheitis Virus from Canada involves routinely used vaccines

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Session: VACCINOLOGY - POULTRY

Objective

Infectious laryngotracheitis virus (ILTV) causes acute upper respiratory infection in chickens affecting weight gain, egg production and respiratory clinical signs. Live attenuated vaccines are routinely used for the control of infectious laryngotracheitis (ILT). Recombination involving vaccinal ILTV strains has been increasingly documented, and such recombinant ILTV isolates have been linked with increased pathogenicity. In this study we looked for recombination signals between vaccine and wildtype ILTV strains present in Canada

Methods

Nucleic acid was extracted from diagnostic samples (n=58) from the Canadian provinces of Alberta, British Columbia, and Quebec. Whole genome sequencing was performed with only 14 samples yielding whole genome sequences. Multiple sequence alignment and phylogenetic analysis were done with the Canadian and other 37 whole genome ILTV sequences from different geographic backgrounds downloaded from public domain. Canadian ILTV sequences were analyzed for potential recombination events, using Recombination Detection *Program* (RDP)4 software and Simplot software.

Results

Evidence of recombination was found in an ILTV sequence belonging to British Columbia involving two ILTV attenuated vaccine strains. This analysis suggested that these two vaccine strains are possible parental strains of the British Columbia ILTV isolate. A second recombination event was identified between another Canadian ILTV sequence and a vaccine ILTV sequence of Australian origin.

Conclusions

Circulating vaccinal and wildtype ILTV strains can recombine complicating vaccine-mediated control of ILT. Further, pathogenicity studies of the ILTV strains including the recombinant ILTV isolate are underway.

Financial Support
Alberta Agriculture and Forestry; Egg Farmers of Canada

Notes:

535 - Comparison of fertility after application of two salmonella vaccines containing different types of oil emulsion

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Session: VACCINOLOGY - POULTRY

Objective

Inactivated vaccines containing bacteria are naturally more reactive compared to vaccines that contain viruses due to the structural pathogen's composition. For this reason, the type of adjuvant and emulsion technology contained in a Salmonella vaccine are extremely important to modulate inflammation at the application site and activate the immune response, all in balance. This is especially important when it comes to breeding flocks, since vaccine application is done in a delicate moment in sexual development.
The objective of this study was to compare fertility rate after application of two salmonella vaccines containing different types of emulsion.

Methods

The trial was conducted in a commercial breeder farm. A total of 108000 females + 15600 males were included in each group, under same nutrition, management and environmental conditions. All animals were vaccinated at 19 weeks of age. One group was vaccinated against Salmonella SE and ST in water-oil-water double emulsion (Avisan® Secure). The other group was vaccinated against Salmonella SE and ST with a single oil emulsion.
Four trays containing 96 eggs were collected per batch per week, for 40 weeks. The fertility rate was assessed by breaking hatching eggs incubated for 3 to 5 days and individually examining their air chamber.

Results

Differences in the fertility rate were observed between the two groups studied throughout the experimental period. The greatest differences were seen up to 10 weeks after vaccination: Avisan Secure Group showed +14,8% at 25 w of age, +12,6% at 26 w of age, +5,95% at 27w of age and at+5,99% at 28w of age.

Conclusions

The most important point related to flock fertility is the growth profile of the males. Any situation that causes stress and consequently weight loss between 16 and 22 weeks of age will directly affect testicular development and weight uniformity.
In the conducted study, relevant differences in fertility were observed and it was demonstrated that the type of emulsion is important when it comes to vaccination management of breeding flocks.

Notes:

537 - Immune responses in pigs following repeated intrauterine vaccination and passive protection in piglets.

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Session: VACCINOLOGY - SWINE

Objective

Evaluate the efficacy of a multi-valent subunit vaccine given via the intrauterine (i.u.) route alone (at the time of insemination) or in conjunction with intramuscular (i.m.) route to induce antigen specific immune response in pigs.

Methods

Experimental pubertal gilts (n=8) in estrus were mock-bred with killed sperm plus i.u. vaccine comprised of F antigen (Lawsonia) and P antigen (Porcine Endemic Diarrhea virus), each formulated with a triple adjuvant (TriAdj). At subsequent estruses, gilts were bred with killed (2nd dose) and live (3rd dose, Day 0) sperm plus P antigen and F antigen with TriAdj by i.m. route. Control group gilts were given PBS (i.u. mock, n=8). Blood samples taken on Day 93, colostrum on the farrowing day and post-slaughter uterine tissue on Day 124 were used to measure the humoral and cell-mediated responses. Piglets (3-d age/21-d age) from gilts of all groups were challenged with PEDV/Lawsonia, respectively, and assessed for weight loss and viral shedding pre and post-challenge.

Results

We observed a significant increase in the P antigen- specific serum and uterine antibody titres, F-specific cell-mediated immunity; and P- and F-specific colostral antibodies in the experimental versus control gilts. Survival scores were higher and PEDV shedding in feces were lower in the piglets born to i.u/i.m. vaccinated gilts compared those born to control gilts. Similarly, Lawsonia challenge showed reduced fecal shedding at days 14 and 19 in the piglets born to i.u/i.m vaccinated. Piglets born to vaccinated gilts had significantly lower birth weight but had weaning weights similar to the piglets of control groups suggesting compensatory growth.

Conclusions

Our results indicate that i.u. /i.m. immunization of a multivalent vaccine initiated humoral and cell-mediated immune responses. Importantly, there was limited but quantifiable passive transfer of immunity to the piglets.

Financial Support
Alberta Agriculture and Forestry; Saskatchewan Agriculture Development Fund

Notes:

538 - Novel Pseudorabies Virus (PRV) vectored bivalent vaccine against Classical Swine Fever and Porcine Circo viruses

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Session: VACCINOLOGY - SWINE

Objective

Classical swine fever (CSFV), porcine circo 2b, and pseudorabies viruses (CSFV, PCV 2, and PRV) are important swine viral diseases worldwide. PCV2b, recently renamed as PCV2d is prevalent in domestic and feral pigs. Even though CSFV and PRV have been eradicated in commercial swine in the U.S. and many European countries, there is a constant risk in the US of PRV spillover from wild/feral pigs and accidental CSFV introduction. Therefore, Our main goal of this research is to develop a safe and protective PRV vectored CSFV and PCV2b subunit vaccine

Methods

We have constructed a triple gene-deleted PRV (PRV-TMV) in which thymidine kinase, gE and gG genes are deleted. PRV-TMV is safe, does not produce any clinical disease but induces a good neutralizing antibody response in piglets. Further, we incorporated a chimeric PCV2b capsid protein (CAP), and chimeric classical swine fever virus E2 and E^{rns} fused with granulocyte-macrophage-colony-stimulating factor (cErns) genes in the TK, gE, and gG-deletion sites, respectively. The resulting PRV TMV-CSFV-PCV2b subunit vaccine virus was characterized for its growth kinetics, chimeric protein expression, and processing *in vitro*. The stability of the vaccine virus was tested for chimeric protein expression after multiple passaging. Currently, we are in the process of testing the safety, two separate vaccine dose-specific immune responses, and the latency-reactivation properties of the vaccine virus in piglets.

Results

The results showed that the PRV TMV-CSFV-PCV2b subunit vaccine virus is stable, replicates with similar growth kinetics but with a slightly reduced titer in swine kidney (SK) cells.

Conclusions

This was the first year of the USDA/NIFA funded project. This year we will complete the latency-reactivation and CSFV/PCV2b-specific immune response in the vaccinated pigs.

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

Notes:

539 - Evaluation of antigen candidates for a *Lawsonia intracellularis* subunit vaccine

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Session: VACCINOLOGY - SWINE

Objective

Lawsonia intracellularis (*LI)* is an economically important bacterium that causes ileitis in pigs. Current vaccines for *LI* do not differentiate between infected and vaccinated animals (DIVA) which is beneficial for disease surveillance. Previously we identified 11 putative surface proteins that were bound by antibodies from infected pigs which could serve as antigens in a subunit vaccine. In this project we tested 5 of the 11 antigens, elucidated the immune response, and assessed whether the vaccines protected against challenge.

Methods

Two vaccines comprised of *LI* surface proteins were formulated. Pigs in Group 1 were vaccinated with Vaccine FOG (proteins F, O, G) and pigs in Group 2 were vaccinated with Vaccine CM (proteins C and M). Groups 3 and 4 were control groups. Experimental groups were vaccinated on day 0 and received a booster dose on day 14. Groups 1, 2, and 3 were challenged on day 27 with a $2.0x10^8$ dose of *LI* in gut homogenate. Group 4 did not receive the challenge dose. Blood was taken on days 0, 14, 27, and 45 for sera collection and peripheral blood mononuclear cells were isolated on day 27 for cytokine analysis. Fecal samples were collected every other day post-challenge for eight time points ending with euthanization on day 45.

Results

The serum antibody, mucosal antibody, and cytokine responses to our antigens were evaluated. We found proteins C and G had significant serum and mucosal antibody responses while proteins F, M and O only had a significant serum antibody response. No measurable cell-mediated immune response was detected amongst any animals for any antigens. qPCR analysis on fecal samples was used to quantify *LI* and showed that Vaccine FOG provided limited protection while Vaccine CM did not provide any protection.

Conclusions

This trial helped us identify promising vaccine candidates and narrow down our list of potential antigens. Future research will focus on identifying whether all three antigens in Vaccine FOG are required for protection.

Financial Support
Government of Canada; Saskatchewan Agriculture Development Fund

Notes:

540 - Investigating the effect of vaccination and diet on post-weaning *E. coli* diarrhea in pigs

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Session: VACCINOLOGY - SWINE

Objective

The objectives of this study were to 1) investigate the effects of a live *E.coli* vaccine on occurrence of diarrhea in nursery pigs and 2) examine if occurrence of diarrhea differs between pigs fed a more easily digestible high complexity (HC) diet or a less expensive low complexity diet (LC) with more plant-based protein that is associated with gut inflammation.

Methods

Two trials were performed at the Arkell swine research station (Guelph, Ontario), with 120 nursery pigs each. The pigs were assigned to 1 of 3 groups: 1) vaccinated, HC diet (VHC, n= 30), 2) non-vaccinated, HC diet (NVHC, n= 60), and 3) non-vaccinated, LC diet (NVLC, n= 30). The pigs were monitored for 5 weeks post-weaning. Fecal consistency was assessed weekly and pigs were considered to have diarrhea if they had loose or watery feces. Fecal swabs were collected and cultured to assess presence of enterotoxigenic *E. coli* (ETEC). A logistic regression method was used to investigate the occurrence of diarrhea and presence of ETEC from rectal swabs between the different groups and diets in trial 1.

Results

Trial 1: No significant difference regarding the occurrence of diarrhea in the pigs was identified between the 3 groups. NVLC pigs were more likely to test positive for presence of ETEC from rectal swabs compared to pigs in NVHC at 2 weeks (OR = 2.5, P = 0.049) and 5 weeks post-weaning (OR = 2.7, P = 0.034). Pigs in the VHC group were more likely to test positive for presence of ETEC from rectal swabs compared to the NVHC group at 2 weeks (OR = 5.0, P = 0.001) and 5 weeks post-weaning (OR = 3.5, P =0.007). Overall, ETEC was more likely to be isolated from pigs with diarrhea at 2 weeks post-weaning (OR = 2.7, p = 0.01).

Conclusions

The use of the *E. coli* vaccine did not appear to provide protection against post-weaning *E. coli* diarrhea and the less expensive LC diet did not increase occurrence of diarrhea under the conditions in this trial. Future studies could examine vaccine efficacy and diet using a controlled ETEC challenge.

Notes:

541 - A universal epitope-based *Streptococcus suis* vaccine induces strong mucosal IgA and serum IgG responses in piglets

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Session: VACCINOLOGY - SWINE

Objective

Streptococcus suis is an important pathogen of swine and an emerging zoonotic pathogen. *S. suis* infection commonly results in meningitis and septicemia leading to death. Co-infection commonly occurs with PRRSv or PCV2. *S. suis* is classified into sequence types (ST), with Types 2 and 3 being most prevalent. Here, using a comparative genomics approach, we developed a universal epitope vaccine based on our *Bacillus*-vectored production platform and tested the immunogenicity of the vaccine by multiple routes of administration.

Methods

Three-week-old pigs (n=30) from a high health farm with no previous *S. suis* concerns were transferred from a farm nursery to weaning boxes. The pigs were individually tagged, weighed, and randomized into one of 10 groups (2 replicates x 5 groups (3 pigs/group)) and allowed to acclimate for 2 days. Standard commercial feed and water were provided *ad libitum*. Treatment groups were as follows: intramuscular (IM), oral gavage (oral), nasal injection (nasal), IM/oral, and oral-saline. On Day 0 of the study, the first dose (2 ml) of treatment was administered. The second dose was administered 14 days later. Serum samples were collected on study days 0, 14, 24, 35. Oral fluids and intestinal sIgA were collected at necropsy. Animals were humanely euthanized.

Results

Vaccine specific IgG and sIgA antibody levels were measured using proprietary ELISAs. Vaccine administration by IM or IM/oral produced the strongest IgG response by D35. All routes of vaccine administration produced intestinal sIgA S/P ratios of >4 by D35. Pooled analysis of oral fluids determined the sIgA S/P was 1.7 (oral), 2.1 (nasal), 4.0 (IM), and 2.2 (IM/oral).

Conclusions

This novel, inactivated, *S. suis* vaccine-induced serum IgG and sIgA antibodies and was well tolerated regardless of the route of administration. Results show the vaccine is highly immunogenic. Further studies are planned to test the efficacy of the vaccine in a larger *S. suis* challenge trial and evaluate additional areas of the immune system for response and indications of protection.

Financial Support
Vetanco

Notes:

542 - Association of SLA haplotypes with B- and T-cell immune response to foot-and-mouth disease virus (FMDV) peptides

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Session: VACCINOLOGY - SWINE

Objective

Dendrimer peptides are a promising strategy to develop new vaccines against FMDV. Several B_2T dendrimers, harbouring the major FMDV antigenic B-cell site in VP1 protein, elicited consistent levels of neutralizing antibodies and IFN-γ-producing cells in pigs. The highly polymorphic nature of the swine leukocyte antigens (SLAs) allows for the presentation of a wide panel of antigenic peptides and thus influences vaccine responsiveness. SLA genotyping was performed in B_2T-immunized animals to assess possible correlations between particular SLA-I or SLA-II haplotypes with peptide-induced neutralizing antibody and T-cell responses.

Methods

Immune responses were assessed in 73 cross-bred Large White/Landrace pigs. SLA-I and SLA-II low-resolution haplotypes (Lr-Hp) were identified by PCR-SSP typing assay. Chi-square ($\chi 2$) correlation analysis, Cramer's value (Cv) and Fisher's exact test were performed.

Results

In 63 pigs immunized with FMDV B_2T dendrimers and 10 non-immunized control animals, 15 SLA-I and 16 SLA-II Lr-Hp were found, reflecting high SLA diversity in farmed pigs. Correlations of T-cell mediated immune response with SLA-I Lr-Hp and between B-cell antibody production and SLA-I or SLA-II Lr-Hp were only found when the sample was reduced to animals with Lr-Hp represented more than once. A significant association with the magnitude of T-cell responses was observed for Lr-Hp 22.0, 37.0, 1.0 and 59.0 (SLA-I) and Lr-Hp 0.15b and 0.27 (SLA-II). Likewise, Lr-Hp 22.0 and 59.0 (SLA-I) and Lr-Hp 0.27 and 0.15b (SLA-II) were found associated with high antibody titers.

Conclusions

The results of 63 immunized pigs point to a robust significant correlation between SLA-II Lr-Hp and T-cell response. Overall, these findings support the contribution of SLA-II restricted T-cells to the magnitude of the T-cell response and, slightly less significantly, to the antibody response evoked by the B_2T dendrimers. This is of potential value for vaccine design against FMDV, as Lr-59.0 and 22.0 (SLA-I) as well as Lr-0.15b (SLA-II) are highly abundant SLA low-resolution haplotypes in European farmed pigs.

Notes:

543 - Activation of porcine dendritic cells by a novel nanoparticle/poly(I:C) combination adjuvant

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Session: VACCINOLOGY - SWINE

Objective

Inactivated and subunit vaccines require adjuvants to induce optimal immune responses. There is a need to develop diverse vaccine adjuvants that can be used for alternative routes of delivery and to promote specific immune responses. The aim of this study was to investigate the adjuvanticity of a novel phytoglycogen-based nanoparticle (Nano-11) in combination with the Toll-like receptor 3 (TLR3) agonist poly(I:C) in porcine monocyte-derived dendritic cells (Mo-DCs).

Methods

The effect of adsorption of poly(I:C) on the size and surface charge of Nano-11 was determined. Monocyte-derived dendritic cells (Mo-DCs) were generated from the blood of domestic pigs and incubated with Nano-11, poly(I:C), or Nano-11/poly(I:C). The effect of the adjuvants on the viability of the cells was determined by the release of lactate dehydrogenase. RNA was isolated for sequencing, expression of costimulatory molecules was determined by flow cytometry, and secretion of TNF and IL-1β by ELISA.

Results

Adsorption of poly(I:C) on Nano-11 significantly increased the size of the nanoparticles with a moderately reduced positive surface charge. The Nano-11/poly(I:C) combination adjuvant was less cytotoxic to porcine Mo-DCs than poly(I:C) only. Nano-11 increased the expression of CD80/86, and this was further increased by the addition of poly(I:C). The Nano-11/poly(I:C) combination enhanced the secretion of TNF and IL-1β compared with either adjuvant alone. Differential gene expression analysis identified an increase in NF-kB and inflammatory pathways induced by Nano-11 and several signature pathways linked to host immune response to viral infections upon incubation with Nano-11/poly(I:C).

Conclusions

We have demonstrated that adsorption of poly(I:C) on Nano-11 enhanced the immunostimulatory effect on porcine Mo-DCs with minimal signs of cytotoxicity. These results support the development of Nano-11 and Nano-11/poly(I:C) as safe and effective vaccine adjuvants.

Financial Support
USDA National Institute of Food and Agriculture

Notes:

544 - Directing suicidal viral replication as a strategy for rapid attenuation

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Session: VACCINOLOGY - SWINE

Objective

With the increasing number of newly emerging infections, the development of improved strategies to shorten the lead development time for attenuating vaccine candidates has become critical. Porcine circovirus 2 (PCV2) is a small DNA virus which is economically important as the causative agent for postweaning multisystemic wasting syndrome (PMWS), in weanling piglets. Although a DNA virus, PCV2 has a mutation rate which is similar to that of RNA viruses, leading to frequent emergence of new subtypes in the field despite the availability of standard vaccines.

Methods

Using PCV2 as a model, in this study, we have explored a strategy for rapid attenuation of viruses which harnesses high viral mutation rates to target the premature termination of the gene expression during viral replication. The rapidly attenuated PCV2 vaccine candidate developed was successfully rescued by transfection.

Results

When tested in a piglet model, the test vaccine elicited strong binding and neutralizing antibody responses. Vaccinated pigs were completely protected against challenge with a heterologous PCV2d strain and had reduced lesion scores compared to pigs administered a commercial vaccine. Importantly, the test vaccine virus was cleared in vaccinated pigs within 2 weeks of exposure and did not produce tissue pathology in vaccinated pigs, indicating that it was both attenuated and safe.

Conclusions

This study is the first demonstration of an effective and safe rapid-attenuation strategy for rapidly mutating DNA viruses, with broad applicability to other animal viruses.

Notes:

545 - Enhancing oral delivery of a minimally replicative porcine epidemic diarrhea virus (PEDV) vaccine

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Session: VACCINOLOGY - SWINE

Objective

Porcine epidemic diarrhea virus (PEDV) is an economically important swine pathogen and the cause of acute diarrhea, vomiting, dehydration in neonatal piglets with mortality rates ranging between 80-100%. Inducing strong lactogenic immunity in pregnant sows by vaccination is critical for protection of neonates against PEDV. This project targets the development of enhanced oral delivery methods for a minimally replicative PEDV vaccine, which combines the safety and efficacy advantages of inactivated and attenuated vaccines.

Methods

The strategy for the development of the minimally replicative rapid-response PEDV vaccine consisted of exposing PEDV virions to low heat to reversibly denature the capsid, followed by digestion of the viral RNA with RNAase, and refolding of the structure at 4^{o}C. The proprietary “green” oral delivery method, designed to protect the vaccine antigen in its passage via the sows’ gastrointestinal tract and deliver the antigen to the enterocytes, was optimized by testing various antigen payloads with the combinations of the ingredients in packaging system using a solvent extraction process. The integrity of the packaging was assessed by a modified nin-hydrin assay and electron microscopy.

Results

The process for the development of the minimally replicative rapid-response vaccine was optimized such that viral replication was not detected until after 3 serial passages in cells while the viral structure remained intact. The oral delivery system was determined to package the virions at an efficiency rate of 50-70%, with the spike proteins projecting from the surface of the amphiphilic vesicles, and no significant cytotoxicity in Vero cells.

Conclusions

Following assessment of the delivery system’s ability to withstand acid and enzymatic degradation, the safety and efficacy of the oral vaccine delivery and vaccine will be tested in a pregnant sow model.

Financial Support
USDA National Institute of Food and Agriculture

Notes:

546 - Development and validation of a real-time RT-PCR for detection of PRRSGard®-like vaccine virus

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Session: VACCINOLOGY - SWINE

Objective

An increasing use of MLV vaccines and doubling of PRRSV PCR tests in last ten years bring challenges to interpret PCR-positive results. ORF5 sequencing or whole genome sequencing can distinguish vaccine-like from wild-type PRRSVs but is relatively expensive and time-consuming. Vaccine-specific PCR for detection of PRRSV vaccine strains is a convenient tool to inform vaccine protocols and differentiate strains. PRRSGard® is a chimeric marker MLV vaccine recently developed by Pharmgate. Here we report the development and validation of a PCR for specific detection of PRRSGard-like vaccine virus.

Methods

PRRSGard® has a unique 23-nucleotide insertion in the ORF1b/2 region. Primers and probe spanning this region were designed. Analytical specificity, sensitivity and diagnostic performance of PRRSGard PCR were evaluated and compared with a commercial PCR.

Results

PRRSGard and commercial PCRs did not cross-react with any of the 27 non-PRRSV swine pathogens. All five commercial MLV vaccines and 33 genetically diverse PRRSV-2 laboratory and field isolates tested negative by PRRSGard PCR but positive by commercial PCR. Sequencing the ORF1b/2 target region of these strains corroborated the PCR results. By testing the serially diluted in-vitro transcribed RNA, PRRSGard PCR and commercial PCR respectively consistently detected up to 10^-6 and 10^-5 dilutions. The limit of detection of PRRSGard PCR was 16.7 genomic copies/reaction with the Ct cut-off of 36. Diagnostic performance of PRRSGard PCR was evaluated using 717 serum samples sequentially collected from PRRSV naïve pigs receiving PRRSGard vaccine. Compared to the commercial PCR, diagnostic sensitivity, specificity, and accuracy of the PRRSGard PCR were 96.7%, 99.8%, and 98.7%, respectively.

Conclusions

In contrast to a commercial PCR that detects all PRRSV strains, the PRRSGard PCR specifically detects PRRSGard-like vaccine virus with comparable performances to the commercial PCR. PRRSGard PCR is a convenient tool to differentiate PRRSGard-like vaccine virus from other PRRSV strains and vaccines as well as to inform PRRSV vaccination protocols.

Notes:

547 - Porcine epidemic diarrhea vaccine evaluation using a newly isolated strain from Korea

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Session: VACCINOLOGY - SWINE

Objective

Evaluate vaccine developed with newly isolated porcine epidemic diarrhea virus

Methods

isolation and develope new PED vaccine

Results

The administration of the inactivated 65-passaged PED-CUP-B2014 to sows greatly increased the survival rate of their offspring and significantly reduced diarrhea severity after PEDV challenge. Higher serum/colostrum PEDV-specific antibodies and higher neutralizing titers were shown in sows vaccinated with PED-CUP-B2014 compared to unvaccinated sows or sows administered commercial PEDV vaccine.

Conclusions

newly isolated PEDV strain conferred critical passive immune protection to pigs against epidemic PEDV infection.

Notes:

548 - The effects of oral antibiotic administration on vaccine-induced H1N1 influenza immune responses in weanling pigs

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Session: VACCINOLOGY - SWINE

Objective

Influenza A virus (IAV) has significant economic impacts on swine production. Recent human studies show that broad-spectrum oral antibiotics before annual trivalent influenza vaccination decreases IgG production and long-term immunity to H1N1 influenza. This study aimed to measure the effects of concurrent oral antibiotic administration on cellular and humoral immune responses to IAV vaccination in swine.

Methods

20 weanling pigs negative for IAV antibodies at enrollment were used for the study. 10 pigs received 5 days of oral chlortetracycline (CTC) per label instructions (10mg/lb), and 10 controls received an equal volume of water. On d5, all received a killed H1N1 swine influenza vaccine per label instructions (FluSure® Pandemic, Zoetis); and were boosted 28 days later. Serum was taken biweekly for eight weeks and analyzed for homologous (CA-2009 pH1N1) antibody titers by hemagglutination inhibition assays. Peripheral blood mononuclear cells (PBMCs) were collected and stimulated with the homologous virus. Stimulated PBMCs were used in ELISpot assays to enumerate influenza-specific memory t-cells (IFNy) and cytokine response (IL-10) at d39 and d60.

Results

At the time of booster vaccine, 1 of 19 pigs had an HI titer of 80, with no detectable titers in 11 of 19 the pigs. On d39, 18/19 pigs had a three-fold or greater increase in HI titer with a mean of 942 for treatment and 824 for controls. On d60, 11/17 pigs had titers >2650, 1 at 1280, 1 at 640, 2 at 320, and 1 at 80 with means of 3450 for treatment and 4880 for controls.
There were no significant differences in mean INFy or IL-10 ELISpot counts between the antibiotic treatment and control groups at any time point. There were no associations between HI titer and INFy cell counts in individuals at 7 or 28 days following booster.

Conclusions

Our study found that concurrent chlortetracycline antibiotic administration at the time of influenza vaccination does not impair cellular or humoral immune responses in weaned pigs.

Notes:

549 - H1N1 G4 swine influenza T cell epitope cross-conservation in swine and human vaccines and circulating strains

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Session: VACCINOLOGY - SWINE

Objective

Pandemic influenza may emerge from animal reservoirs and spread among humans when there is poor antibody protection in the human population, as occurred in 2009 with the emergence of the swine origin H1N1 pandemic. Flu vaccines containing highly cross-conserved T-cell epitopes have been shown to reduce morbidity and limit spread in the absence of antibodies, even when vaccines and emergent strains are poorly matched. Here, we assess the risk of pandemic emergence of G4 in swine populations and the cross-protective potential of swine and human vaccine strains to limit spread using immunoinformatics tools.

Methods

H1N1 HA sequence data, including G4, swine, and human strains circulating in the US were retrieved. European swine flu strains were downloaded to assess their potential to protect the US swine population. Maximum-likelihood phylogenetic trees were constructed for each dataset. Phylogenetic Diversity Analyzer was used to subsample to preserve representative clades and each final reduced dataset combined the G4 data into three single analyses. Swine and human vaccine strains were compared to phylogenetic representative sequences from each host/region using the EpiCC tool which evaluates T-cell epitope relatedness between vaccine and circulating strains.

Results

European swine flu vaccines showed good T-cell cross-conservation with G4 swine virus suggesting that they may protect European swine against G4. T-cell epitope content of US commercial swine vaccines, however, is poorly conserved, indicating the US swine population may be susceptible to emergent G4 and may be protected by vaccination with a European swine flu vaccine. Human flu vaccines were also poorly conserved with G4, which compounds the absence of cross-protective antibody and suggests that G4 has potential to spillover to humans.

Conclusions

Emergent G4 influenza poses a greater threat to the US pork industry. The European swine vaccines could be tested for efficacy against G4. Emergent G4 virus also poses a threat to humans. Further studies are required to better predict the spread of G4.

Notes:

550 - Multifunctional Pathogen-Mimicking Vaccine Delivery System for Influenza Vaccine.

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Session: VACCINOLOGY - SWINE

Objective

The goal of this study is to develop a safe vaccine delivery technology that strongly stimulates the immune response against the Influenza virus. In this regard, previously we have discovered a novel polymer, Inulin Acetate (InAc), which mimics PAMPs as an agonist for TLR4 and stimulate anti-viral immunity. In this study, a particulate-based pathogen-mimicking vaccine delivery system (PMVDS) was designed with InAc as a polymer for the influenza subunit vaccine and tested in mice and pigs.

Methods

Encapsulation of antigen, hemagglutinin (HA)) from H1N1-A/California/07/2009, and M2e peptide (MSLLTEVETPTRNEWECRCSDSSD) in InAc particles was accomplished by double (w/o/w) emulsion-solvent evaporation method. Antigen loading was determined using HPLC analysis. The cellular uptake of PMVDS by dendritic cells was studied using flow-cytometry with FITC-labeled ovalbumin as an antigen. The ability of the PMVDS activating TLR4 was confirmed in murine macrophages. The immunizations were performed on 6-week old BALB/Jc mice (antibody titers), C57/B16 mice (Challenge), and 3-week old piglets. Serum antibody titers were tested using indirect-ELISA.

Results

InAc-PMVDS, which are spherical (1-2µm in diameter) increased the antigen uptake by dendritic cells. This PMVDS stimulated both humoral and cellular immune responses against the immunized antigen as shown by strong antibody and cytokine responses. In mice, PMVDS generated up to ~220 times greater antibody titers than an unadjuvanted antigen. The antibodies inhibited hemagglutination caused by the virus and prevented viral infection in the cell culture model. In mice, PMVDS protected 100% of vaccinated mice from a lethal challenge of influenza. In pigs, a significant increase in anti-HA IgG levels in the serum and IgA antibodies in the lung fluids was observed with InAc-PMVDS vs. an unadjuvanted group

Conclusions

InAc-PMVDS is a multifunctional, platform vaccine delivery system for viral vaccines based on a first plant polymer-based TLR4 agonist, which stimulated both humoral and cellular immunity.

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

Notes:

552 - BVDV compromises fetal immune organ development leading to post-natal predisposition to secondary infections

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Session: VIROLOGY

Objective

Infection of fetuses with BVDV permanently alters immune gene expression through epigenetic mechanisms causing impaired post-natal immune responses that lead to greater susceptibility to bovine respiratory disease (BRD).

Methods

Aim 1 focused on DNA methylation of persistently (PI) or transiently (TI) infected fetal tissues and was deleted due to reduction in the budget. Aim 2 examines epigenetic regulation of genes in postnatal tissues following PI or TI with BVDV. Aim 3 focuses on fetal TI with BVDV followed by a postnatal immune responses to a challenge with BRD at weaning, followed by study of growth to market weight and carcass quality. A combination of methylation

Results

Whole blood (EDTA) samples were obtained from 9 yearling Corriente yearlings including: 3 PI, 3 acutely/transiently infected and 3 uninfected control animals. PI animals were BVDV ACE positive test on ear notches, and RT PCR and virus isolation (VI) positive on whole blood samples. The acutely infected animals were BVDV ACE positive test on ear notches and BVDV RT PCR and VI negative on whole blood taken > 4 weeks later. Control animals were BVDV ACE negative, BVDV RT PCR and VI negative. DNA and RNA have been extracted from PBMCs.
Tissues for methylation studies in Aim 3 will be collected from heifers at slaughter in year 3 of the proposal. Unvaccinated, seronegative, BVDV antigen negative, weaned Hereford heifers were artificially inseminated with X-selected semen, checked for pregnancy at day 32 and sex of fetus on day 60 post-breeding by ultrasound. The first BVDV challenge on day 175 will occur in December.

Conclusions

The TI fetal BVDV model is in progress. The first TI tissue collections will occur in Fall 2021. Concluding statements will be made after the tissue samples have been collected and the analysis has been completed.

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

Notes:

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

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Session: VIROLOGY

Objective

Marek's disease (MD) is a devastating disease in the poultry industry caused by an oncogenic herpesvirus called MD virus (MDV). Though protective against the induction of clinical disease, current vaccines do not induce sterilizing immunity, nor block transmission, that has resulted in driving MDV to increased virulence over the last few decades. Virulent MDV spreads horizontally (chicken-to-chicken) more efficiently than vaccine strains, giving it a tremendous evolutionary advantage within a chicken house. Better strategies need to be developed to block circulation of virulent MDV in poultry houses by targeting transmission.
We have identified 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. We hypothesize that US10 phosphorylation by UL13 is required for incorporation of US10 as part of the MD virion that subsequently results in recruitment of LY6E. LY6E has been shown to enhance viral replication by mediating endosomal escape of the nucleocapsid following entry into cells. We surmise LY6E recruitment protects the MD virion from destruction within the newly infected cell. Importantly, chicken LY6E has been linked to genetic resistance to MD in chickens. To test our hypotheses, we will determine 1) the importance of US10 and its phosphorylation by UL13 for horizontal transmission and 2) the role LY6E plays in horizontal transmission and genetic resistance of chickens to MD. This project is funded through the USDA-NIFA-AFRI grant no. 2020-67015-21399.

Methods

Using recombinant MDV expressing fluorescent proteins and epitope-tagged viral proteins, we will study the interactions between UL13, US10, and LY6E during replication and horizontal transmission in chickens.

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

Notes:

554 - Small molecule inhibitors of rabbit hemorrhagic disease viruses

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Session: VIROLOGY

Objective

Rabbit hemorrhagic disease virus (RHDV) is a member of the *Lagovirus* genus in the Caliciviridae family and infect wild and domestic European rabbits. RHDV infection in rabbits leads to acute liver failure and death. RHDV are classified as RHDV1 and RHDV2 based on the serotype. RHDV1 has been circulating in rabbits since 1984, while RHDV2 was first identified in 2010. Currently, several US states have reported RHDV2 outbreaks in domestic and wild rabbits with a high mortality rate. Spreading of RHD in wild rabbits, unavailability of vaccines in the US and the lack of effective treatment make it challenging to control RHD. Thus, in this study, we screened a library of small molecule compounds against the 3C-like protease (3CLpro) of RHDV1 and 2 to identify inhibitors active against both viruses. The 3CLpro encoded by caliciviruses is essential for virus replication, thus an attractive target for viral inhibition.

Methods

We generated recombinant 3CLpros of RHDV1 and RHDV2 and determined the inhibitory potency of selected compounds using the florescence resonance energy transfer assay. The compounds library was previously generated. In addition, the 3CLpro sequences of RHDV and other lagoviruses in Genbank were used for multiple sequence analysis to investigate the 3CLpro homology among various lagoviruses.

Results

The expressed 3CLpro were proteolytically active in the FRET assay, and their enzymatic kinetics were similar between RHDV1 and RHDV2. Screening of small molecule compounds resulted in the identification of potent inhibitors of 3CLpro of RHDV1 and 2. The inhibitory activities of identified compounds were comparable between RHDV1 and RHDV2, which is in line with a high sequence homology of 3CLpro between RHDV1 and 2.

Conclusions

RHDV is an emerging disease in domestic and wild rabbits with significant ecological and economic impacts. Here, we screened and identified small molecule protease inhibitors with similarly potent activity against RHDV1 and RHDV2. The identified compounds may serve as the platforms for the development of effective treatment options.

Financial Support
USDA National Institute for Food and Agriculture; U.S. National Institutes of Health

Notes:

555 - Ectopic expression of genotype-1 *Hepatitis E virus* ORF4 increases genotype-3 HEV viral replication in cell culture

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Session: VIROLOGY

Objective

Hepatitis E virus (HEV) can account for up to a 25% mortality rate in pregnant women with highest incidences reported for HEV genotype-1 (gt1). Reasons contributing to adverse maternal-fetal outcome during pregnancy in HEV-infected pregnant women remain elusive in part due to the lack of a robust tissue culture model. Open reading frame (ORF4) was discovered overlapping ORF1 in gt1 HEV. ORF4 protein expression is regulated via an IRES-like RNA element. We sought to validate whether HEV gt3 contained a similar ORF4 to gt1 HEV and whether ORF4-mediated replication enhancement could be bestowed upon non gt1 HEV by providing ORF4 *in trans* and observing the replication and infectivity in gt3 HEV.

Methods

Huh7 cell lines constitutively expressing ORF4 were created and used to assess the replication of the Kernow-C1 gt3 and sar55 gt1 HEV in the presence or absence of ORF4. HEV infection was initiated in each cell line via transfection with *in vitro* transcribed capped HEV RNA and replication was assessed. Virus stocks from transfected Huh7 cells with or without ORF4 were harvested and infectivity assessed via infection of HepG2/C3A cells. An immunofluorescence assay (IFA) to detect ORF2 protein using flow cytometry was performed after harvesting cells at 5 days post transfection (dpt) and 7 dpt. Furthermore, we also detected gt1 (sar55) replication in tunicamycin treated ORF4 expressing Huh7 cells.

Results

Gt3 HEV lacks the IRES-like element RNA function and start methionine associated with ORF4 translation. Gt1 ORF4 protein expressed successfully through lentiviral transduction in Huh7 cells. High levels of ORF2 positive cells were seen in ectopically expressed ORF4 cell lines with Kernow P6 being the highest of all three strains. Enhanced fluorescence was seen in ORF4 expressing cell lines with all three strains of virus while maximum antigen was visualized for the P6 strain.

Conclusions

Enhanced viral replication of gt3 HEV was observed when ORF4 was provided *in trans*, suggesting the function of ORF4 protein from gt1 HEV is transferrable.

Financial Support
Ohio State University

Notes:

556 -

Matrix and nucleoprotein segments of a 2016 H5N8 HPAI virus are major drivers of high mortality in mallards

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Session: VIROLOGY

Objective

Highly pathogenic avian influenza (HPAI) viruses of the H5N8 subtype have caused major outbreaks affecting both wild and domestic birds. Typically, waterfowl are readily infected with HPAI viruses but show mild clinical signs. However, viruses from the 2016-2017 European H5N8 outbreak caused high mortality in waterfowl. We thus sought to determine which gene segments are implicated in the high mortality caused by H5N8 HPAI viruses in waterfowl.

Methods

We generated single gene reassortants ($H5N8_{SGR}$) between a H5N8 virus from 2016 that causes high mortality in mallards and a H5N8 virus from 2014 that does not. Groups of ten mallards were inoculated with the $H5N8_{SGR}$ viruses or with the recombinant parental H5N8 virus and monitored for 11 days. Oral and cloacal swabs were collected at various times. At 3 dpi, two mallards per group were necropsied and tissue samples were collected. Virus titers in swabs and tissues were measured using quantitative RT-PCR. Immunohistochemistry for AI antigen was also performed on tissues. To assess virion morphology, electron microscopy was performed on virus preparations.

Results

All mallards inoculated with the parental 2016 H5N8 succumbed to infection, which is consistent with previous findings. All $H5N8_{SGR}$ viruses were able to infect all mallards causing different degrees of morbidity and mortality compared to the parental virus. The $H5N8_{SGR}$ viruses containing segments 5 and 7, encoding nucleoprotein and matrix, had the lowest mortalities among $H5N8_{SGR}$ groups at 38% and 25% respectively. Furthermore, these two $H5N8_{SGR}$ had lower virus levels in tissues, indicating less systemic virus replication. Additionally, electron microscopy showed that the matrix segment from the 2016 H5N8 is associated with filamentous virion morphology.

Conclusions

We have shown that that multiple gene segments contribute to increased morbidity caused by H5N8 HPAI viruses in mallards. Furthermore, the high mortality associated with this virus correlated to extensive viral replication outside the respiratory system and can be largely attributed to segments encoding for M and NP proteins.

Financial Support
U.S. Department of Agriculture

Notes:

557 - Effects of temperature on bluetongue virus evolution after coinfection of Culicoides sonorensis

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Session: VIROLOGY

Objective

Bluetongue virus (BTV) is a segmented, double-stranded RNA virus transmitted by *Culicoides* biting midges. Infection of domestic and wild ruminants with BTV can result in devastating disease and significant economic losses. The global distribution of BTV has expanded recently, perhaps as a consequence of the impact of climate change on Culicoides midges that serve as biological vectors of the virus. While a variety of factors can influence seasonal activity of Culicoides, temperature has an important impact on fluctuations in total populations and rate of BTV virogenesis, with higher temperatures being associated with more rapid BTV replication. While transmission of BTV is optimized during periods of warmer weather, it is presumed more rapid virogenesis could be associated with increased rates of viral reassortment and may enhance BTV's ability to spread to new regions. The objective of this project was to characterize the frequency of reassortment between BTV-10 and BTV-17 in *C. sonorensis* maintained at different temperatures (20°C, 25°C, or 30°C).

Methods

To establish single-virus and co-infections, midges were fed a blood meal containing ~10^5TCID50/ml of BTV-10, BTV-17, or BTV-10+17. Pools of midges (n = 5) collected every other day were processed for BTV qRT-PCR to track virogenesis over time. Co-infected midges collected on days 3, 7, 11, 15, and 19 were processed for BTV plaque-isolation. The complete genotypes of isolated plaques were determined using a novel, amplicon-based sequencing approach.

Results

Preliminary results indicate that coinfected midges maintained at 30°C and 25°C exhibited high BTV copy numbers demonstrating productive virogenesis earlier in infection (day 6 and day 8 respectively) compared to midges maintained at 20°C (day 12). High BTV copy number was of greatest proportion in midge pools that were maintained at 25°C across all infection treatment groups. Additionally, in pools of coinfected midges from day 14, NGS indicated that midges maintained at 25°C had representation of reads for all segments of BTV-10 and BTV-17. Pools of coinfected midges from day 14, reared at 20°C and 30°C, had reads for all segments of BTV -17 but very few reads were present for segments of BTV -10. Midges maintained at 20°C had the longest survival time, followed by midges held at 25°C day and at 20°C and then 30°C.

Conclusions

Our findings demonstrate that temperature and infection status effect both virogenesis and life traits of *C. sonorensis* with potential implications for genetic diversity and transmission dynamics. Warmer temperatures of 25°C and 30°C corresponded to more productive virogenesis in both single infection groups and the coinfection group. Whole genome analysis of day 14 coinfected midges demonstrated a more diverse representation of segments from both BTV -10 and BTV-17 at 25°C compared to 20°C and 30°C temperatures, indicating that 25°C may be an optimal temperature for potential reassortment to occur. Overall, warmer temperatures and coinfection corresponded with lower survival rates of the vector. This suggests that there could be a tradeoff between vector survival and increased virus production due to the devitalizing effects of increased temperature and coinfection. Further research is needed to investigate reassortment events after coinfection. Bluetongue virus reassortment patterns and resulting biological consequences will add an important dimension to the modeling of viral expansion and evolution in the context of climate variability.

Financial Support
USDA National Institute for Food and Agriculture

Notes:

558 - Tissue tropism of 4/91 Infectious Bronchitis Virus variant following infection of laying hens

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Session: VIROLOGY

Objective

Infectious bronchitis is economically important in chickens. The causative agent, infectious bronchitis virus (IBV) primarily infects the respiratory tract and then, spreads to other tissues depending on the strain. During 2012-2016, the 4/91 IBV has been extensively isolated from poultry flocks in Eastern Canada with decreased egg production and quality. To confirm the association of 4/91 IBV with egg production issues, an in vivo study was planned. We hypothesized that the 4/91 IBV variant has a reproductive tract tropism and results in lower egg production and quality. This study aims to determine the tissue tropism of experimental 4/91 IBV infection in laying hens.

Methods

During the peak of egg lay, 6 chickens were infected with 4/91 IBV variant ($1x10^6$ embryo infectious $dose_{50}$) maintaining 6 control hens. Oropharyngeal and cloacal swabs were collected in predetermined time points for the quantification of IBV genome loads. Since egg production or egg quality was not influenced by the 4/91 IBV infection, at 10 days of infection, the chickens were euthanized to observe the lesions in various organs and to collect blood and tissue samples for the quantification of antibody response and IBV genome loads respectively. In addition, we investigated the histological changes and immune cell recruitment in kidney.

Results

No gross lesions were observed in the tissues of infected hens. The IBV genome was quantifiable in swabs, trachea, lung, proventriculus, cecal tonsils, kidney and reproductive (magnum, isthmus and shell gland) tissues. The serum antibody response against IBV was quantified in infected hens. We also observed histological changes in kidney and recruitment of immune cells such as macrophages and T cell subsets. The antibody response in reproductive tract mucosa was not significantly different between 4/91 IBV infected and control chickens.

Conclusions

Overall, the data show that 4/91 IBV is not associated with egg production issues in laying hens with various tissue tropism including kidney where histological lesions and immune cell recruitments were evident.

Financial Support
Agriculture and Agri-Food Canada; Canadian Poultry Research Council; Egg Farmers of Canada

Notes:

559 - Evaluation of bacteria-rotavirus-glycan interactions using a novel porcine intestinal enteroid model

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Session: VIROLOGY - SWINE

Objective

Rotaviruses (RVs) are the leading cause of acute viral gastroenteritis in young children and piglets of significant public health and economic impacts. Probiotics are recognized as health/growth promoters and the best alternative to antimicrobials that reduce the risk of antimicrobial resistance. However, a mechanistic understanding of how probiotics interact with RVs and host-related factors including histo-blood group antigens (HBGAs) is lacking.

Methods

Here, we established *porcine* small intestinal crypt-derived *3D enteroids (PIE) expressing* different HBGAs (A+, H+, and A+/H+). PIEs were infected with human RV (HRV) G1P[8] Wa, porcine RV (PRV) G9P[13], PRV Gottfried G4P[6] or PRV OSU G5P[7] virulent and attenuated strains and virus replication was measured by qRT-PCR.

Results

Our results indicated that virulent G1P[8] and G9P[13]/G5P[7] replicated to highest titers in A^+ and H+ PIEs, respectively. Attenuated HRV and PRVs (except OSU strain) replicated poorly in PIEs, and their replication was not affected by HBGA phenotypes. HBGA synthesis inhibitor treatment demonstrated that HBGAs are essential for G1P[8] Wa replication; but they could be dispensable for G9P[13] and OSU G5P[7] replication. Interestingly, contrasting outcomes were observed following sialidase treatment whereby it significantly inhibited the growth of G5P[7], but enhanced G9P[13] replication. These observations suggest that additional receptors recognized by G9P[13] become unmasked after removal of terminal sialic acids (SA). These results confirm that differential HBGAs-RV and SA-RV interactions determine replication efficacy of virulent group A RVs in PIEs. Further, we demonstrated that out of 10 probiotics/commensal bacteria tested in this study, 6 strains were able to partially block anti-A antibody attachment to A+ PIE, while 3 strains were shown to express HBGA-like antigens and were capable of binding RVs in vitro.

Conclusions

These findings suggest that RV interactions with host or bacterial glycans can affect their pathogenesis.

Financial Support
International Development Research Centre

Notes:

560 - Genotypic characterization of swine influenza virus reassortants in vaccinated and non-vaccinated pigs

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Session: VIROLOGY - SWINE

Objective

Swine are considered an important intermediate host in the ecology of influenza A virus for many reasons. Influenza viruses from various origins can replicate in the respiratory tract of pigs resulting in novel reassortants that may facilitate influenza interspecies transmission. The emergence of novel reassortants not only can result in vaccine failure but also poses a significant risk to public health. However, studies that investigate the emergence of reassortant viruses in naïve and vaccinated pigs are lacking. Here, we want to evaluate the emergence of new reassortant viruses in pigs co-infected with two influenza viruses of different subtypes and lineages.

Methods

We collected bronchoalveolar lavage fluid (BALF) samples from pigs that had been co-infected with an H1N1 and H3N2 virus and had been vaccinated with either a whole inactivated, a live attenuated, or the combination of both and compared the reassortant viruses with those from non-vaccinated pigs. Finally, 242 plaques were randomly picked up from 16 BALF samples during the plaque purification and processed for Illumina Nextseq sequencing.

Results

Two hundred and twenty-six plaques were successfully identified and genotyped and out of these, 40 plaques (17.7%) resulted in novel reassortant viruses that could be categorized in 21 distinct genotypes. In addition, sixteen plaques were classified as mixed genotypes and contained more than 8 segments from both viral isolates. There were 16 (12.6%) reassortants identified in vaccinated pigs, 4 (10%) in seeder pigs and 20 (33.9%) in non-vaccinated pigs. Overall, Pigs receiving the prime-boost vaccination had significantly less reassortant viruses ($p=0.015$) compared to non-vaccinated pigs.

Conclusions

Our study provides initial evidence that vaccination can play a role in reducing the emergence of new reassortant viruses in pigs. These results, if confirmed under field conditions, illustrate the importance of developing effective influenza vaccination control programs that not only improve clinical outcomes but also decrease shedding and genetic diversity in pigs.

Financial Support
Zoetis

Notes:

561 - Dynamics of influenza A virus shedding in the upper and lower respiratory tract of pigs using a co-infection model

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Session: VIROLOGY - SWINE

Objective

Swine influenza affects the growth performance of young pigs and causes significant economic losses to the swine industry. Pigs can replicate influenza A viruses (IAV) from various origins and can be co-infected by multiple strains and subtypes, which contributes to the significant genetic diversity found in US pig farms. However, the dynamics of these co-infections at the individual pig level are poorly understood. In this study, we evaluated the infection dynamics and shedding patterns at the individual pig level in both the upper and lower respiratory tract of pigs co-infected with an H1N1 and an H3N2 subtype following an experimental co-infection model.

Methods

Fourteen naive pigs were experimentally inoculated with either an H1N1 or an H3N2 IAV and were distributed evenly in pairs of 2 in seven rooms at approximately 48 hours post-inoculation. Each room had one H1N1 and one H3N2 inoculated pig. Pigs were housed together to facilitate nose to nose contact and interaction. Nasal swabs were taken daily and bronchoalveolar lavage fluid (BALF) samples were collected at necropsy seven days post-inoculation. Samples were tested by real-time PCR targeting the matrix gene and samples with Ct value under 35 were submitted for whole-genome sequencing using Illumina Nextseq sequencing.

Results

All inoculated pigs tested RT-PCR positive in nasal swabs between 0 and 2 days post-inoculation, and at necropsy 7 out 14 pigs tested positive in BALF. During the 7-day observation period, 4 out of 14 pigs (two H1 and two H3 challenged pigs) were co-infected and shed both viruses in the nasal cavities or lungs. Among them, we found that pig 4480 at 2 dpc, and pig 4497 at 2, 4, and 5 dpc shed both viruses simultaneously in the upper respiratory tract. However, at necropsy (7 dpi), in samples from the lower respiratory tract, all four pigs had no evidence of viral infection with the subtype they had been experimentally inoculated with, but instead had evidence of infection with the other subtype. All pigs displayed mild gross lung lesions (range from 0 to 11%) except pig 4480, an H3H2 inoculated pig with H1N1 only at necropsy, that had 90% lung lesions.

Conclusions

Our study characterized the shedding patterns of IAV at the individual pig level in a co-infection model using 2 subtypes of IAVs and demonstrated complex dynamics of infection that resulted in extended IAV infection patterns overtime. More research is needed to validate these results under field conditions.

Financial Support
Zoetis

Notes:

562 - Transmission of an influenza A virus with human seasonal H3 in pigs resulted in adaptive mutations in the HA gene

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Session: VIROLOGY - SWINE

Objective

Influenza A virus (IAV) is known to cause respiratory disease in pigs and humans. IAV circulates worldwide, with various strains and subtypes being prevalent among the swine population. The hemagglutinin (HA) and neuraminidase (NA) surface proteins are important determinants of host specificity and key factors driving the evolution of IAVs. Interspecies transmission of IAV from humans to pigs is relatively common and many human-origin strains, or gene segments therein, became endemic in pigs, including the triple-reassortant internal gene constellation (TRIG). Although human seasonal strains can become adapted to pigs, usually these strains must undergo molecular changes. However, the evolutionary processes that lead to adaptation in pigs are not fully understood. The purpose of this study was to evaluate the molecular evolutionary dynamics during adaptation of human H3N2 strains to pigs

Methods

To evaluate the adaptation of human seasonal surface genes in pigs, we generated a reassortant H3N2 strain via reverse genetics containing human seasonal HA and NA genes with TRIG backbone and the 2009 pandemic lineage matrix (M) gene. A transmission study was conducted in pigs and nasal swab samples were collected from infected and respiratory contact pigs in multiple time-points. Viral RNA was extracted from the swabs and sequenced by next generation sequencing (NGS) using a Illumina MiSeq platform. Sequences were analyzed by bioinformatic tools (Lofreq, I-TASSER) to identify viral variants.

Results

Four amino acid mutations were identified in the HA1 region of the human seasonal H3 after replication and transmission in pigs (, A154S, V202G,F209Y and synonymous 89D), with one fixed mutation (A154S) that was identified in all respiratory contact pigs. All sites were located in the globular head of the HA.

Conclusions

Our data show that mutations in the HA gene are selected quickly after replication and transmission of human H3 in pigs, suggesting these mutations may be instrumental in the adaptation of human seasonal H3N2 viruses in pigs.

Financial Support
USDA National Institute of Food and Agriculture

Notes:

563 - Mutation in the exoribonuclease of porcine epidemic diarrhea virus causes high genetic instability

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Session: VIROLOGY - SWINE

Objective

Porcine epidemic diarrhea virus (PEDV), a member of alphacoronaviruses, is the causative agent of porcine epidemic diarrhea. It emerged in the US in 2013, causing enormous economic losses to swine industry. Coronavirus nonstructural protein 14 (nsp14) has exoribonuclease activity (ExoN), responsible for proofreading and contributing to replication fidelity. We hypothesized that PEDV mutants with deficient nsp14-ExoN activity replicate poorly, leading to attenuation in pigs.

Methods

Based on infectious cDNA clone of highly virulent PEDV strain (icPC22A), eight mutants targeting nsp14 ExoN catalytic sites, zinc finger or Mg^{2+}-binding site were designed, but only one mutant E191A, carrying the mutation in Mg^{2+}-binding site, was rescued. Both Vero and IPEC-DQ cells were used for *in vitro* characterization. Then 4 or 5-day-old gnotobiotic pigs were experimentally inoculated with the mutant to examine pathogenesis and immunogenicity.

Results

It was very difficult to grow the passage no.1-3 (P1-3) of E191A in Vero or IPEC-DQ cells with peak titers of 1.80±0.12 and 1.42±0.16 $\log_{10}$ $TCID_{50}$/mL, respectively. However, the P4 of E191A grew dramatically to a high infectious titer (5.55±0.35 $\log_{10}$ $TCID_{50}$/mL) in Vero cells, similar to icPC22A. Whole genome sequence analyses revealed that the P4 of E191A has mutated back to the virulent icPC22A. To evaluate the pathogenesis of the E191A, 4 or 5-day-old pigs were orally inoculated with 100 $TCID_{50}$/pig of the P1 of E191A, icPC22A, or mock. All pigs inoculated with icPC22A showed severe diarrhea and died within 6 days post-inoculation (dpi). In comparison, only 2 pigs (2/3, 66.7%) in E191A group showed mild diarrhea at 6 dpi. However, sanger sequencing showed that the viral genome in the fecal samples from E191A group at 2 dpi has lost the mutation, suggesting the instability of that mutant.

Conclusions

In summary, mutations at the essential functional sites within nsp14-ExoN domain of PEDV were either lethal or genetically unstable. The nsp14-ExoN domain is not an appropriate target for the design of PEDV live attenuated vaccines.

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

Notes:

564 - Porcine epidemic diarrhea virus nsp1 is an interferon antagonist and a determinant of virulence

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Session: VIROLOGY - SWINE

Objective

Since the highly virulent porcine epidemic diarrhea virus (PEDV) emerged in the US in 2013, it has caused enormous economic losses to US swine industry. Although efforts have been made, no efficacious and safe vaccines are available to prevent and control porcine epidemic diarrhea. Our project aims to develop safe, effective, and recombination-resistant live attenuate vaccines (LAVs). PEDV nonstructural protein 1 (nsp1) has been shown *in vitro* as an antagonist to host innate immunity, including interferon (IFN) responses. We hypothesized that recombinant PEDV with inactivated nsp1 induces robust host antiviral innate immune responses, leading to virus attenuation. The objective of this study is to generate PEDV nsp1 mutants using reverse genetics and evaluate their pathogenicity and immunogenicity *in vitro* and *in vivo*.

Methods

We generated a PEDV nsp1 mutant icPC22A-nsp1-N93/95A by replacing its critical sites N93 and N95 with alanine, using the infectious cDNA clone of a virulent PEDV strain, icPC22A. The growth curve of icPC22A-nsp1-N93/95A was characterized in Vero cells. Then, we examined its sensitivity to IFN-β treatment in Vero cells. We plan to investigate its pathogenicity and immunogenicity in pigs.

Results

The recombinant PEDV nsp1 mutant icPC22A-nsp1-N93/95A formed similar sizes of plaques to icPC22A. However, it grew to significantly lower infectious titers (5.7 ± 0.8 $\log_{10}$ $TCID_{50}$/mL) than icPC22A (6.8 ± 0.0 $\log_{10}$ $TCID_{50}$/mL). In cells pre-treated with high concentration (200 units / 10^5 cells) of IFN-β, the replication of icPC22A-nsp1-N93/95A mutant was significantly inhibited. Its inhibition rate (0.8510±0.2107) was significantly higher than that (0.5170±0.0010) of icPC22A.

Conclusions

The icPC22A N93/95A mutant replicated less efficiently and was more sensitive to IFN-β pre-treatment than icPC22A in Vero cells. It may be less resistant to host innate anti-viral defense and a target for rational design of PEDV LAVs. We plan to test its pathogenicity and immunogenicity *in vivo* soon.

Financial Support
USDA National Institute of Food and Agriculture

Notes: