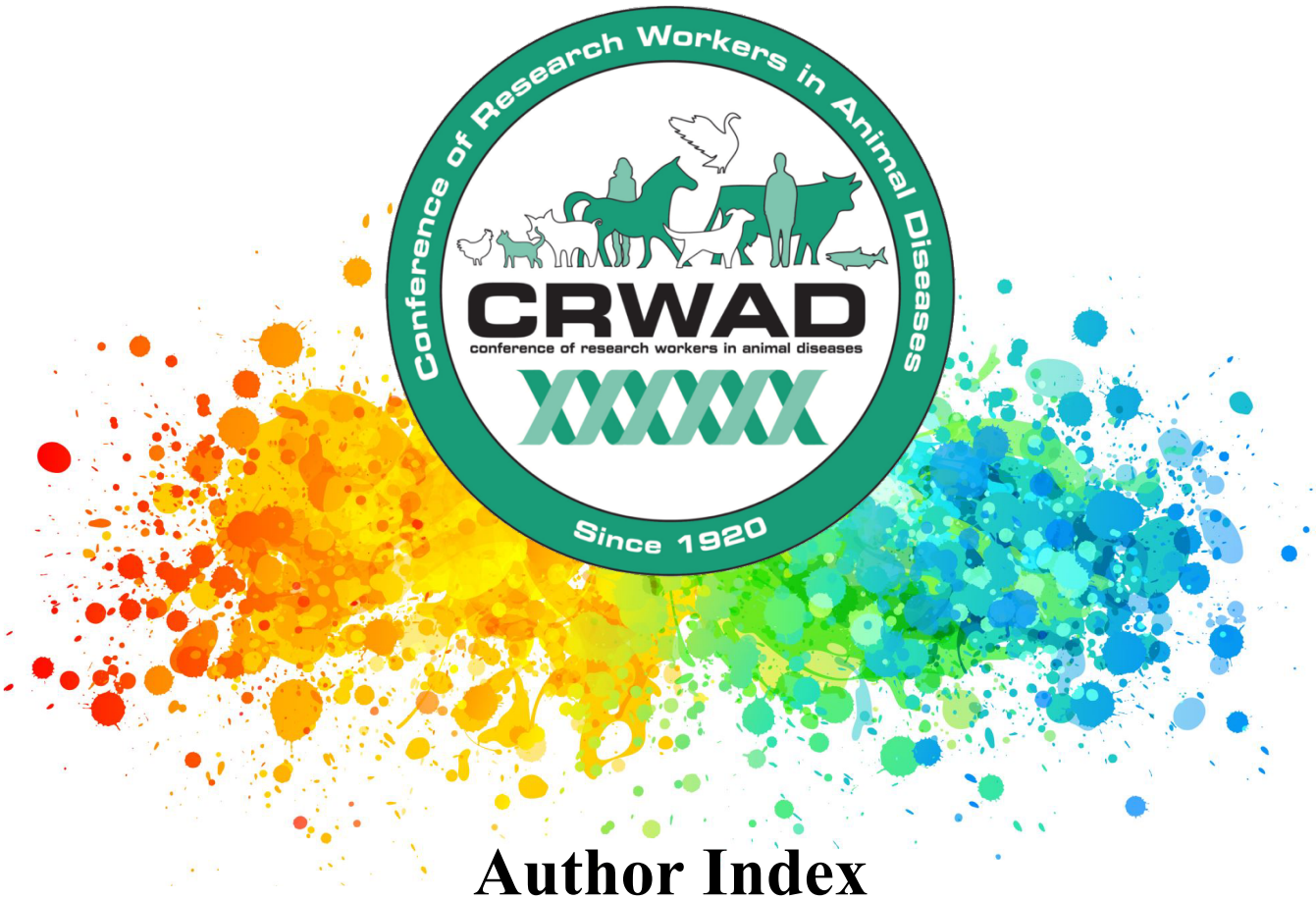

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&
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104th Conference of Research Workers in Animal Diseases

January 20-23, 2024

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Chicago, IL

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CRWAD 2024 President's Message

January 2024

It is with immense pleasure and great enthusiasm that I welcome each of you to the Conference of Research Workers in Animal Diseases – an annual gathering that serves as a nexus for our global community of researchers and veterinarians. As the president of this esteemed Conference, I am honored to lead an event that has become a cornerstone in the field, fostering collaboration, sharing groundbreaking research, and propelling innovation in veterinary science. This year's Conference promises to be an exciting one. With 20 featured speakers and more presentations than ever, our program committee has worked tirelessly to curate a collection of presentations that span a diverse array of topics, addressing the latest advancements, challenges, and opportunities in the field of animal health. From emerging infectious diseases to advancements in diagnostic techniques, this year's Conference will be one to remember.

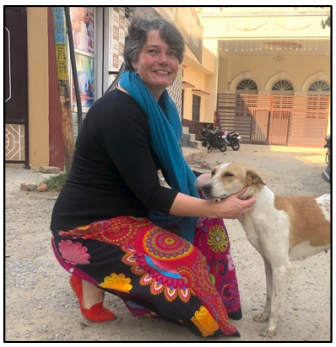
I encourage each of you to actively participate, share your insights, and engage in the vibrant discussions that will unfold during the Conference. Your presence and contributions are vital to the success of this event, and I have no doubt that the knowledge shared and connections forged will have lasting impact on the future of animal health. On behalf of the entire organizing committee, thank you for your commitment to advancing the frontiers of knowledge in animal science. Together, let us continue to make the Conference of Research Workers in Animal Diseases a resounding success and a beacon for future generations of researchers and veterinarians.

Thank you, and have a great Conference.

Sincerely,

Dr. Annette O'Connor, BVSc, MVSc, DVSc, FANZCVs
President, Conference of Research Workers in Animal Diseases

CRWAD 2024 Featured Speakers



"Global One Health efforts to eliminate vector-borne zoonoses"

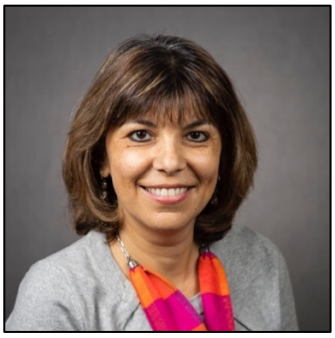
Christy Petersen, MS, PhD, DVM
University of Iowa

CRWAD Keynote Speaker
Sunday, 1/21/2024 2:00 PM

"Food's journey in the last mile – managing food waste as untapped resources"

Zhengxia Dou, MS, PhD
University of Pennsylvania

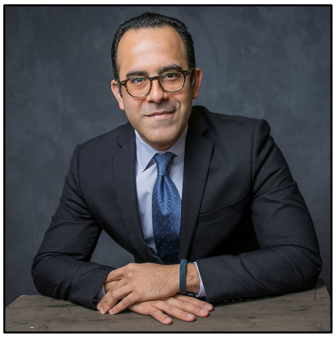
CRWAD Special Symposium:
Novel Solutions to Problems in Animal Health
Sunday, 1/21/2024, 4:15 PM



"Genomic solutions for sustainable beef production: Climate-smart cattle breeding"

Raluca Mateescu, MS, PhD
University of California, Davis

CRWAD Special Symposium:
Novel Solutions to Problems in Animal Health
Sunday, 1/21/2024, 3:00 PM

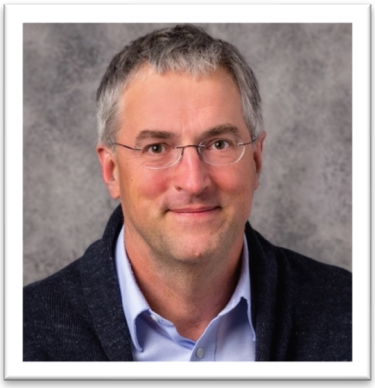


"R&D needs for US agriculture in a changing climate"

Ariel Ortiz-Bobea, MS, PhD
Cornell University

CRWAD Special Symposium:
Novel Solutions to Problems in Animal Health
Sunday, 1/21/2024, 5:00 PM

CRWAD 2024 - Distinguished Career Awards



"Before there was COVID, there was Bovine Respiratory Disease"

Christopher Chase, DVM, PhD
South Dakota State University

AAVI Distinguished Veterinary Immunologist
Sunday, 1/20/2024, 8:30 AM

"Coronaviruses: Past, present, future threats to animals & humans"

Linda Saif, MS, PhD, DACVM
Ohio State University

ACVM Distinguished Veterinary Microbiologist
Tuesday, 1/23/2024, 8:30 AM

"What question are we trying to answer? Embracing causal inference"

Jan M. Sargeant, DVM, MSc, PhD, FCAHS
University of Guelph

AVEPM Calvin Schwabe Award
Monday, 1/22/2024, 8:30 AM

"Prevention and control of diseases in aquaculture species"

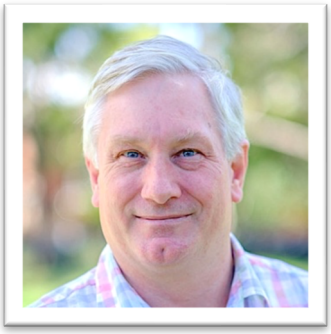
Benjamin Beck, MA, PhD
USDA

Animal Vaccinology Research Network Featured Speaker
Tuesday, 1/22/2024, 2:00 PM

"SARS-CoV-2 spillovers across the human animal interface"

Andrew Bowman, MS, DVM, PhD, DACVPM
Ohio State University

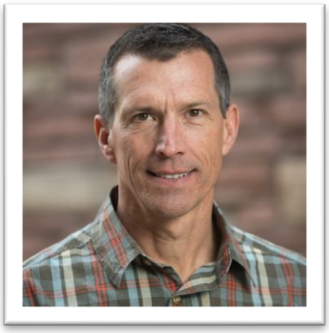
ACVM Featured Speaker
Tuesday, 1/23/2024, 10:30 PM



"Vaccines – critical components of effective veterinary antimicrobial stewardship"

Glenn Browning DVM, PhD
University of Melbourne

Animal Vaccinology Research Network Featured Speaker
Monday, 1/22/2024, 2:00 PM



"Utilizing aptamer-based proteomics to investigate feline infectious peritonitis pathogenesis and discover biomarkers"

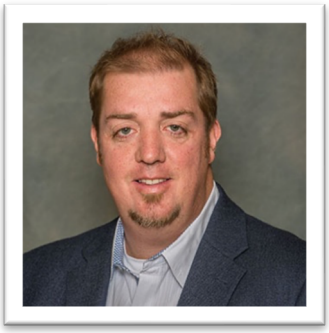
Gregg Dean, DVM, PhD, DACVP
Colorado State University

ACVM Featured Speaker
Tuesday, 1/23/2024, 11:15 AM

"Progresses and challenges in the development of vaccines against intracellular intestinal parasites in poultry"

Hyun Lillehoj, PhD
USDA

Animal Vaccinology Research Network Featured Speaker
Monday, 1/22/2024, 2:45 PM



"Stressed from the start: Implications of early life stress for lifelong health and disease resistance"

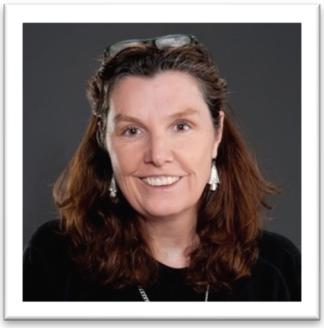
Adam Moeser, MS, PhD, DVM
Michigan State University

AAVI Featured Speaker
Sunday, 1/20/2024 10:30 AM

"Does vaccination prevent antimicrobial resistance?"

Paul S. Morley, DVM, PhD, Diplomate ACVIM
Texas A&M University

Animal Vaccinology Research Network Featured Speaker
Monday, 1/22/2024, 5:00 PM



"Rethinking variable selection and study design approaches"

Annette O'Connor BVSc, MVSc, DVSc, FANZCVSc
Michigan State University

AVEPM Featured Speaker
Monday, 1/22/2024, 9:15 PM

"Pathogenesis of murine and human coronavirus infections"

Stanley Perlman, MD, PhD
University of Iowa

ACVM Featured Speaker
Tuesday, 1/23/2024, 9:15 AM

"Host responses during influenza A virus adaptation to a new species"

Daniela Rajao, MS, PhD, DVM
University of Georgia

AAVI Featured Speaker
Sunday, 1/20/2024 11:15 AM

"Aligning valid research outcomes with stakeholder values"

David Renter, DVM, PhD
Kansas State University

AVEPM Featured Speaker
Monday, 1/22/2024, 11:15 PM

"Do our exposure variables tell us what we think they are telling us?"

Audrey Ruple, DVM, DACVPM, MRCVS
Virginia Tech

AVEPM Featured Speaker
Monday, 1/22/2024 10:30 AM

"A tribute to our friend and colleague Dr. John D. Lippolis"

Randy Sacco, MS, PhD
USDA-ARS

AAVI Featured Speaker
Sunday, 1/20/2024 9:15 AM

CRWAD Fellows

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

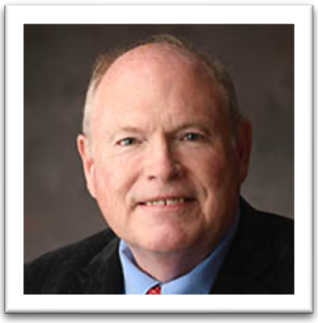
Scientists recognized as CRWAD Fellows have distinguished research careers evidenced by the outstanding impact and importance of their work, and their ability to communicate and interpret science to stakeholders and the public. Fellows have made significant contributions to scientific literature reflecting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

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

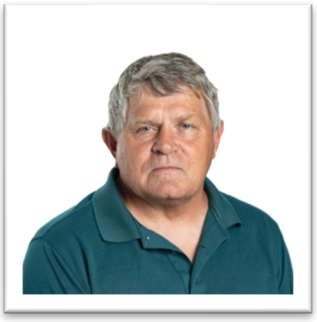
2024 CRWAD Fellow Inductees

Christopher Chase, DVM, PhD, DACVM
Department of Veterinary and Biomedical Sciences,
South Dakota State University

M.M. Chengappa, BVSc, MVSc, MS, PhD, DACVM
University Distinguished Professor,
College of Veterinary Medicine,
Kansas State University



Scott McVey, DVM, PhD, DACVM
School of Veterinary Medicine and Biomedical Sciences, University of Nebraska



Steven Olsen, DVM, PhD, DACVM
Infectious Bacterial Diseases Research Unit, National Animal Disease Center, USDA-ARS

James Roth, DVM, MS, PhD, DACVM
College of Veterinary Medicine, Iowa State University

Amelia Woolums, DVM, PhD, DACVM, DACVIM
College of Veterinary Medicine, Mississippi State University

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Professor Emerita, University of Massachusetts Amherst

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Professor Emeritus, The Ohio State University

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Professor, College of Veterinary Medicine, Cornell University

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Dean Emeritus, College of Veterinary Medicine, Iowa State University

Carol Chitko-McKown, PhD (2023)
U.S. Meat Animal Research Center, Clay Center, Nebraska

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Professor Emeritus, University of California, San Diego

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Professor, University of Florida

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Professor Emeritus, University of California, Davis

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Professor Emeritus, University of California, Davis

Laura Hungerford, DVM, MPH, CPH, PhD, FNAP (2021)
Professor and Department Head, Virginia-Maryland College of Veterinary Medicine

Jun Lin, PhD (2021)
Professor, University of Tennessee

Joan Lunney, PhD (2023)
USDA-ARS Beltsville, MD

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Distinguished Professor, Kansas State University

Stuart W.J Reid, BVMS, PhD, DVM, DipECVPH, FRSB, FRSE, FRCVS (2021)
President, Royal Veterinary College, University of London

David Renter, DVM, PhD (2023)
Professor, Kansas State University

Richard Ross, MS, PhD (2021)
Distinguished Professor Emeritus, Iowa State University

Linda J. Saif, PHD (2021)
Professor, College of Veterinary Medicine, Ohio State University

Y. M. Saif, DVM, PhD (2021)
Professor and Head Emeritus, Ohio State University

Janice ("Jan") Merrill Sargeant, DVM, MSc, PhD (2021)
Professor, University of Guelph, Ontario

Patricia Shewen, BSc, DVM, MSc, PhD (2023)
Professor Emerita, University of Guelph, Ontario

Lorraine Sordillo-Gandy, BS, MS, PhD (2021)
Meadow Brook Chair, Michigan State University

Subramaniam Srikumaran, BVSc, MS, PhD (2023)
Professor Emeritus, Washington State University

Thomas Wittum, MS, PhD (2023)
Professor, The Ohio State University

Please visit https://crwad.org/fellows_directory/ for biographical information about CRWAD Fellows.

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1 - Before COVID there was BRD

Chris Chase[1]

[1]Department of Veterinary and Biomedical Science, South Dakota State University. Christopher.Chase@SDSTATE.EDU

Session: AAVI - Featured Speakers, 2024-01-21, 8:30 - 9:15

Bovine respiratory disease (BRD) has long been the predominate cause of morbidity and mortality in cattle. COVID brought attention to the reality that when it comes to the immune system you can have "too much of a good thing". Co-morbidities such as obesity, autoimmune diseases, nutritional plane, age and other factors that induce stress exacerbated the disease. In spite of the development of new and improved BRD vaccines and antimicrobial therapies, the incidence of bovine respiratory disease has increased. These strategies, directed at the pathogen alone, have failed to improve prevention and control of BRD. In considering the battle against BRD what hasn't changed is the manner in which cattle are "managed" to "minimize" the stressors and the "cytokine storms" associated with the various stressors that occur in cattle.

In this talk I will discuss BRD in relation to host responses to bovine viral diarrhea virus and bovine herpesvirus 1, the resulting proinflammatory responses and immune dysfunction in the weaned animal. In addition, cardiac-respiratory syndrome associated with "late deads" in the feed yard is another "link" to COVID. Maintaining homeostasis in the animal through nutritional and management interventions needs to be part of our approach to limit BRD to improve productivity and enhance animal welfare.

Notes:

2 - A tribute to our friend and colleague Dr. John D. Lippolis

Randy Sacco[1]

[1]Ruminant Diseases and Immunology Research Unit, National Animal Disease Center, USDA ARS. randy.sacco@usda.gov

Session: AAVI - Featured Speakers, 2024-01-21, 9:15 - 10:00

As Dr. John Lippolis was to be a featured speaker for the AAVI Mini-symposium, we thought that it would be fitting to provide a tribute to our friend and colleague who passed away on Saturday, August 26, 2023, by highlighting his research accomplishments. During his 21 years of service at the USDA/ARS/National Animal Disease Center (NADC), Dr. Lippolis developed an internationally recognized research program in mastitis. His program focused on immunology, biochemistry, molecular biology, genetics, and mass spectrometry. Among his numerous accomplishments, John demonstrated that vitamin D has therapeutic potential in the treatment of mastitis, identified bacterial virulence factors which play critical roles in the pathogenesis of mastitis, and he established that Holsteins with the genetic background found in 1964 dairy cattle have significantly better disease outcome in experimental mastitis than contemporary Holsteins. Dr. Lippolis authored or co-authored 83 peer-reviewed publications, five book chapters, and one patent. For his work in mastitis and mass spectrometry, he received the West Agro, Inc., Award from the American Dairy Science Association. The award recognizes outstanding milk quality research as affected by the control of mastitis, management of milking, and practices in milk production. Twice his manuscripts were selected as the "Editor's Choice" for the Journal of Dairy Science. He received more than 40 speaking invitations in the US, Italy, Norway, and Spain. Recently, John had served as Acting Associate Director at NADC. Prior to accepting a position at the NADC, he was the Technical Director, National Institutes of Health MHC Tetramer Core Facility, Emory Vaccine Research Center, Emory University, Atlanta, GA. Dr. Lippolis received a B.Sc. from BYU and Ph.D. from Pennsylvania State University College of Medicine.

As my talk will highlight some of Dr. Lippolis' research, it is of note that we co-authored 18 manuscripts, 13 of which covered research related to mastitis, lactation, and proteomics. Our other manuscripts involved research examining aspects of the bovine immune response to respiratory pathogens.

Notes:

3 - The potential role of veterinary technicians in promoting antimicrobial stewardship

Laurel Redding[1], Stephen Cole[1]

[1]University of Pennsylvania School of Veterinary Medicine. lredding@upenn.edu

Session: Antimicrobial Use & Resistance 1, 2024-01-21, 8:30 - 8:45

Objective: A core principle of antimicrobial stewardship (AMS) in veterinary settings is the need for engagement of all stakeholders; however, no studies have addressed the role of veterinary technicians in AMS specifically. The objective of this study was to qualitatively assess knowledge, opinions, and practices related to AMS among veterinary technicians.

Methods: Semi-structured interviews were conducted with 33 veterinary technicians from 25 clinics with varied backgrounds, experience and roles. Interviews centered on participants work experience and interactions with their employer, perceptions of antimicrobial resistance and overuse in veterinary medicine, observed application of AMS principles, opinions on potential opportunities for technicians to contribute to AMS and concomitant potential barriers to these opportunities. Transcripts of interviews were coded thematically by two authors. Qualitative methods were used to analyze the data: briefly, themes were organized into a hierarchical framework, and the characterization of codes was compared across different categories of respondents.

Results: Most veterinary technicians were knowledgeable about antimicrobial drugs but could not provide a complete definition of antimicrobial resistance or AMS. Most veterinary technicians could identify examples of antimicrobial misuse. Participants identified areas of client education and discussion with veterinarians as potential areas to contribute to AMS. Barriers identified included hierarchical structures of veterinary practices and time constraints. Most participants expressed a personal interest in participating in AMS.

Conclusions: There is a possible appetite among some veterinary technicians to participate in AMS and they already play applicable roles in practices. Barriers such as educational needs, hierarchical structures of veterinary practices and time constraints will need to be addressed if technicians are included in AMS efforts.

Financial Support: This research was supported by internal funding from the University of Pennsylvania School of Veterinary Medicine.

Notes:

4 - Assessing the challenges and factors influencing Illinois small animal veterinarians' antimicrobial use practices

Setyo Yudhanto[1], Csaba Varga[1,2]

[1]College of Veterinary Medicine, University of Illinois-Urbana, [2]Carl R. Woese Institute for Genomic Biology. setyoy2@illinois.edu

Session: Antimicrobial Use & Resistance 1, 2024-01-21, 8:45 - 9:00

Objective: Appropriate antimicrobial use is essential to preserve the availability and effectiveness of antimicrobials to treat bacterial infections. Several guidelines exist to promote prudent antimicrobials use in small animal practice. However, companion animal veterinarians face several challenges when prescribing antimicrobials that can impact their decisions. By evaluating these issues, we aim to provide insights into the challenges and factors influencing their decision when prescribing antimicrobials to dogs and cats to promote judicious antimicrobial use.

Methods: We conducted an online survey to assess the challenges encountered by companion animal veterinarians in Illinois when prescribing antimicrobials. A descriptive analysis evaluated factors influencing companion animal veterinarians' antimicrobial use practices. Text analysis examined their responses given to an open-ended question regarding their challenges when prescribing antimicrobials. The frequency and a correlation network among the words based on a pairwise correlation test were assessed at the text analysis step by using "tidyverse", "tidytext", and "widyr" libraries in R.

Results: A total of 95 responses were received from the survey, and 77 responders completed the questions about factors influencing the companion animal veterinarians' decision when prescribing antimicrobials. At the same time, 66 responders completed the open-ended question and were included in the text analysis. Companion animal veterinarians considered the severity of clinical signs (46.2% extremely influential, and 44.9% very influential), culture and antimicrobial susceptibility test results (73.1% extremely influential, and 14.1% very influential); and patients' medical and previous antibiotic use history (28.6% extremely influential, and 45.5% very influential) as influential to their decision when prescribing antimicrobials. The text analysis indicated that owner (n=32), compliance (n=20), antimicrobials (n=19), cost (n=15), culture (n=14), and administration (n=11) were the most common words mentioned by the responders. Meanwhile, the network illustrated correlation of the words "owner" and "compliance"; "administration" and "route"; and "cost" and "culture" were among the most correlated words.

Conclusions: Understanding factors and challenges impacting Illinois companion animal veterinarians' antimicrobial prescription choices will support the development of effective antimicrobial stewardship programs to mitigate the emergence of antimicrobial resistance.

Notes:

5 - Trends of feline *Escherichia coli* antimicrobial resistance over 14 years illustrate the need for judicious antimicrobial use in cats

Claudia Cobo Angel[1], Abdolreza Mosaddegh[1], Melissa Aprea[1], Cassandra Guarino[1], Kevin Cummings[1], Casey Cazer[1]

[1]Cornell University. cga54@cornell.edu

Session: Antimicrobial Use & Resistance 1, 2024-01-21, 9:00 - 9:15

Objective: Regional antimicrobial resistance (AMR) data can be used to direct rational early therapeutic decisions and to develop antimicrobial stewardship guidelines to tackle the development and spread of resistant microorganisms. This study aims to assess the antimicrobial resistance (AMR) trends among *Escherichia coli* isolated from cats between 2008 and 2022, utilizing minimum inhibitory concentration (MIC) data.

Methods: We analyzed MIC results from 1477 feline *E. coli* isolates, obtained from samples submitted to the Cornell University Animal Health Diagnostic Center, primarily from the northeastern United States. MIC values were obtained using the Sensititre™ Complete Automated System. MICs were categorized as susceptible or not susceptible using the Clinical and Laboratory Standards Institute breakpoints. Multidrug resistance (MDR) was defined as not susceptible to at least one agent in three or more antimicrobial categories and was analyzed using a Poisson regression model. Additionally, accelerated failure time (AFT) survival models were employed to model MIC values. All the models included year as a categorical variable (reference level: 2008 - 2011), site of isolation (classified into urinary tract isolate (UTI) as the reference and non-UTI), and whether the isolate exhibited beta-hemolysis.

Results: Not-susceptibility to only one antimicrobial was observed in 192 isolates (13%); and 565 (38%) isolates were not susceptible to two or more antimicrobials. The antimicrobials with the highest susceptibility rates were antibiotics typically reserved for human use imipenem (99%), amikacin (98%) and piperacillin-tazobactam (95%). High susceptibility to trimethoprim-sulfamethoxazole (94%) and ceftiofur (94%) was also observed. Excluding penicillins for non-UTI, ampicillin (74%) and cefazolin (69%) had the lowest susceptibility rates. In total 414 (29%) isolates were classified as MDR. The interaction between UTI and year was significant, evidencing that non-UTI had an increasing risk of exhibiting an MDR pattern over the years. Results from the AFT models indicated a decrease in MICs for fluoroquinolones and gentamicin in recent years. However, MICs for cephalexin increased from 2016 to 2022 and cefovecin from 2012 to 2019. The predicted MIC_{50} for urinary tract isolates is significantly higher for all the antimicrobials analyzed except for ampicillin, enrofloxacin, and tetracycline. Beta-hemolytic isolates were associated with reduced MICs for cephalexin, ceftiofur, enrofloxacin, marbofloxacin, orbifloxacin, pradofloxacin, and tetracycline.

Conclusions: This study highlights the growing challenge of AMR in feline medicine, emphasizing the critical importance of responsible antimicrobial use and ongoing surveillance to address *E. coli* AMR effectively.

Financial Support: This study was funded by the Cornell Feline Health Center Research Grants Program, a grant made available to the College of Veterinary Medicine, Cornell University.

Notes:

6 - Whole-genome analysis of New Delhi metallo-β-lactamase (NDM)-producing Enterobacterales from Korean companion dogs

Su Min Kyung[1], Jun Ho Lee[1], Eun-Seo Lee[1], Han Sang Yoo[1]

[1]Departments of Infectious Disease, College of Veterinary Medicine, Seoul National University. stevekyung25@snu.ac.kr

Session: Antimicrobial Use & Resistance 1, 2024-01-21, 9:15 - 9:30

Objective: Carbapenemase-producing Enterobacterales (CPE) is one of the most significant multidrug-resistant (MDR) pathogens in modern society, given its widespread circulation in the nosocomial environment. Although New Delhi metallo-β-lactamase (NDM)-producing CPE pathogens are emerging as a public threat, their epidemiology in our society remains largely unknown. Furthermore, the dissemination of CPE among companion animals, which are closely associated with human beings, is still under consideration by major diagnostic and surveillance systems. In this study, whole genome analysis was conducted to gain genomic understanding of CPE strains isolated from companion animals in our society.

Methods: In order to isolate CPE strains, screening investigations were conducted on a total of 1,197 samples (swabbed samples or stored bacterial isolates) from 562 companion animals (canine or feline) visiting four Korean animal clinical hospitals between 2018 and 2022. CPE isolates were obtained using meropenem impregnated MacConkey (MIM) agar, and the minimum inhibitory concentration (MIC) was determined. Whole bacterial DNA was isolated, sequenced, and assembled using Oxford Nanopore long reads, which were corrected with short reads from the Illumina NovaSeq 6000 platform. The whole-genome structure and positions of antimicrobial resistance (AMR) genes were identified and visualized using CGView. Worldwide datasets were downloaded from the NCBI GenBank database and used for whole-genome phylogenetic analysis and to determine the molecular genetic epidemiology of CPE isolates.

Results: The whole-genome analysis was conducted on six isolates of New Delhi metallo-β-lactamase (NDM)-producing CPE strains, which were identified as a result of the screening. A total of 3 *Escherichia coli* and 2 *Klebsiella pneumoniae* isolates harboring NDM-5 were identified, along with a *Proteus mirabilis* isolate harboring NDM-1. All isolates were confirmed as MDR strains through minimum inhibitory concentration (MIC) determination. As a result of the study, the whole-genome structures and multiple novel structures, including various AMR genes, were identified and visualized for each isolate. Genomic regions, including AMR genes, were identified with multiple variations. In the whole-genome epidemiological analysis, multiple phylogroups were identified, revealing the genetic relationship of CPE isolates with other strains, whether novel or not, carrying various AMR genes.

Conclusions: This study identified multiple novel genetic variations of AMR genes and unidentified phylogroups from companion animals, indicating that the circulation of CPE pathogens in companion animals is also a serious concern for modern society. These genetic findings would provide useful background information for the establishment of urgent control measures and surveillance programs. The findings from this study indicate that the issue of CPE is not confined to human health and should be addressed from the perspective of the "one health approach."

Financial Support: This study was supported by National Research Foundation (NRF- 2020R1A2C200879414), BK21 FOUR Future Veterinary Medicine Leading Education and Research Center and Research Institute for Veterinary Science, Seoul National University, Seoul, Republic of Korea.

Notes:

7 - Detection of antimicrobial resistance in *Escherichia coli* isolated from retail meat products in North Carolina

Mabel K. Aworh[1], Megan Jacob[1], Catherine Gensler[2], Erin Harrell[1], Lyndy Harden[1], Siddhartha Thakur[1], Paula J. Fedorka-Cray[1]

[1]Department of Population Health and Pathobiology, College of Veterinary Medicine, North Carolina State University, Raleigh, NC, [2]Department of Agricultural and Human Sciences, College of Agriculture and Life Sciences, North Carolina State University, Raleigh, NC. maworh@ncsu.edu

Session: Antimicrobial Use & Resistance 1, 2024-01-21, 9:30 - 9:45

Objective: *Escherichia coli* is often used as an indicator for antimicrobial resistance (AMR) surveillance in food animal products. Our study aimed to determine the prevalence of AMR in *E. coli* isolates from retail meat purchased from grocery stores in North Carolina, USA.

Methods: During 2022, we purchased retail chicken, ground turkey, and pork chops from different counties in North Carolina. Different antibiotic use claims were noted. *E. coli* was isolated using standard culture and characterized using whole genome sequencing. Multi-locus sequence typing, phylogroups, and single nucleotide polymorphism (SNP)-based maximum-likelihood phylogenetic tree were used to determine the genetic relatedness of *E. coli* isolates. Multidrug resistance (MDR; defined as genotypic resistance to ≥3 classes of antimicrobials) was also determined. Data were analyzed by computing proportions and using the chi-square test.

Results: Of 312 samples, 138 (44.2%) were positive for *E. coli*. Of these, *E. coli* isolates were recovered from ground turkey (n=78; 56.5%), chicken (n=41; 29.7%), and pork chops (n=19;13.8%). Only 117/138 (84.8%) of the isolates comprising 72 (61.5%) from ground turkey, 27 (23.1%) from chicken, and 18 (15.4%) from pork chops had quality sequence data and were further characterized genotypically. AMR genes were detected in 91 (77.8%) of the isolates of which 42 (35.9%) were MDR. Collectively, commonly observed AMR genes were *tetB* (41/117; 35%), *tetA* (29/117; 24.8%), *aph(3")-lb* (29/117; 24.8%), and *bla*TEM-1 (24/117; 20.5%), the majority of which originated from ground turkey isolates [*tetB* (36/117), *tetA* (23/117), *aph(3")-lb* (24/117) and *bla*TEM-1 (20/117)]. Antibiotics use claims had no effect on MDR *E. coli* isolates from the different meat types (X^2=2.21, p=0.33). MDR was observed in isolates from meat products with labels indicating "no claims" (n=29; 69%), "no antibiotics ever" (n=9; 21.4%), and "organic" (n=4; 9.5%). *E. coli* isolates consisted of 81 known sequence types (STs) and ST117 (n=10; 8.5%), ST297 (n=6; 5.1%), and ST58 (n=4; 3.4%) were the most prevalent STs across retail meat types. The most prevalent phylogroups were B1 (n=34; 29.1%) and A (n=33; 28.2%). Thirty-four different replicon types were observed with IncFIB (AP001918) (n=73, 62.4%); Col (MG828) (n=50, 42.4%); IncFIC (FII) (n=43, 36.4%); Col156 (n=37, 31.4%); Incl1 (n=31, 26.3%); p0111 (n=26, 22%); IncFII (n=25, 21.2%); IncHI2 (n=18, 15.3%) and IncHI2A (n=18, 15.3%) as the most prevalent. AMR genes were carried on plasmids in 17 *E. coli* isolates of which 15 (88.2%) were recovered from turkey and two (11.8%) from chicken. IncHI2 and IncHI2A harbored AMR gene *aph(3")-Ib* in eight (47.1%) isolates recovered from ground turkey. Five clonal patterns were detected among the isolates.

Conclusions: We detected AMR and MDR *E. coli* from retail meat types in North Carolina. Most AMR genes and MDR were observed from turkey *E. coli* isolates. Our observation of detecting MDR *E. coli* in retail meat with no indication of antimicrobial use suggests that additional research is required to understand the origin of resistance. The presence of ST117, an emerging sequence type implicated in human pathogens, warrants further surveillance. The isolates were distinctly diverse suggesting an instability in population dynamics.

Financial Support: The authors would like to appreciate the support of Jacob Lab, Cray Lab and Thakur Lab for providing the resources required for this research. The authors received funding from FDA GenomeTrakr program grant 1U18FD00678801 for whole-genome sequencing of the isolates.

Notes:

8 - Antimicrobial susceptibilities of *enterococcus* from calves treated with chlortetracycline for anaplasmosis control

Naemi P. Bickmeier[1], Alyssa R. Toillion[1], Brandt C. Skinner[1], Tippawan Anantata[1], Raghavendra G. Amachawadi[2], Calvin Liu[3], Emily J. Reppert[2], Johann F. Coetzee[4], Kathryn E. Reif[1]

[1]Kansas State University, Department of Diagnostic Medicine, [2]Kansas State University, Department of Clinical Sciences, [3]Kansas State University, Department of Statistics, [4]Kansas State University, Department of Anatomy and Physiology. Naemibickmeier@ksu.edu

Session: Antimicrobial Use & Resistance 1, 2024-01-21, 9:45 - 10:00

Objective: In the U.S., chronic bovine anaplasmosis is commonly managed by protracted use of chlortetracycline (CTC)-medicated (0.5-2 mg/lb/BW/day) feed products, with no limit on duration of use. Prolonged antibiotic use may have unintended consequences, including development of antimicrobial resistance in off-target microbes. Among zoonotic pathogens of livestock origin, enterococcal species pose a risk to human health, a concern which is exacerbated by reports of their resistance to antibiotics important for treating human diseases, including vancomycin (VAN). The objective of this study was to evaluate changes in enterococcus antimicrobial susceptibilities from cattle provided CTC for chronic anaplasmosis control.

Methods: Holstein-Jersey cross cattle with chronic anaplasmosis were blocked by weight, randomly allocated to one of the CTC treatment groups (0-, current FDA-approved doses 0.5-, and 2-, and experimental dose, 10 mg/lb/BW/day) and fed their respective treatment for 120 days. Enterococcus were isolated from fecal samples collected pre-treatment, after 58 and 114 days of consecutive treatment, and 21-days post-treatment cessation. The isolates were speciated using Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight Mass Spectrometry (MALDI-TOF MS). Sensititre™ NARMS gram positive plates were used to evaluate enterococcus antimicrobial susceptibility to 14 antibiotics using CLSI breakpoints. The log-transformed minimum inhibitory concentration (MIC) data were subjected to linear mixed model analysis. Tests were performed at the 0.1 level with Tukey's multiplicity adjustment.

Results: The 222 isolates included *E. hirae* (61.8%), *E. casseliflavus* (19.8%), *E. durans* (6.3%), *E. mundtii* (4.9%), E. *faecium* (5.8%), *E. gallinarum (1.3%), E. aquimarinus* (0.5%) and *E. avium* (0.4%). Of all isolates, 214 were resistant to at least one antibiotic while 95 (44.3%), 89 (41.5%), 26 (12.1%), 4 (1.8%), and 1 (0.4%) isolates were resistant to one, two, three four and five antibiotics respectively. In total, 89.6% (199/222) of isolates were tetracycline (TET) resistant, of which 92.9% (53/57) were resistant before CTC administration. One isolate was VAN resistant. Long-term administration of CTC did not significantly change the median MIC for TET by treatment or over time. Median MICs for tigecycline, daptomycin, erythromycin, gentamycin, ciprofloxacin, vancomycin, ampicillin, quinupristin/dalfopristin, chloramphenicol, streptomycin, linezolid, nitrofurantoin and avilamycin also did not change or minimally changed.

Conclusions: The most isolated enterococcus species were *E. hirae* and *E. casseliflavus*. In total, 96% of the isolates were resistant to at least one antibiotic of which 90% were TET resistant, while one isolate was VAN resistant. Under the conditions of this study, FDA-approved CTC dosages for active anaplasmosis control had no or only minimal effect on increasing enterococcus TET resistance, however, most isolates were already TET resistant. Associations between TET resistance and resistance to other antibiotics were not observed. It is possible that changes in antimicrobial susceptibility may differ by enterococcus species; however, further studies with greater power are needed to address changes in antimicrobial susceptibility at the species level.

Financial Support: This project was supported by Agriculture and Food Research Initiative Competitive Grant no. 2018-68003-27463 from the USDA National Institute of Food and Agriculture.

Notes:

9 - Biosecurity education for livestock producers: A data-driven evaluation with google analytics

Isha Agrawal[1], Rima D. Shrestha[1], Hamidreza Sodagari[1], Csaba Varga[1]

[1]University of Illinois at Urbana-Champaign. ishaa3@illinois.edu

Session: Biosecurity & Infection Control 1, 2024-01-21, 8:30 - 8:45

Objective: Educating livestock producers on biosecurity is imperative for ensuring the health, sustainability, and profitability of swine and beef farms. A biosecurity-focused website is an excellent educational tool for livestock producers to emphasize the critical role of biosecurity on their farms. Adding Google Analytics to the website allows educators to evaluate, refine, and update its content to address the evolving educational needs of swine and beef farmers. The study objectives was to track website utilization and traffic data, including website acquisition, user engagement, and demographics, to obtain valuable insights into website use patterns for future enhancements.

Methods: We designed two biosecurity educational websites, one for swine and one for beef cattle producers. To evaluate the website's outreach and engagement, we established a framework utilizing Google Analytics to collect website use and traffic data. For this study, we evaluated Google Analytics data from the website's launch day (swine website: July 5th, 2022; beef cattle website: February 15th, 2023) until August 31st, 2023.

Results: For the swine biosecurity website, in approximately 14 months, we recorded 1421 users, involving 1053 new users and 368 returning visitors and an aggregated event count of 12938 (754 downloads of resources). The average engagement time of the website was 1min 48s. With a global outreach to 68 countries, the countries with the most users were the US (622), the Philippines (118), and Canada (47). Within the US, most website users were reportedly from the leading pork-producing states, including Illinois, Iowa, and Minnesota. The swine diseases page gained the highest user interest with the most prolonged engagement, while the resources page had the highest website traffic.

For the beef biosecurity website, in 6.5 months, we recorded 364 users, involving 352 new users and 12 returning visitors and an aggregated event count of 3182 (43 downloads of resources). The average engagement time of the website was 1min 31s. With a global outreach to 48 countries, the countries with the most users were the US (209), Canada (32), and Australia (12). Within the US, most website users were reportedly from Illinois, Texas, and California. The highest user interest was drawn to the beef diseases page, where engagement was most prolonged, while the biosecurity practices module acquired the highest website traffic.

Conclsuions: The study findings support the utility of the biosecurity educational website as an effective tool to reach a broad livestock farmer audience. Leveraging the data from Google Analytics helped us examine the behavioral patterns of the website users and facilitated deeper comprehension of user preferences and engagement tendencies. The outcomes of this study served as a roadmap, guiding us toward potential enhancements for our educational website in the future.

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

Notes:

10 - Enhancing food security: Assessing biosecurity attitudes and decision-making strategies of swine producers

Richmond S Baye[1], Scott Merrill [1], Asim Zia[1], Julie Smith[1], Eric Clark [1], Christopher Koliba[2]

[1]University of Vermont, [2]University of Kansas. rbaye@uvm.edu

Session: Biosecurity & Infection Control 1, 2024-01-21, 8:45 - 9:00

Objective: The 2018 African swine fever (ASF) outbreak has raised the profile of biosecurity. The ensuing consequence on decision making are often understood through the fiscal lens despite lingering socio-psychological impacts. While existing studies have solely focused on either economic or socio-psychological factors as predictors of biosecurity adoption and disease reporting, this study seeks to identify and classify livestock producers into distinct subgroups based on their attitudes towards biosecurity and risk profiles. We conducted a survey in which producers were presented with 2 different scenarios and tested producers' willingness to immediately report suspected ASF cases, their trust in government agencies to effectively manage an outbreak, their knowledge about biosecurity, willingness to buy livestock insurance, motivation to self-invest in biosecurity, readiness to report suspected infections on their farms, and their intention to contact a veterinarian to examine their herd.

Methods: We designed and launched a survey between March and June 2002, targeting swine producers across the US. The survey yielded 535 responses and was designed to gauge swine producers' intent to adopt preventive practices on their farms and to understand their underlying behavioral attitudes and risk profiles. To gain a nuanced understanding of the biosecurity profiles and latent attitudes within this population, we employed latent class analysis. This approach enables us to understand the multifaceted dimensions of swine producers' attitudes and behaviors towards biosecurity adoption and disease reporting.

Results: Our results revealed three distinct classes of producers: 1) producers who are biosecurity aware but with limited engagement, 2) biosecurity skeptics, and 3) biosecurity optimists. We examined how these groups differ and share similarities in their attitudes, risk profiles, and decision-making to implement biosecurity. For instance, Class 1 producers show zero readiness to report suspected cases of infected livestock on their farms, even though they are knowledgeable about biosecurity practices. However, they demonstrate low trust in government agencies' ability to manage disease outbreaks. Class 2 producers possess a moderate level of knowledge about biosecurity practices on their farms. They also exhibit high motivation to invest in such practices, with an increased readiness to report suspected cases of infected livestock on their farms. Additionally, they have strong trust in government agencies' ability to effectively manage disease outbreaks. On the other hand, Class 3 producers significantly deviate from those in Class 1. Nonetheless, they exhibit limited intention to contact veterinarians to examine their herd. This is also reflected in their distrust of government agencies' effectiveness in managing infectious disease outbreaks and their reduced interest in purchasing livestock insurance.

Conclusions: The heterogenous nature of these groups provide valuable insights into how individuals perceive disease risk and reporting. Consequently, our research provide insight towards risk communication strategies targeted at different producers aimed at encouraging biosecurity adoption, and reduced likelihood of incursion of a Tier 1 disease.

Financial Support: USDA | NIFA

Notes:

11 - Biosecurity gaps within commercial swine production systems using the secure pork supply framework

Marissa Hall[1], Magnus Campler[1], Kelsey Mills[2], Jason Galvis[2], Gustavo Machado[2], Andréia G. Arruda[1]

[1]Department of Veterinary Preventative Medicine, College of Veterinary Medicine, The Ohio State University, [2]Department of Population Health and Pathobiology, College of Veterinary Medicine, North Carolina State University. hall.3043@osu.edu

Session: Biosecurity & Infection Control 1, 2024-01-21, 9:00 - 9:15

Objective: Preventing the incursion of foreign animal diseases (FADs) in the United States swine industry is paramount with re-emerging threats such as African Swine Fever close to North American borders. Secure Pork Supply (SPS) plans offer swine producers tools to make biosecurity plans customized to their production systems to better prepare for disease outbreaks. As training and knowledge often differ between companies and individuals, the objective of this study was to characterize biosecurity gaps in SPS plans and maps made by personnel within production companies using the SPS guidelines.

Methods: Biosecurity managers from three commercial swine production companies agreed to create outlined SPS plans and maps (n=224) using only online SPS training materials prior to assessment. Each site map had to include features such as a perimeter buffer area, a line of separation between the building and outdoor environment, access points, animal loading areas, site entries, designated parking and disinfection areas, as well as carcass disposal routes and disposal areas. Written SPS plans had to outline site procedures in case of disease outbreaks which needed to correspond to site maps. Biosecurity gaps for individual SPS maps and plans were evaluated based on adherence to SPS guidelines, and map feature placement.

Results: Out of the 224 plans, 98.0% provided a site map in conflict with their written SPS plan. 62.9% of the maps had misplaced or missing map features, 36.6% failed to disclose animal loading areas, 26.3% failed to provide a designated disinfection area, 17.8% failed to provide a designated carcass disposal area, and 6.7% misplaced their perimeter buffer area. All sites failed to provide information regarding loading areas designated for animals only.

Conclusions: Despite access to training materials, standardized in-person biosecurity training workshops and available consulting resources may be beneficial in educating commercial swine producers and aid in preventing disease outbreaks including FADs.

Notes:

12 - Testing an extended theory of planned behavior to explain cattle producers' intent to comply with FMD controls

Asim Zia[1], Amy H. Delgado[2], Gabriela Bucini[1], Scott C. Merrill[1], Christopher Koliba[3], Gemma Del Rossi[4], Richmond S. Baye[1], Bo Norby[5], Julie M. Smith[1]

[1]University of Vermont, [2]Animal and Plant Health Inspection Service, U.S. Department of Agriculture, [3]University of Kansas, [4]University of California, Santa Barbara, [5]Veterinary Medical Center, College of Veterinary Medicine. azia@uvm.edu

Session: Biosecurity & Infection Control 1, 2024-01-21, 9:15 - 9:30

Objective: Improved understanding of the socio-psychological determinants and mediating factors of compliance with disease control measures at the producer level can better inform the design of livestock risk management strategies. By postulating and applying an extended theory of planned behavior, this study seeks to empirically understand and quantify the effects of socio-psychological determinants of a stratified random sample of Texas cattle producers' intent to comply with foot and mouth disease (FMD) control measures. We tested the following hypothesis: Risk perceptions about foot and mouth disease, perceptions about other producers' behavior, trust in regulatory agencies, and moral norms about the behavior have significant effects in explaining the intent to comply with animal disease control practices, mediated through standard Theory of Planned Behavior variables - attitude, perceived behavioral control and social norms.

Methods: Building upon a unique survey data set collected by Delgado *et al*., this study deploys a structural equation modeling (SEM) approach to empirically test the study hypotheses. A total of 2,018 (~2%) producers received Survey 1, and 2,022 (~2%) additional producers received Survey 2. Of those selected, 524 (27%) who received Survey 1 and 574 (29%) who received Survey 2 fully completed the survey and were included in the analysis. The questions regarding behavioral responses to voluntarily request veterinary examination or comply with stop-movement orders were introduced with a short scenario providing contextual information. For testing the study hypotheses, we estimated six SEMs that predict producer compliance with disease management practices to request veterinary examinations or to hold cattle when an FMD outbreak has not occurred yet (SEM #1 and SEM #4), a FMD outbreak has already occurred (SEM #2 and SEM #5), and regardless of FMD outbreak status (i.e., combined data) (SEM #3 and SEM #6).

Results: We discovered that standard Theory of Planned Behavior is necessary but not sufficient in explaining cattle producers' intent to comply with animal disease control practices. Additional latent variables such as risk perception, trust in regulatory agencies, moral norms and perceptions about other producers' behaviors contribute significantly to explaining producer intentions to comply with certain biosecurity measures. Overall, social norms that mediate the effects of trust in regulatory agencies, moral norms, and other producers' behaviors have relatively smaller yet positive effects on increasing regulatory compliance. Both attitude and perceived behavioral control have relatively large and positive effects on regulatory compliance.

Conclusions: From a practical standpoint, this study points out the value of clear communication of animal disease risk by regulatory agencies, professionals, and veterinarians; which in turn can shape producer risk perceptions and the subsequent effects on producer attitudes and perceived behavioral control towards compliance with regulations and practices. Policy interventions that increase positive attitudes and perceived behavioral control of producers towards animal disease control behaviors will likely increase both regulatory and voluntary compliance and increase system-wide resilience when faced with livestock disease.

Financial Support: This work was funded by USDA-NIFA AFRI grant # 2021-67015-35236 as part of the joint USDA-NSF-NIH-UKRI-BSF-NSFC Ecology and Evolution of Infectious Diseases program.

Notes:

13 - Using Association Rule Learning algorithms to target recommendations more likely to be implemented by dairy farmers

Faustin Farison[1, 2], Vitoria Régia Lima Campêlo[1, 2], Marie-Ève Paradis[3, 4], Sébastien Buczinski[5], Gilles Fecteau[2, 5], Jean-Philippe Roy[2, 5], Pablo Valdes Donoso[2, 5], Simon Dufour[1, 2], Juan Carlos Arango Sabogal[1, 2]

[1]Département de pathologie et microbiologie, Faculté de médecine vétérinaire, Université de Montréal, [2]Chaire de recherche de biosécurité en production laitière, Université de Montréal, [3]Association des médecins vétérinaires praticiens du Québec (AMVPQ), [4]DSAHR Inc., [5]Département de sciences cliniques, Faculté de médecine vétérinaire, Université de Montréal. faustin.farison@umontreal.ca

Session: Biosecurity & Infection Control 1, 2024-01-21, 9:30 - 9:45

Objective: Our objective was to explore the usefulness of Association Rule Learning (ARL) to 1) describe the information collected through biosecurity Risk Assessment Questionnaires (RAQ) on dairy farms and 2) predict the biosecurity practices that will be more likely to be implemented by producers based on their responses to the RAQ.

Methods: The RAQs completed by 3825 Québec dairy producers as part of the Canadian milk quality insurance program (proAction), between 2018 and 2021, were analysed using ARL algorithms. This non-supervised machine learning technique has been widely used in marketing for consumer segmentation based on buying patterns and to identify products most frequently purchased together. All non-redundant questions of the RAQs that evaluated biosecurity practices (n=31) were included in the analysis. Before applying the algorithm, we assumed that each biosecurity practice represented an item that can be purchased by the farmer. Likewise, the RAQ represented the supermarket ticket which includes the items purchased by a given producer.

Initially, the Apriori algorithm was used to generate all possible association rules (i.e., all possible combination of practices frequently applied together). A rule is composed by a set of practices forming an antecedent (X) that is associated to a consequent (Y), where X and Y are two independents sets of practices. Then, the best rules were retained based on three metrics: support (frequency of the rule), lift (strength of association between X and Y) and confidence (conditional probability to implement Y given that X is already implemented). Graph-based visualizations were used to illustrate the network of relationships between the practices. For each practice predicted by the algorithm, the composition and metrics of the corresponding rules were described.

Results: Of the 22 million rules generated by the algorithm, the best 63, based on the metrics, were retained. The best rules predicted, with a confidence ≥70%, 13 biosecurity practices that evaluated the risk of introduction of pathogens into the farm (external biosecurity; n=3) or their transmission within the farm (internal biosecurity; n=10). For instance, we observed that farmers who have visible biosecurity signage on their farm and disinfect pens that have housed sick cattle had a 91% probability to require visitors to use clean coveralls and boots. Further, farmers who require visitors to use clean coveralls and boots, have a designated area to house sick cattle, and prevent contact of calves with lactating cows had a 75% probability of not feeding non-sealable milk to their calves.

Conclusions: ARL is an interesting methodology to analyse information collected through RAQ and study the relationships between biosecurity practices on dairy farms. Since ARL identifies the practices more likely to be implemented by a given producer, it allows veterinarians to provide targeted recommendations. This is a personalized approach that might improve producers' uptake of prevention and control programs moving from a descriptive to a predictive use of the RAQ.

Financial Support: Project funded by the Natural Sciences and Engineering Research Council of Canada (NSERC)-Alliance grant (#ALLRP554326-20), Dairy Farmers of Canada, Novalait and the Agri-Food Innovation Partnership Program under the Canadian Agricultural Partnership, an agreement between the governments of Canada and Quebec.

Notes:

14 - Eliciting behavioral responses to alternate biosecurity risk management strategies in a stochastic game

Asim Zia[1], Richmond Baye[1], Scott Merrill[2], Eric Clark[2], Gemma Del Rossi[3], Jackson Dean[4], Samuel Rosenblatt[4], Nick Cheney[4], Laurent Hebert-Dufresne[4], Julia Smith[5]

[1]Department of Community Development & Applied Economics, University of Vermont, [2]Department of Plant & Soil Science, University of Vermont, [3]University of California, [4]Department of Computer Science, University of Vermont, [5]Department of Animal & Veterinary Sciences, University of Vermont. azia@uvm.edu

Session: Biosecurity & Infection Control 1, 2024-01-21, 9:45 - 10:00

Objective: Human decision-making and behavior play significant roles in the introduction, spread, recognition, reporting and containment of new, emerging or foreign diseases and pests. Detection and mitigation strategies against the introduction of disease are commonly termed "biosecurity." While there are generally accepted biosecurity risk management strategies to support herd health in food animal production systems before, during and after a disease outbreak, an improved understanding of the producers' behavioral responses in response to differential media of stochastic information and incentive manipulation treatments can inform the design of biosecurity risk management strategies.

Methods: In this study, we developed a Partially Observed Stochastic Game (POSG) to test the role of information uncertainty, risk messaging and economic incentives on producer behaviors pertaining to the adoption of biosecurity risk management strategies. The POSG is mounted online and more than 1000 Amazon Turks are invited to play the game containing 32 treatments over 5 rounds, leading to 1000x32x5 = 192,000 observed decisions. In addition, about 100 hog producers played the game in a field setting, generating 19,200 observations. In both online and field environments, subjects received differentiated payments matched with their performance in the games. The gaming data (N=211,200 observations) are analyzed with panel data models and machine learning algorithms to learn the strategies that both maximize producer profits and enhance biosecurity.

Results: We discover that both Amazon Turkers and Hog Producers adopt very similar strategies to maximize producer profits and enhance biosecurity under stochastic disease transmission and environmental uncertainty conditions. Further, we find that high profit makers in both populations of players are "risk takers," who are less likely to adopt biosecurity. Conversely, net losers in both populations tend to be risk averse, who are more likely to adopt biosecurity. Visual gauges induce risk aversion. More information about the disease in the network induces risk aversion, while information about biosecurity adoption in the network induces risk taking behaviors.

Conclusions: A combination of fine-tuned communication strategies (e.g., more visual network wide information) and policy incentives can induce higher adoption of biosecurity measures, yet this also results in net redistribution of losses and profits across market players. Additional data from stochastic games under variety of competitive and cooperative rule structures could provide useful information to design biosecurity investment policies and risk bearing strategies between public and private sectors.

Financial Support: This work was funded by USDA-NIFA AFRI grant # 2021-67015-35236 as part of the joint USDA-NSF-NIH-UKRI-BSF-NSFC Ecology and Evolution of Infectious Diseases program.

Notes:

15 - Assessment of a subunit vaccine candidate formulated with various adjuvants against *Mycoplasma gallisepticum*

Jeremy Miller[1, 2], Rosemary Ozyck[1, 2], Patrick Pagano[2], Esmeralda Hernandez[2], Megan Davis[2], Anton Karam[2], Edan Tulman[1, 2], Steven Szczepanek[1, 2], Jessica Malek[1, 3], Lawrence Silbart[1, 2, 3], Steven Geary[1, 2]

[1]Center of Excellence for Vaccine Research, [2]University of Connecticut Department of Pathobiology, [3]University of Connecticut Department of Allied Health Sciences. jeremy.miller@uconn.edu

Session: Vaccinology 1, 2024-01-21, 8:30 - 8:45

Objective: *Mycoplasma gallisepticum* is the etiologic agent of Chronic Respiratory Disease (CRD) in chickens, infectious sinusitis in turkeys, and Conjunctivitis in house finches. Infections of chickens may result in reduced egg laying and downgrading of carcasses. Those two conditions have been estimated to cause an economic loss of over $700 million annually in the United States alone. A subunit vaccine could provide advantages over currently utilized vaccines including no risk of reversion to virulence, improved adaptability to emerging strains, and ability to distinguish between vaccinated and naturally infected animals. To meet this need, we designed a subunit vaccine containing two primary adhesion proteins (GapA and CrmA) along with four early-expressed phase variable lipoprotein hemagglutination A proteins (VlhAs 3.03, 3.06, 4.07, 5.05). Previous work from our lab determined that virulent *M. gallisepticum* strain R_{low} undergoes ordered, non-stochastic switching of VlhAs in-vivo. The function of these VlhAs is not confirmed, but they are believed to play a secondary role in attachment and switch expression in response to changes in tissue morphology as infection progresses. We hypothesized that if we vaccinated with primary adhesions and known early-phase VlhAs, we could disrupt bacterial adherence and disease progression.

Methods: All proteins were produced recombinantly in E. coli. 5-week-old SPF female white leghorn chickens were vaccinated in a prime-boost schedule with 21 days between doses and 21 days between boost vaccination and challenge. Groups of 15 chickens received either saline, or all six proteins combined with an adjuvant. Vaccine doses included 50ug of each specified protein. Formulations were mixed with Montanide ISA 78VG (v/v 3:7), Addavax (v/v 1:1), Alhydrogel 2% (v/v 1:1), MPLA (20ug), CpG ODN 2007 (20ug), Pam2CSK4 (5ng), or Pam3CSK4 (5ng). Chickens were bled prior to vaccination and prior to challenge to assess seroconversion against vaccine antigens by systemic antibody response. Chickens were challenged intratracheally with $1*10^8$ CFU *Mycoplasma gallisepticum* strain R_{low} on both D0 and D2. Chickens were sacrificed on D14. Tracheal sections were taken for bacterial recovery and to assess histopathology. Excess trachea was washed with phosphate buffered saline to analyze mucosal antibody responses.

Results: Multiple adjuvants achieved significant reductions in bacterial recovery from chicken tracheas (Alum, P=.0448; MPLA, P=.0076; CpG ODNs P=.0021; Pam2CSK4, P=.0134;). Two adjuvants achieved significant reductions in thickest tracheal section (MPLA, P=.0049; CpG ODNs, P=.0050). One adjuvant achieved significant reductions in average tracheal thickness (CpG ODNs, P=.0002).

Conclusions: We have identified a promising vaccine candidate and shown that it has efficacy with numerous adjuvants. One adjuvant (CpG ODNs) resulted in improvements in all three outcome measures. This is the first report of an efficacious subunit vaccine candidate against *M. gallisepticum*.

Financial Support: Funding was received from: USDA NIFA Award # 2022-67016-37222 and Center of Excellence for Vaccine Research (CEVR).

Notes:

16 - A Platform-based approach to development and deployment of an effective vaccine to protect against an emerging disease outbreak.

Alan Young[1, 2], Amber Schueler[2], Ashley Petersen[2], Sara Erickson[2], Tom Halbur[2], Gary Anderson[2]

[1]South Dakota State University, [2]Medgene. alan.young@sdstate.edu

Session: Vaccinology 1, 2024-01-21, 8:45 - 9:00

Objective: Vaccines are critical to support the Animal Agriculture industry. The current USDA regulatory process ensures a pure, safe, potent and efficacious product, but does take years and significant expense to develop and license which limits availability against emerging diseases. The USDA recently created new subcategories for platform and prescription platform vaccines. These regulatory pathways can be used to rapidly respond with products that address pathogens with high diversity as well as new and emerging diseases. Vaccines developed under these guidelines must use a well-established, highly uniform production process, must be non-replicating subunit approaches, and demonstrate safety in the target species of interest. Multiple prescription vaccine products can be generated based off this backbone to respond to targets of commercial interest, as well as Foreign Animal Diseases (FADs).

Methods: Using this platform, an effective vaccine was developed to respond to an outbreak of Rabbit Hemorrhagic Disease virus (RHDV2) in the United States. The target VP60 protein was formulated into vaccine using a proprietary Baculovirus expression system, and deployed as a 2-dose inactivated vaccine under emergency use authorization. An in-house serological ELISA was developed in conjunction with a live-animal challenge study to evaluate effectiveness of the vaccine in preventing disease following live virus challenge.

Results: Initial studies already reported demonstrated that the vaccine was capable of protecting vaccinated rabbits from clinical disease. As a component of the full licensing pathway, we have recently completed studies that demonstrate strong antibody titers lasting greater than 6 months following vaccination. Furthermore, vaccinated rabbits were protected from live virus challenge at this time point, whereas virtually all non-vaccinated rabbits succumbed to disease. The vaccine is currently proceeding towards licensing, and can form the basis under these regulatory guidelines for more rapid responses using the platform for future disease targets.

Conclusions: This approach provides a roadmap for generation of new vaccines for animal health that can be implemented rapidly in response to immediate need. A future goal is to expand the utility of our platforms through development of new subunit vaccine approaches to address animal health issues of commercial relevance or national security. Most importantly, once fully licensed, this and other Medgene-licensed platforms can rapidly respond to new disease challenges threatening the health of US animals.

Financial Support: This study supported by Medgene, 1006 32nd Avenue, Brookings, SD. 57006

Notes:

17 - Development of bird flu mucosal-deliverable nanovaccine

Olaitan C. Shekoni[1], Jennifer Schrock[1], R. Suresh[1], D. Bugybayeva, [1], S. Dolatyabi[1], F. Akter[1], M. Singh[1], Gourapura J. Renukaradhya[1]

[1]Dept. of Animal Sciences, Centre for Food Animal Health, The Ohio State University. shekoni.2@buckeyemail.osu.edu

Session: Vaccinology 1, 2024-01-21, 9:00 - 9:15

Objective: The Avian influenza virus (AIV) is a significant threat to the poultry industry. AIV infection causes massive influenza outbreaks, high mortality, and devastating economic loss to the poultry industry globally. AIV is categorized into highly pathogenic (HPAI) and low pathogenic (LPAIV) strains. This study was aimed at developing a mucosal-deliverable nanovaccine for bird flu, with the goal of decreasing illness, death, and virus transmission in poultry. It also sought to assess how well the nanovaccine stimulates the cross-reactive mucosal immune response and reduces the viral load in the respiratory tract and feces of homologous and heterologous AIV-challenged poultry.

Methods: In this vaccine trial, H5N3 strain of LPAI was selected to prepare mannose-conjugated chitosan encapsulated inactivated AIV nanovaccine, which is also surface coated with Salmonella flagella protein (mCS-AIV-NP/F). In addition, a potent mucosal adjuvant c-di-GMP was entrapped in mCS separately (mCS-GMP-NP/F). The vaccine formulation was analyzed for physiochemical properties such as particle size, shape, and charge using Malvern zetasizer and scanning electron microscopy. For the in vivo study, one-week-old 78-layer chicks (13 groups, n=6/group) were administered with mCS-AIV-NP/F along with or without mCS-GMP-NP/F, twice at 2- and 3-week intervals via the mucosal route (oral/conjunctival/nasal), and birds were challenged with H5N3 or H5N2 strain of AIV 3-weeks later. The samples collected for analysis includes cloacal swabs, oropharyngeal swabs, bile, and serum samples at day post-vaccination (DPV) 0 and 21 and day post-challenge (DPC) 0, 3, and 5. AIV-specific homologous, heterologous, and heterosubtypic mucosal IgA and systemic IgG antibodies were analyzed by Enzyme-Linked Immunosorbent Assay (ELISA). Challenge virus load in the airways was measured by quantitative Polymerase Chain Reaction (qPCR). The IgA and IgG antibody data were presented as mean values from 2-6 chickens with standard error of the mean (± SEM). Statistical analysis included two-way ANOVA followed by Bonferroni Post-test.

Results: Our data indicated that the vaccine candidate had ideal physiochemical properties for mucosal delivery. The particles were spherical in shape, 100-400nm in size, with a Polydispersity Index value (PDI) of <0.4 and zeta potential > +30mV, suggesting the formulated vaccine was monodisperse with a high positive surface charge. Immunologically, the candidate vaccine-induced IgG and mucosal IgA antibody secretion. Also, co-administration of mCS-AIV-NP/F with mCS-GMP-NP/F induced higher levels of homologous and cross-reactive AIV-specific IgG and IgA antibody response. Furthermore, the 150 and 300 hemagglutination units (HAU) doses of the mCS-AIV-NP/F vaccine were administered both with and without c-di-GMP adjuvant-induced higher mucosal IgA antibody responses.

Conclusions: This study demonstrates the efficiency of mucosal delivery of whole inactivated AIV vaccine using mCS-NP as a delivery vehicle in poultry. In addition, coadministration of mCS-AIV-NP/F with a STING adjuvant-induced both cell-mediated and humoral immune responses in birds. Although, the formulated AIV nanovaccine failed to substantially reduce the viral load in the respiratory tract of birds. However, lowering the doses of HAU in the mCS-AIV-NP/F vaccine will address potential immune tolerance at mucosal sites due to excessive antigen mass. Furthermore, spacing out the vaccination timepoints to 3-4 boosters could enhance immune responses in birds.

Financial Support: This research was fully supported by CFAES, The Ohio State University.

Notes:

18 - Development a viral vectored vaccine against equine rotavirus A

Chandika Gamage[1, 2], Diane Cryderman[3], Come J. Thieulent[1, 2], Wellesley Dittmar[1, 2], Udeni B. R. Balasuriya[1, 2], M. Aldana Vissani[4, 5, 6], Viviana Parreño[5, 6], Maria E Barrandeguy[4, 5], Mariano Carossino[1, 2]

[1]Department of Pathobiological Sciences, School of Veterinary Medicine, Louisiana State University, [2]Louisiana Animal Disease Diagnostic Laboratory, School of Veterinary Medicine, Louisiana State University, [3]Viral Vector Core Facility, Carver College of Medicine, University of Iowa, [4]Escuela de Veterinaria, Universidad del Salvador, [5]Instituto de Virología, CICVyA, Instituto Nacional de Tecnología Agropecuaria (INTA), [6]Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). cgamage@lsu.edu

Session: Vaccinology 1, 2024-01-21, 9:15 - 9:30

Objective: Equine rotavirus A (ERVA) is a non-enveloped virus with eleven double-stranded RNA genome segments. ERVA is a major cause of diarrhea in foals worldwide, generating significant economic losses to the equine breeding industry. ERVA strains are classified into G-types based on their outer capsid glycoprotein VP7, encoded by segment 9. ERVA genotypes G3 and G14 are the predominant causes of outbreaks in foals around the globe. The currently available ERVA vaccines only include a G3 genotype strain and do not elicit complete protection, particularly against heterologous strains. Hence, the objective of the study is to develop a vector-based vaccine for the prevention of ERVA.

Methods: The modified vaccinia virus Ankara (MVA) was used as a vector platform to express the VP7 glycoprotein of ERVA genotypes G3 and G14, which contains the major neutralizing epitopes. Two shuttle plasmids were designed for MVA recombination containing the following elements: (1) green fluorescent protein coding sequence (GFP) flanked by LoxP sites, and (2) nucleotide sequences corresponding to ERVA VP7 of either G3 or G14 with a downstream His-tag and under a vaccinia-specific promoter (namely MVA-G3 and MVA-G14). Following transfection of MVA-infected immortalized chicken fibroblast (UMNSAH/DF-1) cells, GFP+ cells were sorted and re-plated. GFP from recombinant viruses recovered from GFP+ cultures were excised using a Cre recombinase-expressing plasmid and plaque purified to isolate GFP negative recombinant viruses. Recombinant viruses were plaque-purified and confirmed by PCR and sequencing. The generated recombinant MVA-G3 and MVA-G14 virus stocks were finally propagated in DF-1 cells, sucrose cushion-purified and titrated by plaque assay. One-step growth curve analysis of the wild-type MVA, and the recombinant viruses was performed on DF-1 cells at a multiplicity of infection of 5. The expression of G3 and G14 VP7 glycoproteins was confirmed by Western blotting and immunofluorescence staining. To assess immunogenicity, eight-week-old male and female BALB/c mice were immunized intraperitoneally with a 2-dose regime containing 10^7 PFU of MVA-G3, MVA-G14 or MVA-G3+MVA-G14 at a 14-day interval. Recombinant virus shedding was monitored in bedding and feces via qPCR spanning 21 days following initial vaccination.

Results: Two recombinant MVA viruses expressing VP7 of ERVA G3 and G14 genotypes were developed. Comparative one-step growth curve analysis on DF-1 cells demonstrated that the MVA-G3 and MVA-G14 replicate *in vitro* similarly to the wild-type MVA up to 48 hours post-infection (hpi). However, a significant reduction in the titer of the MVA-G14 was observed at 72 hpi, when compared to wild-type MVA ($p < 0.05$). Expression of VP7 was confirmed via immunofluorescence staining and Western blotting (~37 kDa) using an anti-His-tag antibody. No clinical signs were observed in immunized mice and no shedding of MVA-G3 or MVA-G14 was detected following vaccination and up to 21 dpv.

Conclusions: The MVA-vectored vaccine constructs expressing the VP7 glycoprotein of ERVA G3 and G14 are promising vaccine candidates against ERVA. Immunogenicity and efficacy evaluation of these recombinant vector-based vaccine candidates are in progress.

Financial Support: This study is funded by the Grayson Jockey Club Research Foundation, Lexington, KY, US and the US Department of Agriculture 1466 Formula Funds at the School of Veterinary Medicine, Louisiana State University.

Notes:

19 - Mt-10 vaccine protects Diversity Outbred mice from CVB3 infection

Mahima T Rasquinha[1], Meghna Sur[1], Kiruthiga Mone[1], Ninaad Lasrado[1, 2], David Steffen[1], Jay Reddy[1]

[1]School of Veterinary Medicine and Biomedical Sciences, University of Nebraska-Lincoln, [2]Center for Virology and Vaccine Research, Beth Israel Deaconess Medical Center, Harvard Medical School. mrasquinha2@huskers.unl.edu

Session: Vaccinology 1, 2024-01-21, 9:30 - 9:45

Objective: Enteroviruses such as group B coxsackieviruses cause a wide range of diseases in humans. Among these viruses, Coxsackievirus B3 (CVB3) is known to cause myocarditis and pancreatitis. Previously we have demonstrated that a live attenuated vaccine virus Mutant 10 (Mt-10), offers protection from multiple CVB infections as evaluated in various inbred mouse strains, but these studies may not necessarily translate in human settings due to the outbred nature of the human population. To address this issue, we sought to use Diversity Outbred (DO) mice bearing the genetic background representing 8 different inbred mouse strains, to determine the efficacy of Mt-10.

Methods: Groups of male and female DO mice received two doses of $1x10^6$ $TCID_{50}$ Mt-10, 21 days apart and were challenged with $1x10^7$ $TCID_{50}$ CVB3, seven days post-vaccination. Blood was collected for serum at 0-, 21-, 28- and 48-days post vaccination, and hearts and pancreata were collected for histological evaluation at termination.

Results: Data revealed that the sera from vaccinated and CVB3 infected animals had CVB3 specific-VP1 antibodies predominantly of the IgG2a and IgG2C isotype. Although hearts from infected and vaccinated/challenged animals did not reveal lesions, the pancreata had varying levels of atrophy and infiltration in infected, but not in vaccinated/challenged animals.

Conclusions: Together, the data suggests that Mt-10 vaccine virus can offer protection against CVB3 infections in DO mice, taking one step closer to evaluate responses in other preclinical models that includes using non-human primates.

Financial Support: Transformational Project Award by the American Heart Association [18TPA34170206]; Jackson Laboratory's Diversity Outbred Pilot Grant Program; ARD funds from the University of Nebraska-Lincoln.

Notes:

20 - A BoHV-vectored vaccine is protective against RVFV in calves and lacks nasal virus-shedding upon reactivation

Selvaraj Pavulraj[1], Rhett W Stout[1], Elise D Barras[1], Daniel B Paulsen[1], Shafiqul I Chowdhury[1]

[1]Department of Pathobiological Sciences and Louisiana Animal Disease Diagnostic Laboratory, School of Veterinary Medicine, Louisiana State University. pselvaraj1@lsu.edu

Session: Vaccinology 1, 2024-01-21, 9:45 - 10:00

Objective: Rift Valley fever virus (RVFV) is an emerging pathogen that maintains high biodefense priority based on its threat to cattle and sheep and its ability to cause human hemorrhagic fever. RVFV-infected livestock are a significant risk factor for human infection by direct contact with contaminated blood, tissues, and aborted fetal materials. Therefore, livestock vaccination in the affected regions has the direct dual benefit and one-health application of the vaccine; protection of livestock against RVFV and thereby eliminating the risk of severe and sometimes lethal human RVFV disease. In this study, we have two objectives. i) Construct a bovine herpesvirus type 1 (BoHV-1) vectored subunit RVFV vaccine and test the protective efficacy by assessing humoral and cell-mediated immune response against RVFV in the vaccinated calves. ii) Determine the latency-reactivation and nasal virus shedding property of the vaccine virus in calves.

Methods: Previously, our lab has developed a BoHV-1 quadruple gene-deleted vector that lacks virulence and immunosuppressive properties (UL49.5, glycoprotein G, gE cytoplasmic tail [gE-CT] and US9; BoHV-1qmv). In the BoHV-1qmv vector, we have incorporated RVFV envelope proteins Gn ectodomain sequence fused with bovine granulocyte-macrophage colony-stimulating factor [GMCSF] and Gc and expressed it as a proteolytically cleavable polyprotein (designated as BoHV-1qmv Sub-RVFV). We have characterized the vaccine virus in vitro and confirmed the chimeric Gn-GMCSF and Gc proteins expression by immunoprecipitation/immunoblotting. To determine the vaccine's protective immunogenicity against RVFV, calves (n=8) were immunized with our vaccine, both intranasally and subcutaneously. In addition, we determined the latency-reactivation and nasal virus shedding property of the BoHV-1qmv Sub-RVFV in the immunized calves.

Results: The BoHV-1qmv Sub-RVFV vaccine virus replicated as like BoHV-1 wild-type (wt) but has a smaller plaque phenotype. Both the Gn-GMCSF and Gc chimeric proteins were expressed with their expected molecular mass of 72 kD and 62 kD, respectively. The vaccine induced moderate RVFV-specific neutralizing antibody titers in the vaccinated calves. Additionally, the peripheral blood mononuclear cells (PBMCs) isolated from the vaccinated calves, when stimulated with heat-inactivated RVFV MP12 antigen *in vitro*, produced six-fold increased levels of interferon-gamma transcripts compared with that of the unvaccinated control calves. In the latency reactivation study, both BoHV-1 wt and BoHV-1qmv Sub-RVFV established latency in the trigeminal ganglion (TG) following intranasal inoculation. Upon dexamethasone-induced latency-reactivation, BoHV-1 wt-infected calves shed the virus in nasal secretions and there was a memory B-cell response following the reactivation. However, in the case of vaccinated calves, the vaccine virus reactivated in the TG neurons and replicated but as expected, due to the defective anterograde neuronal transport (gE-CT and US9 deletion), there was no nasal virus shedding and no memory B-cell response.

Conclusions: A single dose of BoHV-1qmv Sub-RVFV vaccine is highly immunogenic and efficacious against RVFV in calves. The BoHV-1qmv vector is safe and does not shed nasal secretions upon latency reactivation. This property of the BoHV-1qmv Sub-RVFV prevents the likelihood of vaccine virus circulation in cattle and the risk of reversion to virulence. The efficacy and immunogenicity of BoHV-1qmv Sub-RVFV vaccine against RVFV in sheep is currently in progress.

Notes:

21 - Analyzing key factors and dynamics of African Swine Fever spread: Philippine insights

Jesper Chia-Hui Hsu[1], Rachel Schambow[1], Maximino Montenegro [2], Ruth Miclat-Sonaco [3], Andres Perez [1]

[1]Center for Animal Health and Food Safety, College of Veterinary Medicine, University of Minnesota, [2]Pig Improvement Company (PIC) Philippines, [3]National Livestock Program, Office of the Undersecretary for Livestock, Department of Agriculture. hsu00124@umn.edu

Session: Epidemiology 1, 2024-01-21, 8:30 - 8:45

Objective: This research addresses the urgent concern of African swine fever (ASF), a highly fatal disease affecting domestic pigs and wild boar. Since its introduction to the Philippines in July 2019, the rapid spread of genotype II ASF virus has posed a significant threat to the swine industry. Through spatial-temporal analysis and quantitative risk factor prioritization, this research aims to identify spatial and temporal patterns and key factors influencing ASF spread in commercial and backyard farms in the Philippines.

Methods: Data on reported ASF outbreaks from August 2019 to July 2022 were obtained from the International Training Center on Pig Husbandry, Department of Agriculture of the Philippines. The data included event ID, administrative levels, and dates of reporting. Outbreak locations were approximated using the centroid (latitude and longitude) of the smallest administrative level. Descriptive statistics were computed using R software, and spatial clustering was assessed using the global Moran's *I* test in ArcGIS Pro (ESRI Inc., Redlands, CA, USA). Local space-time clusters were identified using the SaTScan software v10.0.2 (Kulldorff, Cambridge, MA, USA) [1] with Monte Carlo simulation.

A committee evaluation process selected 25 participants with expertise in the Philippine swine industry who attended a May 2023 educational workshop in Batangas. The participants included 10 members from the Philippine veterinary services and 15 from the swine industry. During the workshop, the spatial-temporal results were presented and interpreted locally. Additionally, a conjoint analysis was conducted within the group to identify and prioritize risk factors associated with ASF introduction and transmission in the Philippines. To estimate the relative weighted value for each risk factor, logistic regression and ordinal logistic regression models were performed in RStudio version 4.2.2 using the MASS package [2].

Results: This study analyzed 19,697 ASF farm outbreaks in the Philippines from August 2019 to July 2022. Spatial and temporal clustering was observed, with Central Luzon being the most affected region. Outbreaks were highest from August to October and lowest in April and May, potentially influenced by environmental factors and cultural practices. In addition, the conjoint analysis identified swill or contaminated feed, inadequate biosecurity protocol, and personnel movement as significant risk factors for ASF spread among farms in general. Swill-fed or contaminated feed had the highest odds ratio of 19.84 (95% CI 12.09-33.22), followed by personnel movement (11.11, 95% CI 6.88-18.26), and absence of a disinfection protocol (7.54, 95% CI 4.73-12.21). Commercial farms faced additional risks from contaminated vehicles and personnel, while backyard farms were vulnerable to introduction of new pigs, environmental contamination, and poor feeding quality.

Conclusions: Space-time analyses revealed annual clusters of ASF outbreaks occurring over three-month periods in the second half of the year, enhancing our understanding of temporal patterns. Human behavior, including swill feeding, inadequate biosecurity, and lack of disinfection protocols, significantly influenced ASF spread. Preventing vehicle and individual access to susceptible farms was crucial, outweighing the impact of wild boar populations. Strict biosecurity measures and targeted interventions are vital for controlling and preventing ASF, safeguarding the swine industry and public health in the Philippines.

Financial Support: 2023-2024 MnDRIVE Global Food Ventures (GFV).

Notes:

22 - Using MALDI-TOF mass spectrometry to determine *Staphylococcus chromogenes* strain types

Pamela R. F. Adkins[1], Ridwan Adesola[1], John W. Barlow[2]

[1]University of Missouri, [2]University of Vermont. adkinsp@missouri.edu

Session: Epidemiology 1, 2024-01-21, 8:45 - 9:00

Objective: The objective of this study was to determine if MALDI-TOF mass spectrometry can be used to identify the multilocus sequence typing (MLST) clonal complex (CC) of *Staphylococcus chromogenes* isolates collected from dairy farms in the United States.

Methods: A collection of *S. chromogenes* isolates (n = 46) with known MLST CC types were used in this study. A total of 15 *S. chromogenes* isolates were selected as representatives of each CC type included within the collection, including at least 2 isolates of each CC type. The selected isolates were prepared using tube extraction methods and then added to the MALDI-TOF database, following the manufacturer's guidelines (Bruker Daltonics, Billerica, Massachusetts) to create the *S. chromogenes* study MALDI library. Next, all study isolates were tested using the newly created library. First, four MALDI-TOF spots were plated for each isolate, including two tube extraction spots and two plate extraction spots. Collected data was evaluated in 4 ways and in all evaluation methods, 2 MALDI-TOF data points were used. The MALDI-TOF data collection methods included collecting 1) the top 2 matches within 1 spot run for the tube extraction method, 2) the top 2 matches within 1 spot run for the plate extraction method, 3) the top match for each of the 2 spots run for the tube extraction method, and 4) the top match for each of the 2 spots run for the plate extraction method. Results were defined as known if both CC types identified using the two MALDI evaluation points matched and then defined as correct if both CC types within the MALDI evaluation method matched the known test isolate CC type. Known results were defined as incorrect if the CC types within the MALDI evaluation method were the same but did not match the test isolate CC type. Results were defined as unknown if 2 different CC types were identified within the MALDI evaluation method.

Results: Data was successfully collected for all methods and all isolates except for two isolates using the 1 spot plate extraction technique. Overall, most samples were classified as unknown when using the 1 spot methods, including 76% (70/92) with the tube extraction method and 96% (86/90) with the plate extraction method. Most samples were classified as known using the 2 spot methods, including 61% (28/46) using the tube extraction method and 52% (24/46) using the plate extraction method. Overall, using 2 spots and the plate extraction method resulted in the most correctly identified isolates, with 87.5% (21/24) of the classified isolates being identified as the correct CC type.

Conclusions: MALDI-TOF is capable of determining the MLST CC types of *S. chromogenes* isolates. Among evaluation methods used to date, using the top match for each of 2 spots run and the direct plate method of MALDI-TOF mass spectrometry provided the most correct matches with MLST CC types. Using MALDI-TOF data to strain type isolates is fast, simple, and inexpensive. This technique may help provide data necessary to understand strain differences among *S. chromogenes* isolates.

Financial Support: This research was funded by the USDA-NIFA award #2022-67015-37123.

Notes:

23 - The impact of ecogeographical variables on the potential distribution of *Ornithodoros* spp. in Texas

Alexa K. Mendoza[1], Cora Garcia[1], Leila Akhand[1], Dee Ellis[2], Christopher Butler[3], Meriam Saleh[1]

[1]School of Veterinary Medicine and Biomedical Sciences, Texas A&M University, [2]Texas A&M AgriLife Extension Service, [3]College of Arts & Sciences, Texas A&M University. akmendoza@cvm.tamu.edu

Session: Epidemiology 1, 2024-01-21, 9:00 - 9:15

Objective: *Ornithodoros turicata* is an argasid tick and competent vector for African swine fever virus (ASFv) in laboratory settings. If ASFv was introduced to the United States, this re-emerging global swine disease would cause severe economic consequences. The widespread presence of feral hogs and warthogs coupled with *O. turicata* in Texas, cultivates a greater risk of ASFv becoming established within the state. Establishment of ASFv within *Ornithodoros* spp. would complicate eradication efforts because of the ticks' nidicolous ecology, longevity, and indiscriminate feeding behaviors. The specific aims of this study were to determine the ecogeographical variables that most influence the potential distribution of *Ornithodoros* spp. and to conduct field surveillance for these ticks in Texas.

Methods: A literature review was conducted to identify suitable tick habitats and survey locations. Surveillance sites were focused along the US-Mexico border and near swine operations, with 16 counties selected. Ticks were captured using dry ice-baited traps near animal habitats. Ticks were identified using standard morphologic keys and a subset were confirmed molecularly via PCR and sequencing of the 16S rRNA gene fragment. Ecological niche modeling was used to determine the bioclimatic variables associated with *O. turicata* presence. Nineteen bioclimatic variables in addition to elevation were used to determine which bioclimatic factors most influence the distribution of *O. turicata*, which were used to create maps of predicted suitability based on where in the United States these variables occur.

Results: Collections have been completed in 13/16 counties, with *Ornithodoros* spp. ticks identified in 11/13 counties. All ticks (n=1552) were identified as *O. turicata*. Six bioclimatic variables of importance were identified: precipitation seasonality, maximum temperature of warmest month, minimum temperature of coldest month, mean temperature of wettest quarter, mean temperature of warmest quarter, and mean temperature of coldest quarter. A map of potential *O. turicata* distribution was constructed using these six variables and depicted a distribution of *O. turicata* that stretched from southern California to Texas, with an allopatric population in Florida. The suitability map of Texas depicted the vast majority of Texas as being a potential suitable habitat for *O. turicata* except for the easternmost quarter.

Conclusions: Establishing the current and projected distribution of *O. turicata* is essential to understanding the potential sylvatic cycle of ASFv. Our model shows an expanded distribution than previously known in the Northeast. As ASFv is in North America and as the feral hog population continues to grow, the variables needed to establish ASFv in the United States are present. Should ASFv become established in the United States and enter our pork industry, the industry would suffer massive economic devastation with rippling effects to other industries in the agricultural system and our ability to export pork products to our trading partners. Continued surveillance in these geographical ranges is a crucial proactive measure needed to address the threat this re-emerging transboundary animal disease poses to the United States.

Financial Support: This material is based upon work supported by the U.S. Department of Homeland Security under Grant Award Number, Award No 18STCBT00001 through the Cross-Border Threat Screening and Supply Chain Defense Center of Excellence.

Notes:

24 - Quantifying equine influenza transmission in Mongolia

Andrew W. Park[1], Ayanna Johnson[2], Carlos Molinero[1], Annakate M. Schatz[1], Mafalda Viana[3], Daniel R. Perez[1], Daniela S. Rajao[1], Batchuluun Damdinjav[4], Pablo R. Murcia[3]

[1]University of Georgia, [2]Norfolk State University, [3]University of Glasgow, [4]Mongolian State Central Veterinary Laboratory. awpark@uga.edu

Session: Epidemiology 1, 2024-01-21, 9:15 - 9:30

Objective: To quantify exposure risk of horses to influenza from wild bird populations and estimate rate of transmission between horses following exposure.

Methods: Equine influenza positivity rates were calculated from field data collected at three separate time points between 2021-2022 and collated along with each population's herd size and risk group status (high or low based on proximity to water sources used as stopover sites by wild birds). A stochastic SIR model of pathogen transmission was constructed and partly parameterized from existing literature. Less well identified parameters were varied between plausible ranges and model output was compared to field data to estimate scenarios most consistent with observed data.

Results: Parameters reflecting equine-adapted strains best captured the range of positivity rates (0-70%). High risk groups representing a four-fold risk of exposure to influenza captured observed differences between low and high-risk groups in field data. Inclusion of seasonal exposure risk was required in models to generate observed patterns in data. Interactions between waning immunity and seasonal exposure risk mean that low and high-risk groups are reliably discernable from positivity rate data at certain times of year but not others.

Conclusions: Simulation of equine influenza transmission with stochastic SIR models generates dynamics consistent with data and implies that circulating strains are well-adapted to horses, exhibit seasonal risk of exposure and that populations proximate to wild bird stopover sites may experience up to a four-fold increase in exposure risk. Additionally, the research reveals that sampling throughout a year (versus at the same time each year) can be vital in animal disease research to detect underlying differences in risk of exposure between populations that might otherwise be masked by interactions between waning immunity and seasonal transmission.

Financial Support: Support for this research was provided by the National Science Foundation (grant #1659683) through the Population Biology of Infectious Diseases Undergraduate Research program at UGA

25 - Non-invasive sampling strategies for the molecular surveillance of EHV-2 in naturally shedding yearling horses

Amjad Khan[1], Edward Olajide[1], Madeline Friedrich[2], Anna Holt[2], Lutz S. Goehring[1]

[1]Department of Veterinary Science, University of Kentucky, [2]Richard A. Gillespie College of Veterinary Medicine, Lincoln Memorial University. akh242@uky.edu

Session: Epidemiology 1, 2024-01-21, 9:30 - 9:45

ObjectiveS: Equid alphaherpesvirus 1 (EHV-1) is a highly contagious respiratory tract pathogen of horses, and initial infection may be followed by myeloencephalopathy or (last trimester) abortion. Surveillance and early detection have focused on PCR assays using nasal swabs collected from nasal passages, which is cumbersome and not well tolerated by the horse. This study aimed to assess non-invasive sampling techniques as surveillance tools using a cohort of 11 horses naturally infected with Equid gammaherpesvirus 2 (EHV-2). EHV-2 served as a low pathogenicity surrogate for EHV-1 with presumably a similar shedding behavior.

Methods: Horses were individually housed indoors for 10 hours periods on 2 consecutive days. Daily sampling involved rayon swabs, with nasal swabs as the gold standard diagnostic sample. Alternative samples included nostril wipes (NW), environmental surface samples (smear option); droplet catching devices which stayed outside the stall bars (indirect transmission), and air sampling (AS), with the latter done via 2 strategies: individual air samples collected going from stall to stall (200L/horse), and collective air sample collected at a stationary central (aisle) point for 6 hours (total volume: 18m^3 air) using an air sampler (Bertin™ Coriolis Micro Air Sampler). Initial screening of the samples was done through quantitative PCR (qPCR; QuantStudio7 Life Technologies) using specific primers and probes. Absolute quantitation of the samples was performed through digital PCR (dPCR; Absolute QTM, Life Technologies) quantify the number of glycoprotein B (gB) genome copies per microliter of extracted DNA template.

Results: On day 1, nine horses, followed by eleven on day 2, showed detectable EHV-2 genome copies through qPCR in nasal swabs. dPCR analysis revealed 95.5% positivity for nasal swabs, 81.8% for environmental surfaces, and 90.1% for droplet-catching devices. Nasal swabs showed significant concordance with nostril wipes (100%, $P = 0.90$), with catching devices at 94.7% and environmental surfaces at 78.9%, displaying insignificant variation ($P > 0.05$). In total, all air samples tested positive via PCR, with an average genomic DNA concentration of 5.91 DNA copies L^{-1} of air (5.9×10^3 / m^3 of air) for individual samples. For combined air samples, the mean genomic DNA concentration was 0.67 copies L^{-1} of air (6.67×10^2 copies / m^3 meter of air). An agreement of 100% was observed between air samples and nasal swabs in terms of detection

Conclusions: While air sampling has reliably detected EHV-2 genome copies using 2 different sampling techniques, its use as a successful surveillance tool will require threshold determinations and most likely protocol adjustments to detect single shedding animals in a closed barn situation. Individual nostril wipes are considered less invasive for the horse, and provide a good alternative to nasal swabs, However, collection is still labor-intensive and requires strict barrier precautions to avoid indirect (fomite) transmission between horses.

Financial Support: This study was funded by International Equestrian Federation (FEI), Lausanne Switzerland.

Notes:

26 - Investigating the role of nilgai antelope in the transmission cycle of bovine babesiosis in Texas, USA

Tammi L. Johnson[1], Kelly A. Persinger[1], Naomi S. Taus[2], Sara K. Davis[2], Lowell S. Kappmeyer[2], Jacob M. Laughery[2], Karen C. Poh[2], Massaro W. Ueti[2], Pia U. Olafson[3]

[1]Texas A&M AgriLife Research, [2]USDA, ARS, Animal Disease Research Unit, Washington State University, [3]USDA, ARS, Knipling-Bushland United States Livestock Insects Research Laboratory. tammi.johnson@ag.tamu.edu

Session: Epidemiology 1, 2024-01-21, 9:45 - 10:00

Objective: In the mid-1950s cattle fever ticks, *Rhipicephalus* (*Boophilus*) *microplus* and *R.* (*B.*) *annulatus,* were considered eradicated from the US except for an area along the Texas-Mexico border, known as the Tick Eradication Quarantine Area (TEQA). In this region, nilgai antelope (*Boselaphus tragocamelus*) and white-tailed deer (*Odocoileus virginianus*) are two common alternative hosts for cattle fever ticks, having been found infested in Texas and Mexico. To date, there have been no studies evaluating the role that nilgai antelope may play in the bovine babesiosis disease transmission cycle. The objective of this research was to evaluate susceptibility of nilgai antelope to infection by one of the causative agents, *Babesia bovis*, as a means of assessing their propensity to serve as sources of infections to tick vectors.

Methods: Wild-captured nilgai calves were hand-reared and acclimated to human interaction until 4-5 months of age. In each study, nilgai calves and a *Bos taurus* calf (positive control) were challenged with either *B. bovis* blood-stabilate (merozoite stage, n = 4 nilgai) or *B. bovis*-infected *R.* (*B.*) *microplus* larval tick homogenate (sporozoite stage; n = 4 nilgai) via intravenous inoculation. Animals were monitored for clinical and molecular signs of infection, including elevated body temperature and anemia, as indicated by decreased packed cell volume, and via PCR, serology (ELISA), histopathology, *in vito* culturing, and sub-inoculation of exposed nilgai blood into naïve *B. taurus* calves.

Results: None of the nilgai calves showed any evidence of clinical infection over the course of the study when inoculated with either life-stage of *Babesia* parasites; body temperature and PCV remained normal and stable. Further, there was no signs of infection being established in any of the diagnostic assays performed. In contrast, both *B. taurus* positive control animals exhibited clinical signs of infection (increased temperature, anemia) and were PCR-positive using a diagnostic assay.

Conclusions: Given the overlapping habitat of these alternative hosts with primary cattle hosts, it is imperative to understand the impacts of these exotic species, as they are a new addition to the ecology and epidemiology of this disease system. If nilgai antelope are not susceptible to infection with *B. bovis*, as has been shown for white-tailed deer, they may act as dilution hosts by eliminating *Babesia* from the ticks and breaking the parasite transmission cycle, thus decreasing the likelihood of disease re-emergence and establishment in the United States.

Financial Support: This research was funded by USDA-ARS Non-Assistance Cooperative Agreements 58-3091-9-014 and 58-2090-017 and USDA National Institute for Food and Agriculture Hatch Project #1019784.

Notes:

27 - The respiratory pathogen *Mycoplasma ovipneumoniae* forms biofilms that increase resistance to antibiotic treatment

B. Tegner Jacobson[1], Jessica DeWit[1], Eli Selong[1], Noah Adams[1], LaShae Zanca[1], McKenna Quirk[1], Katrina Lyon[1], Chris Corona[1], Michael Throolin[1], Erika Schwarz-Collins[2], Mark Jutila[1], Diane Bimczok[1]

[1]Montana State University, [2]Montana Veterinary Diagnostic Laboratory. bry.tegner@gmail.com.

Session: Bacteriology, 2024-01-21, 8:30 - 8:45

Objective: *Mycoplasma ovipneumoniae* (*M. ovi*) is a respiratory pathogen associated with mild atypical pneumonia in domestic sheep, or severe pneumonia in bighorn sheep. *M. ovi* can be transmitted by asymptomatic carriers with *M. ovi* colonization of the upper airways. In our recent challenge experiment in specific pathogen free (SPF) domestic lambs, *M. ovi* established persistent colonization of the nasal passages that was resistant to antibiotic treatment. Other *Mycoplasma spp.* have been found to form biofilms, and biofilm formation increases antibiotic resistance in many organisms. The objective of this study was to determine whether *M. ovi* can form biofilms and whether such biofilms would have an increased resistance to antibiotic treatment.

Methods: Biofilm formation by *M. ovi* was tested using the *M. ovi* reference strain (Y98, ATCC 29419) and a *M. ovi* clinical isolate (MSU-NW4) on multiple growth surfaces such as glass, glass-like polymer, and plastic and with different atmospheric conditions such as 5% CO_2, aerobic and microaerobic treatments. Biofilms were stained with either crystal violet or a SYTO9/PI live-dead stain for confocal imaging to observe the thickness and the structure of the biofilms. 96-well glass bottom plates were used to perform an antibiotic susceptibility test for the biofilms and were compared to the planktonic *M. ovi* grown in plastic 96-well plates.

Results: Both the reference Y98 strain and the MSU-NW4 clinical isolate showed robust biofilm formation *in vitro* with high levels of confluence observed by Y98 that were comparable to *M. bovis,* which is a known biofilm-forming *Mycoplasma* species. The optimal growth for the biofilms was on glass coverslips in liquid broth media or on glass bottom 96-well plates incubated at aerobic conditions. The Y98 reference strain showed an increase in resistance to gentamicin treatment from 4 µg/mL in the planktonic phase to 64 µg/mL as a biofilm.

Conclusions: Our data show strong support for *in vitro M. ovi* biofilm formation and provide evidence that these biofilms can result in increased antibiotic resistance. Biofilm formation by *M. ovi* could contribute to bacterial persistence in the ovine upper respiratory tract of asymptomatically infected carriers, contributing to spread of the organism. Moreover, biofilm formation could also contribute to the failure of antibiotic treatment regimens that target *M. ovi*. The optimization of growth conditions that allow us to form *in vitro* biofilms will provide a foundation for further studies and understanding of the biofilm-associated effects on infection.

Financial Support: This work is supported by the following: USDA-NIFA award 2023-67011-40356, USDA award 2022-67016-36503, and Montana Agricultural Experiment Station.

Notes:

28 - Characterization and resolution of bacterial polymicrobial biofilms by bovine respiratory disease pathogens

Thomas J. Inzana[1], Padma Rajagopalan[2], Bindu Subhadra[1], Weiman Weng[1], Pablo Sobrado[2]

[1]Long Island University, [2]Virginia Tech. thomas.inzana@liu.edu

Session: Bacteriology, 2024-01-21, 8:45 - 9:00

Objective: Chronic bovine respiratory disease (BRD) is a biofilm disease, which makes the bacterial pathogens associated with BRD more resistant to antibiotics and host immune defenses. We have previously characterized biofilm formation by *Histophilus somni* and *Pasteurella multocida*, and are now characterizing the biofilm of *Mannheimia haemolytica*, and polymicrobial biofilm formation. To further study and characterize biofilm formation by these pathogens, 3D bovine tissue culture (organoids) will be used. Due to the enhanced resistance of bacterial biofilms to antibiotics and host immune responses, compounds will be identified that remove most of the biofilm matrix.

Methods: Detailed analysis of the *M. haemolytica* biofilm and of polymicrobial biofilms will be studied by confocal laser scanning microscopy (CLSM). Biofilm quantification is measured by crystal violet staining. Biofilm formation will be studied from attachment through maturation on bovine 3D tissue culture organoids by CLSM. Compounds identified that remove most of the *H. somni* biofilm are being further examined for their effect on biofilms of other bacterial species and enhancing bacterial susceptibility to antibiotics.

Results: *M. haemolytica* forms a poor biofilm individually, but biofilm formation by a capsule-deficient mutant is improved. Nonetheless, *M. haemolytica* does form a polymicrobial biofilm with *H. somni* and *P. multocida*. The physical properties of the polymicrobial biofilm was more dense and rougher than the single species biofilm. Bovine epithelial cells (BT cells) and endothelial cells (CPA cells) were efficiently grown in a 3:1 ratio in a 3D collagen matrix. *H. somni* strain 2336 added to the 3D cell cultures at various concentrations (10^7, 10^6, or 10^5 colony forming units/ml) grew on and formed a biofilm in these organoid cell cultures. Three compounds from a library of over 5000 effectively removed >80% of an established *H. somni* biofilm matrix. At least one compound was also highly effective at removing the biofilm matrix of *P. multocida* and *M. haemolytica*. There was negligible toxicity of these compounds for bovine tissue culture cells, although such toxicity varied depending on the concentration of the compound and cell type.

Conclusions: Bacterial biofilms become established in chronic infections, and are often polymicrobial. We have confirmed that BRD pathogens effectively form a polymicrobial biofilm, which may confer advantages to each individual species. Bovine 3D tissue cultures can be established and used to more effectively study bacterial biofilms *in vitro* than in tubes or flow cells. Compounds with little toxicity have been identified that can remove bacterial biofilms, and may be able to be used in conjunction with antibiotics to enhance treatment of BRD and other diseases.

Financial Support: This work was supported by USDA-NIFA grant 2019-67015-29916 to TJI and from funds from Long Island University.

Notes:

29 - Alternative approach for antibiotic susceptibility determination in *Mycoplasma* biofilms

Eli Selong[1], Jessica DeWit[1], McKenna Quirk[1], Chris Corona[1], Michael Throolin[1], LaShae Zanca[1], Diane Bimczok[1], B. Tegner Jacobson[1]

[1]Montana State University. eli1selong@gmail.com

Session: Bacteriology, 2024-01-21, 9:00 - 9:15

Objective: *Mycoplasma bovis* is a respiratory pathogen that is strongly associated with bovine respiratory disease. *M. bovis* and can form biofilms *in vitro,* and biofilms are associated with increased antibiotic resistance in many other biofilm forming organisms. A decrease in acid production has been observed in the biofilm state by *M. bovis*, which makes interpretation of the standard microdilution antibiotic susceptibility assay difficult. We propose an alternative approach for antibiotic susceptibility testing in biofilm-forming *Mycoplasma* that utilizes flow cytometry to determine the percentage of live to dead organisms.

Methods: A reference strain for *M. bovis* (ATCC 25523) was used for the development of the live/dead percentage assay. Both planktonic and biofilm growth of *M. bovis* were analyzed using the standard broth microdilution method. The acid production of the biofilms at gentamicin concentrations below the minimum inhibitory concentration (MIC) for planktonic *Mycoplasma* was assessed to determine whether there was a reduction in acid production at these subinhibitory concentrations. To perform the live/dead percentage assay, pre-formed biofilms and planktonic cultures were disrupted via sonication, subjected to a wide range of gentamicin concentrations (0.25 µg/mL - 128 µg/mL), incubated for 6 days, and then were stained with SYTO9 and propidium iodide (PI). The stained cells were then analyzed with flow cytometry to observe the percentage of live versus dead cells in the biofilms. The MIC of the biofilms was defined as the concentration that led to a significant drop in this percentage.

Results: Results from the standard broth microdilution assays performed with *M. bovis* biofilms were difficult to interpret due to the lack of visible acid production. The acid production assays allowed us to quantify this decrease in acid production at low levels of gentamicin with no significant difference in pH between the uninoculated controls and the biofilms, even as biofilm biomass increased. Using the live/dead ratio percentage to determine susceptibility to gentamicin, we observed an MIC of 4 µg/mL for planktonic *M. bovis* and an MIC of more than 32 µg/mL for *M. bovis* grown as a biofilm.

Conclusions: The reduction of acid production by the *M. bovis* biofilms renders MIC results from standard broth microdilution assays uninterpretable. Our new live/dead percentage method enabled a more accurate determination of MICs for *Mycoplasma* biofilms. The flow cytometry method and the standard method resulted in similar MICs for the planktonic *Mycoplasma,* and an increase in gentamicin MIC was observed with the *M. bovis* biofilms. The new live/dead percentage method could also be applied to other low-acid producing *Mycoplasma spp.* as a complement to current methods.

Financial Support: This work is supported by the following: USDA-NIFA award 2023-67011-40356, USDA award 2022-67016-36503, and Montana Agricultural Experiment Station.

Notes:

30 - The effects of microbiota, bile acids, and pH on *Campylobacter jejuni* in vitro growth

Bilal Alrubaye[1], Tahrir Alenezi[1], Ying Fu[1], Janashrit Shrestha[1], Hong Wang[1], Xiaolun Sun[1]

[1]University of Arkansas. alrubaye@uark.edu

Session: Bacteriology, 2024-01-21, 9:15 - 9:30

Objective: The prevalent foodborne pathogen *Campylobacter jejuni* asymptomatically colonizes chicken intestine, yet it doesn't colonize conventionally raised mice. The underlying mechanism of the differential colonization by *C. jejuni* remains incompletely understood. Interestingly, chickens and mice have different intestinal microbiota and bile. We aimed to investigate the effect of microbiota and bile acids on *C. jejuni in vitro* growth.

Methods: The small intestinal content (SC) from chickens and mice were collected and anaerobically cultured in Brain Heart Infusion broth (BHI) in the presence of 1.5 mM cholic acid (CA) or chenodeoxycholic acid (CDCA) for 24h. The pre-cultured media were autoclaved, pH measured, and named m/ch-SC-CA or m/ch-SC-CDCA. BHI pH was adjusted to 5.5 and 5.75, 6, 6.5 and 7, and CA, CDCA, deoxycholic acid (DCA) and lithocholic acid (LCA) were added to 1.5 mM. *C. jejuni* 81-176 at around 7 log10 CFU/ml was anaerobically cultured in the pre-cultured media or the BHI with bile for 24h. *C. jejuni growth* was enumerated by serial dilution and plating under a microaerobic condition for 48h.

Results: *C. jejuni* growth was 8.7 log10 CFU/ml after 24 h anaerobic culture in BHI. Notably, *C. jejuni* growth was 0, 0, and 6.7 log10 CFU/ml in m-SC, m-SC-CA or m-SC-CDCA, respectively, while its growth was 5.1, 5.3, and 5.7 log10 CFU/ml in ch-SC, ch-SC-CA or ch-SC-CDCA, respectively. We then reasoned that mouse and chicken microbiota might differentially influence pH. Indeed, the pH value of m-SC, m-SC-CA, m-SC-CDCA, ch-SC, ch-SC-CA and ch-SC-CDCA was 5.47, 5.46, 7.08, 6.44, 6.42, and 6.25, respectively. Based on the pH value, *C. jejuni* was cultured in BHI with various pH and 1.5 mM CA or CDCA. Notably, *C. jejuni* growth was 0, 2.4, 3.6, 5.4, and 6.0 log10 CFU/ml BHI with pH of 5.0, 5.5, 6.0, 6.5, and 7.0, respectively. Interestingly, *C. jejuni* growth was 0, 3.9, 5.1, 6.2, and 6.0 log10 CFU/ml of 1.5 mM CA BHI with the four pH, respectively, while its growth was 0, 0, 3.4, 6.3, and 5.3 log10 CFU/ml of 1.5 mM CDCA BHI with the four pH, respectively. We then examine the effect of inoculum levels on *C. jejuni* growth with different pH and bile acids. *C. jejuni* growth was 5.4 vs. 4.5, 5.4 vs. 4.5, and 5.1 vs. 4.3 log10 CFU/ml at pH of 6.0, 1.5 mM CA, or 1.5 mM CDCA with inoculum of 8.0 vs. 7.0 log10 CFU/ml. Notably, *C. jejuni* growth was 1.6 vs. 3.2, 4.3 vs. 3.2, and 3.0 vs. 2.5 log10 CFU/ml at pH of 5.75, 1.5 mM CA, or 1.5 mM CDCA with inoculum of 8.0 vs. 7.0 log10 CFU/ml. Furthermore, *C. jejuni* growth was 4.4 vs. 3.3 and 3.0 vs. 3.1 log10 CFU/ml at pH of 1.5 mM DCA or LCA with inoculum of 8.0 vs. 7.0 log10 CFU/ml.

Conclusion: These data suggest that *C. jejuni* growth and colonization might be influenced by microbiota-modulated pH, metabolized bile acids, and the colonization levels. Unraveling the complete mechanism may provide new intervention strategies to control *C. jejuni* chicken and human infection.

Financial Support: This research was supported by grants from Arkansas Biosciences Institute, USDA National Institute of Food and Agriculture (NIFA) Hatch project 1012366, NIFA Hatch/Multi State project 1018699, NIFA SAS 2019-69012-29905, and NIFA project 2020-67016-31346 to X.S and B. H.

Notes:

31 - Identification of *Anaplasma marginale* adhesins that bind tick cells and bovine erythrocytes using phage display

S. M. Noh[1], S. M. Jarvis[1], M. S. M. Solyman[2], O. C. Akinsulie[2], R. Koku[2], E. H. Hoffmann[2]

[1]Animal Disease Research Unit, USDA-ARS, [2]Department of Veterinary Microbiology and Pathology, Washington State University. susan.noh@usda.gov

Session: Bacteriology, 2024-01-21, 9:30 - 9:45

Objective: Bovine anaplasmosis, one of the most common production-limiting, tick-borne diseases of cattle found worldwide, is caused by *Anaplasma marginale*. Methods for disease control and prevention are limited and thus a subunit vaccine that prevents disease and reduces tick transmission would improve cattle health globally. As an obligate intracellular bacterium, host cell entry is an essential component of the pathogen lifecycle. Thus, identification of molecules involved in host cell adhesion and entry are high priority vaccine candidates. The goal of this work is to use phage display in an unbiased screen to identify *A. marginale* proteins that bind bovine erythrocytes or *Dermacentor andersoni* cells, a natural tick vector of *A. marginale*.

Methods: Overall, 66 *A. marginale* genes, including nearly all annotated outer membrane proteins and other vaccine candidates were cloned into T7 phage which express the insert on the viral capsid. The phage were mixed with erythrocytes, ticks cells or empty wells to allow binding (biopanning). Following four rounds of biopanning, the phage that bound the respective host cells were recovered and quantified using real-time PCR. To verify the function of a subset of these candidate adhesins, proteins were expressed, purified, and used in *in vitro* assays to determine if the presence of the recombinant adhesin candidate would reduce *A. marginale* entry into tick cells. One-way ANOVA with Dunnett's Multiple Comparison was used to determine statistical significance.

Results: During phage display, 76% and 68% of screened proteins were eliminated as adhesins for erythrocytes and tick cells, respectively. Eleven and 16 adhesin candidates were identified in erythrocytes and tick cells, respectively. For both host cell types, OmpA and multiple proteins from the Msp1 superfamily were recovered, including Msp1b, Mlp2, Mlp3, and Mlp4. Thus OmpA, Msp1a, Msp1b, and Mlp2 were expressed as recombinant protein and tested for their ability to block *A. marginale* entry into tick cells. *A. marginale* levels following treatment with OmpA were statistically significantly reduced by 3.7-fold ($p = <0.0001$). The presence of Msp1a-$_{1\text{-}300}$ and Msp1b reduced *A. marginale* levels by 4.7-fold ($p = <0.0001$) and 2.3-fold ($p = 0.0018$), respectively. The addition of Mlp2 resulted in a 2.2-fold ($p = 0.0009$) decrease in *A. marginale* levels. In contrast, treatment with Msp1a$_{301\text{-}623}$ did not reduce *A. marginale* entry.

Conclusions: The data presented here indicate that OmpA, Msp1a, Msp1b and Mlp2 play a role in *A. marginale* entry into red blood cells and tick cells with possible contributions from Mlp3, and Mlp4. Further testing is required to determine the role of additional identified adhesin candidates for tick cells and erythrocytes. Toward vaccine development, the next steps will be to determine if antibodies against the binding domains of these proteins can block *A. marginale* entry into host cells *in vivo*.

Financial Support: USDA-ARS CRIS: 2090-32000-043-000D.

Notes:

32 - Cross-colonization of segmented filamentous bacteria between broilers and layers

Jared Meinen-Jochum[1], Melha Mellata[1]

[1]Iowa State University. jmjochum@iastate.edu

Session: Bacteriology, 2024-01-21, 9:45 - 10:00

Objective: Segmented filamentous bacteria (SFB) are unique immunomodulating bacteria that are crucial for the maturation of the gut immune system in humans, mice, and chickens in early life. SFB are host-specific and will not colonize hosts belonging to different species. In chickens, different genetic lines are used for either meat (broilers) or egg (layers) production. Our lab has demonstrated the ability of introducing layer-sourced SFB to layers after hatch to increase early SFB gut colonization, immune maturation, and resistance to *Enterobacteriaceae*, like *Salmonella*. The objective of this study was to demonstrate whether layer-sourced SFB could also colonize broilers and promote benefits by reducing *Enterobacteriaceae.*

Methods: One-day-old Ross 308 broilers (n = 23 per group) were treated with either PBS (CON), Dekalb-derived SFB (layers; D-SFB), or Ross-sourced SFB (broilers; R-SFB) via oral gavage. At 5-, 10-, 17-, and 24-days post inoculation (dpi), the presence of SFB and the amount of total *Enterobacteriaceae* were examined in feces from 6 birds per group. The levels of SFB were determined in ilea scrapings in the proximal, medial, and distal ileum at 8, 15, 22, and 29 dpi and post-necropsy. *Enterobacteriaceae* were evaluated by plating on MacConkey agar and SFB via microscopy and qPCR. Individual birds were massed at each necropsy and compared to their starting mass to calculate weight gain.

Results: At 8 dpi, D-SFB birds demonstrated a significantly higher level of SFB compared to CON and R-SFB birds ($p < 0.0001$). At 22 dpi, all birds were colonized with SFB throughout all sections of the ileum. At 24 dpi, a significant decrease in total *Enterobacteriaceae* was observed in the feces of the D-SFB group ($p < 0.05$). No significant difference in weight gain was observed between groups at any timepoint in the experiment.

Conclusions: The introduction of SFB to chickens at hatch is important to reduce bacteria that are concerning for both poultry and human diseases. Our data show that SFB derived from layers are able to colonize both broilers and layers. Current studies are being undertaken to measure the immune response caused by SFB treatment.

Financial Support: This research was supported by the United States Department of Agriculture (USDA) Hatch projects (IOW05700-NC1202 and IOW04202) and Kent corporation.

Notes:

33 - Stressed from the start: Implications of early life stress for lifelong health and disease resistance

Adam J. Moeser[1]

[1]Dept. Large Animal Clinical Sciences. Michigan State University. moeserad@msu.edu

Session: AAVI - Featured Speakers, 2024-01-21, 10:30 - 11:15

The first three months of postnatal life are a critical period for the ontogeny of multiple physiological systems, including the gastrointestinal (GI), immune, nervous, and endocrine systems. This period is characterized by significant plasticity, wherein environmental cues play a pivotal role in sculpting these systems for optimal lifelong functionality. However, in animal production, this developmental window coincides with the most significant production stressors, which can alter developmental trajectories. Early life stress is a robust predictor of health outcomes, disease susceptibility, and mortality across species; however, there remains a paucity of knowledge regarding its long-term developmental and pathophysiological consequences.

Early weaning is the most stressful management practice employed in production animal systems and involves multiple concurrent stressors, including maternal and littermate separation. Transport stress, changes in diet, and immune activation caused by stress, vaccination, and exposure to novel antigens and pathogens. Despite the production and economic necessity, early weaning is clearly linked with adverse effects, including compromised performance, increased morbidity, and mortality throughout the lifespan of the animal. There is a critical need to understand the mechanisms by which early life stressors like early weaning impair long-term health. Our research conducted at Michigan State University is investigating the mechanisms that underlie the heightened disease susceptibility linked with early life stress. This presentation will cover how early life stress impacts the developing GI, immune, and nervous systems and sensitivity to later life stress and immunological challenges. Further, host and genetic factors, such as biological sex, shape and influence these critical developmental processes and stress sensitivity, ultimately shaping gut health, immunity, and the lifelong health trajectory and disease resistance of these animals. This understanding is crucial for developing targeted management strategies and therapeutic interventions to optimize developmental outcomes or ameliorate the adverse effects of early life stressors in animals and humans.

Notes:

34 - Host responses during influenza A virus adaptation to a new species

Daniela Rajao[1]

[1]Dept. of Population Health, University of Georgia. daniela.rajao@uga.edu

Session: AAVI - Featured Speakers, 2024-01-21, 11:15 - 12:00

Influenza A virus (FLUAV) is one of the most important pathogens of swine and can cause outbreaks in swine herds throughout the year. FLUAV is prevalent across swine herds, with several genetically and antigenically diverse FLUAV strains endemically circulating in swine worldwide, mostly of the H1N1, H3N2, and H1N2 subtypes. In the United States, four major lineages of H1 and H3 FLUAV circulate in swine populations, with considerable genetic diversity between and within these lineages. This great genetic diversity is due, in part, to the spillover and subsequent adaptation of viruses from other species, particularly humans.

While FLUAV host jumps occur frequently, sustained transmission in the new host is more limited, highlighting that the virus has to evolve to effectively replicate, transmit, and become endemic in the new host. As an example, multiple reverse-zoonotic events (from humans to pigs) have been detected globally but only few human-origin FLUAV lineages have become endemic in swine, typically with marked genetic differences from the precursor strain. Our research is focused on understanding virus-host interaction and the selective factors that affect virus evolution and adaptation during replication and transmission of human viruses in pigs. Several selective factors may affect FLUAV evolution and persistence of genetically diverse viral progeny during the course of replication, such as the individual's immunity. Some components of the innate immunity are involved in non-specific virus neutralization activity that varies between hosts, imposing different levels of pressure that may affect virus evolution. Similarly, the antibody-mediated immunity creates pressure that drives selection of antigenic variants and promotes antigenic evolution. My presentation will discuss the process of adaptation of influenza A viruses in pigs and how host factors shape virus evolution and transmission.

Notes:

35 - Can we reliably quantify blood vitamin E cattle-side? A method comparison study

Eric Owczarzak[1], Nick Grotenrath[2], Barry Bradford[2], Angel Abuelo[1]

[1]College of Veterinary Medicine, Michigan State University, [2]College of Agriculture and Natural Resources, Michigan State University. owczar12@msu.edu

Session: Diagnostic Testing 1, 2024-01-21, 11:15 - 11:30

Objective: Vitamin E is essential in mitigating the impact of oxidative stress on periparturient dairy cows and neonatal calves. Therefore, it is essential to accurately measure circulating vitamin E concentrations. Currently, the only method to reliably measure vitamin E is liquid chromatography-mass spectrometry (LC-MS), which is an expensive and time-consuming procedure that requires highly-specialized equipment. However, a cheaper and faster method has been developed, which allows the quantification of circulating vitamin E animal-side using a handheld fluorometric analyzer (HFA). Our objective was to compare the accuracy of the HFA to the gold standard LC-MS method for measuring vitamin E in bovine samples.

Methods: A total of 178 samples collected for other studies were used: 99 newborn calf serum samples from a vitamin E supplementation study (including treated and control animals) and 79 whole blood samples from cows in the 1-6 days post-calving. Vitamin E concentrations were measured on thawed calf serum and fresh cow EDTA blood using an HFA, following manufacturer instructions. Whole blood was then centrifuged to obtain plasma. Vitamin E was also quantified in calf serum and cow plasma at the Michigan State University Veterinary Diagnostic Laboratory using LC-MS. Calf and cow results were analyzed separately as they represent different biological matrixes and physiological times. In each dataset, results between HFA and LC-MS determinations were compared using Passing-Bablok regressions and Bland-Altman plots.

Results: The vitamin E concentrations that were determined by the HFA ranged from 0 µg/mL to 8.9 µg/mL, whereas those determined by LC-MS ranged from 0.28 µg/mL to 30.75 µg/mL. The HFA showed a poor linear relationship with LC-MS for both calves and cows (intercept = 0.33 and 0.67 µg/mL, respectively). The HFA unreliably estimated vitamin E, with mean bias of -3.17 and 0.64 µg/mL for calves and cows, respectively. Moreover, 40.4% of the calf samples read below the acceptable range for the HFA, making it unsuitable for this age group.

Conclusions: Under the conditions of our study, the HFA yielded unreliable results and cannot be recommended for field use.

Notes:

36 - Quantification of calprotectin and myeloperoxidase in equine feces by ELISA: a validation study

Rebecca C. Bishop[1], Sarah Graham[1], Sara Connolly[1], Pamela A. Wilkins[1], Annette M. McCoy[1]

[1]University of Illinois Urbana-Champaign. rb17@illinois.edu

Session: Diagnostic Testing 1, 2024-01-21, 10:45 - 11:00

Objective: Inflammatory intestinal conditions, such as colitis, are common in horses and cause significant morbidity and mortality across breeds and disciplines. Unfortunately, there are few reliable diagnostic modalities for inflammation of the equine large colon and therefore there is a need to evaluate novel diagnostic markers of intestinal inflammation that can be used clinically in equine patients. Fecal inflammatory biomarkers are a direct product of intestinal inflammation and enter the feces when mucosal barrier function is lost. In humans, fecal inflammatory biomarkers are used for diagnosis and monitoring of intestinal inflammation. However, the use of fecal inflammatory biomarkers in equine feces as a non-invasive diagnostic test for colitis has not been evaluated. The objective of this study was to validate commercial ELISA kits for the detection of the inflammatory biomarkers myeloperoxidase (MPO) and calprotectin (CP) in equine feces.

Methods: Fecal samples were obtained from horses that were either clinically healthy or had large colon inflammation (defined as measurable right dorsal colon wall thickness ≥ 5mm on abdominal ultrasound). Feces were suspended in buffer to create fecal supernatant. Serum and fecal supernatant were analyzed using ELISA kits validated for detection of MPO and CP in equine serum. Variations in sample handling protocols (centrifugation speed, extraction buffer, filtration) were evaluated. Assay validation steps included intra- and inter-assay variability (CV), dilution linearity, spike recovery, and sample type correlation.

Results: Seventeen paired fecal and serum samples were used (10 healthy horses, 7 colitis). Previously reported sample handling protocols resulted in detectable MPO and CP, but poor CV, linearity, and spike recovery. There was linear correlation between serum and fecal samples for CP (P=<0.001) but not MPO (P=0.06). Alternate sample handling protocols that affected biomarker recovery included decreased centrifugation speed, creation of fecal suspensions from fresh rather than frozen feces, use of an alternate fecal extraction buffer rather than PBS, and the addition of a filtration step. There was a significant difference between alternative sample handling protocols for CP (P<0.001) and MPO (P=0.002) with improved CV for CP (2.1-18.6%) but not MPO (14.4-53.4%). Processing fresh feces with a fecal extraction buffer and filtration of supernatant resulted in the best CV (0.5-3.8%) and recovery (45-64%) for CP. Detection of MPO was inconsistent regardless of method.

Conclusions: Evaluated as a whole, our data suggest that the commercially available MPO ELISA kit is not suitable for use with equine feces using any of the sample preparation methods evaluated. Using the modified sample preparation protocol, the CP ELISA kit is appropriate for use to quantify CP in equine fecal samples.

Financial Support: Research funding from American Quarter Horse Foundation. Student support from NIH T35 0D011145.

Notes:

37 - Evaluation of a rapid test strip, PCR, and enriched aerobic culture for the detection of *Salmonella* in equine feces

Emily C. Herring[1], Kristy L. Pabilonia[2], Rebecca S. McConnico[3], Ann M. Chapman[4], Helen Aceto[5], Nathan M. Slovis[6], Paul S. Morley[7], Binu T. Velayudhan[8], Brandy A. Burgess[1]

[1]Dept. of Population Health, University of Georgia, [2]Dept. of Microbiology, Immunology, and Pathology, Colorado State University, [3]Dept. of Agricultural Sciences and Forestry, Louisiana Tech University, [4]Dept. of Veterinary Clinical Sciences, Louisiana State University, [5]Dept. of Clinical Sciences, New Bolton Center, University of Pennsylvania, [6]Hagyard Equine Medical Institute, [7]Veterinary Education, Research, and Outreach Program, Texas A&M University/West Texas A&M University, [8]Athens Veterinary Diagnostic Laboratory, University of Georgia. echerring@uga.edu

Session: Diagnostic Testing 1, 2024-01-21, 11:00 - 11:15

Objective: *Salmonella enterica* is one of the most common causes of healthcare-associated infections in horses, and outbreaks often have substantial clinical and financial impacts on affected animals and facilities. Rapid, accurate detection of this pathogen is critical to prevention and control efforts in equine facilities. However, estimation of diagnostic accuracy measures for both current and novel tests is hindered by the lack of a gold standard test for *Salmonella* detection in horses. This challenge can be overcome through the application of Bayesian latent class models, which combine existing knowledge of test performance and disease prevalence parameters with observed test results to generate unbiased estimates of test sensitivity and specificity. Using this approach, in this study we aimed to estimate the sensitivity and specificity of four tests for the detection of *Salmonella* in horses: tetrathionate-enriched culture, selenite-enriched culture, qPCR, and a novel rapid test (Reveal® 2.0 - a lateral flow immunoassay designed for food safety applications).

Methods: All four tests were applied in three populations of horses with different *Salmonella* prevalences: horses presenting to an equine referral hospital with a condition associated with a high (n = 142) or low (n = 418) risk of *Salmonella* shedding, respectively, and horses that were previously identified as test-positive for fecal *Salmonella* shedding at an equine general practice or referral hospital (n = 106). A Bayesian latent class model was used to estimate the sensitivity and specificity of each test. Informative prior distributions for disease prevalence and test accuracy measures were generated via expert opinion.

Results: Preliminary results indicate that qPCR is both sensitive (median [95% CI]: 90.7% [83.5%, 95.7%]) and specific (median [95% CI]: 97.2% [95.5%, 98.5%]). Tetrathionate- and selenite-enriched culture were similarly specific (median [95% CI]: 99.3% [98.4%, 99.8%]; 92.9% [90.7%, 94.7%]) but less sensitive (median [95% CI]: 81.4% [73.4%, 88.1%]; 71.7% [57.6%, 83.8%]). Reveal® 2.0 was both moderately specific and sensitive (median [95% CI]: 66.0% [62.5%, 69.4%]; 67.1% [54.9%, 78.0%]).

Conclusions: Given its low cost and ease of use, these results suggest that Reveal® 2.0 may be useful as a point-of-care screening test for fecal *Salmonella* shedding in horses, especially if used in a parallel testing strategy.

Financial Support: Grayson-Jockey Club Research Foundation, Morris Animal Foundation, Foundation for the Horse. Boehringer Ingelheim Advancement in Equine Research Award.

Notes:

38 - Infection status discrimination in livestock biofluids using near infrared spectroscopy analysis

Mariana Santos-Rivera[1], Florencia Meyer[1], Carrie K Vance[1], Amelia R Woolums[1], Merrilee Thoresen[1]

[1]Mississippi State University. florencia.meyer@msstate.edu

Session: Diagnostic Testing 2, 2024-01-22, 3:15 - 3:30

Objective: Bovine Respiratory Disease (BRD) is a complex condition caused by the combined effects of one or more viral/bacterial agents and stress. We used near-infrared spectroscopy (NIRS) to develop predictive models for infection status.

Methods: Calves (n=5) were infected with *Mannheimia haemolytica* or Bovine Respiratory Syncytial Virus (BRSV) and saliva, nasal secretions, breath condensate, and blood were obtained daily before and after challenge. NIR spectra (350-2500nm) were collected from each sample using a portable FieldSpec3 spectrometer and 1mm quartz cuvettes, collecting 10 independent spectral signatures per sample. For each biofluid, multivariate analysis and predictive modeling were built from balanced spectral datasets using a leave-one-animal out approach; data from one animal was removed as the external validation and the remaining data randomly split into a 80/20 distribution for the calibration and internal validation sets. Principal component analysis (PCA) and Linear Discriminant Analysis (LDA) were used to generate the predictive models applied to the external dataset.

Results: NIRS predictive models built from spectra of plasma, nasal secretions and breath condensate were able to discriminate diseased state from healthy state in cattle, for both *M. haemolytica* and BRSV. Preliminary results indicate that PCA-LDA prediction models of plasma could classify animals as healthy vs. infected with M. haemolytica with an accuracy, sensitivity and specificity of 83.4%, 79.2% and 87.5%, respectively, for the external validation. Similarly, BRSV infected calves could be identified by NIRS models built on breath condensate with an accuracy, sensitivity and specificity of 74%, 71% and 76%, respectively, while models built from nasal secretions presented an accuracy, sensitivity and specificity of 79%, 72% and 86%.

Conclusions: These results are encouraging and provide the foundational knowledge to aid in the development of a non-invasive diagnosis tool for BRD.

Notes:

39 - Bayesian latent class analysis to estimate the diagnostic performance of the bull breeding soundness evaluation

Todd Gunderson[1], Karen Shuck[2], Brian Vander Ley[2], David Smith[1, 3]

[1]Department of Pathobiology and Population Medicine, College of Veterinary Medicine, Mississippi State University, [2]Great Plains Veterinary Education Center, School of Veterinary Medicine and Biomedical Sciences, University of Nebraska-Lincoln, [3]Mississippi State University. tg1256@msstate.edu

Session: Diagnostic Testing 1, 2024-01-21, 11:30 - 11:45

Objective: The purpose of this study was to estimate the sensitivity and specificity of the bull breeding soundness evaluation (BSE) for classifying *Bos taurus* bulls as sub-fertile.

Methods: BSEs were performed on 500 beef bulls at the U.S. Meat Animal Research Center in Clay Center, Nebraska. Semen from these bulls was evaluated using eosin-nigrosin stained slides at 1000X magnification by a trained technician on-site, then independently by a veterinarian off-site. Bulls with slides that had <70% morphologically normal sperm were classified as sub-fertile. Interobserver agreement was assessed using Cohen's kappa coefficient. Sensitivity, specificity, and true prevalence were estimated using Bayesian latent class analysis. Sensitivity was defined as the probability of an observer correctly identifying a bull as sub-fertile, and specificity was defined as the probability of correctly identifying a bull as satisfactory.

Results: Of 478 bulls evaluated for semen morphology, 68 (14%) were classified as sub-fertile on-site and 96 (20%) were classified as sub-fertile off-site. The kappa coefficient for inter-observer agreement was 0.5. The estimated true prevalence of sub-fertile bulls was 16% (95%C.I. 2-35%). The estimated sensitivity and specificity for on-site observations were 52% (95%C.I. 22-85%) and 93% (95%C.I. 86-99%) respectively, and for off-site observations were 62% (95%C.I. 30-89%) and 88% (95%C.I. 80-97%).

Conclusions: These results show similar test sensitivity and specificity between observers. Furthermore, they indicate that the BSE may have a relatively low positive predictive value at our estimated pre-test probability, and that the negative predictive value of the BSE may not be much more informative than prevalence.

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Notes:

40 - Optimization and application of PCR for the detection of pathogenic Leptospira in bovine semen

Camila Hamond[1], Ellie J. Putz[2], Karen LeCount[1], Tammy Anderson[1], Patrick Camp[1], Jessica Hicks[1], Tod Stuber[1], Darrell O. Bayles[2], Linda K. Schlater[1], Jarlath E. Nally[2]

[1]National Veterinary Services Laboratories, APHIS, [2]Infectious Bacterial Diseases Research Unit, Agricultural Research Service, United States Department of Agriculture. camila.hamond@usda.gov

Session: Diagnostic Testing 1, 2024-01-21, 11:45 - 12:00

Objective: Bovine leptospirosis is a global zoonotic disease that causes infertility, abortions, stillbirths, weak offspring, and decreased milk production and growth rates. Currently, the routine diagnosis of bovine leptospirosis for the export/import of bovine semen relies on the microscopic agglutination test (MAT), a serological assay that detects agglutinating antibodies in serum from bulls exposed to leptospires. However, a positive MAT titer in bovine sera does not differentiate antibodies present due to infection versus immunization, nor diagnose shedding of leptospires from urine or semen. In this study, *lipL32* qPCR protocols were optimized to directly detect pathogenic *Leptospira* in bovine semen samples.

Methods: Bovine isolates of *L. borgpetersenii* serovar Hardjo strain TC129, strain S014J, and *L. santarosai* serogroup Pyrogenes strain DCP-017, were used to spike bull semen samples, which did or did not contain semen extender. DNA was extracted from spiked semen samples before or after dilution (1:2, 1:3, 1:5, 1:10) with PBS or 1% bovine serum albumin (BSA) using the Maxwell RSC Purefood Purification Pathogen kit to determine the lower limit of detection. Lower limits of detection were also determined using spiked semen that was frozen at -80°C for 24 hours prior to extraction of DNA. All experiments were performed twice, independent of each other. An internal positive control was used on all PCR samples. Two hundred and sixty-eight randomly selected commercial bovine semen samples were screened for *Leptospira* with the PCR protocols optimized in this study. Culture using HAN media was attempted for PCR-positive bovine semen samples. An isolate cultured from a PCR-positive semen sample was characterized by serotyping and genome sequencing.

Results: Spiked semen that was not diluted prior to DNA extraction was negative for target DNA and an internal positive control. Spiked semen that was diluted 1:5 or 1:10, in either PBS or 1% BSA, prior to extraction of DNA was optimal for detection of target DNA and an internal positive control. The lower limit of detection for leptospires in semen containing extender was 10 leptospires/ml compared to 100 leptospires/ml in semen without extender. No difference in lower limits of detection was observed between fresh versus frozen samples, or between different strains of leptospires. Of two hundred and sixty-eight randomly selected frozen bovine semen samples tested by *lipL32* rt-PCR, four (1.5%) were positive with Ct values less than 40 (S74, Ct=36.1; S118, Ct=36.5; S130, Ct=37.6; & S112, Ct=39.2). One sample was culture positive and identified as *L. borgpetersenii* serogroup Sejroe.

Conclusions: The *lipL32* qPCR can detect as low as 10 leptospires/ml in frozen bovine semen samples. Over 1.5% (4/268) of commercial bovine semen tested in this study contained DNA derived from pathogenic leptospires. Our findings highlight the need to consider bovine semen in the transmission of bovine leptospirosis, and the potential of a *lipL32* qPCR for the detection of bulls positive for leptospires compared to currently used serological assays (MAT).

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Notes:

41 - Canaries for the modern age - dogs as sentinels of human health and wellbeing

Courtney Sexton[1], Audrey Ruple[1]

[1]Virginia-Maryland College of Veterinary Medicine at Virginia Tech. sextonc@vt.edu

Session: One Health / Public Health 1, 2024-01-21, 10:30 - 10:45

Objective: Understanding the totality of an individual's experiences and exposures at different stages of life and over the lifespan is crucial to improving and maintaining quality of life during the aging process. While many of the factors that influence when, how, and how well we age are difficult to assess via longitudinal studies in humans, our canine companions offer an under-realized opportunity to learn how our shared exposome (physical and social environments) contributes to aging, health, and wellbeing, for both people and dogs. Recent and emerging research underscores the important role companion animals serve in being uniquely positioned sentinels of human health, and here we discuss why dogs should be considered appropriate and valuable sentinels in terms of public health, individual disease and aging, and social welfare.

Methods: We reviewed the recent literature (2020 - present) in which companion animals, specifically dogs, are reported as biomarkers of human health, environmental exposures, and aging. Google Scholar and Web of Science search terms include "companion animal sentinels" and "dogs as sentinels of human health." In our review, we analyzed studies according to which element of the shared environment they investigate: vector-borne pathogens; VOCs, EDCs, and heavy metals; reproductive health; and social adversity.

Results: Among studies published within the last three years, we find substantial evidence from across the spectrum of inquiry (and across the globe) that dogs can indeed offer insight into certain shared elements of risk in our exposomes. Though our review indicates that there is substantial data supporting the validity of recognizing dogs as sentinels of human health, we are lacking a system for capturing the different capacities in which dogs are representative of the exposomal influences on diverse human populations.

Conclusions: As we know, human environments and the impacts of environmental factors can vary drastically, and this should be captured by future studies to more accurately assess exposure risks across diverse human populations. It is standard practice in veterinary and canine cognition, behavior, and welfare studies to include study population demographics. We recommend the implementation of a standardized system to report human-level data in companion dog studies. Capturing such data will not only elevate the role of dogs as valid animal sentinels, but will also enable us to assess whether we as a community of scientists are capturing the diversity of exposures about which our sentinel pets can provide early outcome information.

Financial Support: NIH

Notes:

42 - Predicting human and dog Lyme disease incidence using Google Trends data

Lauren Wisnieski[1], Karen Gruszynski[1], Vina Faulkner[1], Barbara Shock[1]

[1]Lincoln Memorial University. lauren.wisnieski@lmunet.edu

Session: One Health / Public Health 1, 2024-01-21, 10:45 - 11:00

Objective: Google Flu Trends (GFT) was a service operated by Google to predict outbreaks of flu and was discontinued in 2015 due to inaccurate predictions. GFT trends overestimated flu prevalence by over 50% in 2011-2012, which some researchers attributed to the increased media coverage of “swine flu” and “bird flu”. However, Google Trends may still have potential to be an affordable, timely, robust, and/or sensitive surveillance system given refinement of search terms, monitoring and updating of the algorithm, and use of additional data streams. A previous study found that there were similar patterns between Google searches for Lyme disease symptoms and Lyme disease incidence among humans. The objective of our study was to validate the use of Google Trends search data for predicting Lyme disease incidence among humans and dogs by comparing models with varying search terms.

Methods: We requested Lyme disease data for the years 2010-2021 from state health departments for human data and from IDEXX for canine data. We downloaded Google Trends search data on terms for Lyme disease, symptoms of Lyme disease, and diseases with similar symptoms as Lyme disease for humans and for dogs using the ‘gtrendsR’ package in R version 4.0.2. We built a 12-month expanding window negative binomial model for each search term for the human and for the dog models. A 12-month lag of each search term was included as a predictor to account for seasonality. Models were evaluated for predictive ability using Root Mean Squared Errors (RMSEs) and plots of observed and predicted case counts.

Results: The final sample for human Lyme disease included data from 16 states. Results from the human models indicated that terms for Lyme disease, including "Lyme disease", "Lymes", and "Lyme", were the most predictive search terms (as indicated by the lowest RMSEs) of Lyme disease case counts, indicating specificity of these search terms. The best performing model was for the search term "Lyme disease", but model performance varied across states. For some states, the predicted case counts closely followed the observed case counts over time. We will also present results from our dog Lyme disease models, which will include human and dog Lyme disease related search terms and human Lyme disease case counts.

Conclusions: With this study, we demonstrate the potential use of Google Trends search data for prediction of monthly human Lyme disease case counts at the state-level. The models produced accurate predictions for some states, but not for other states. We will also present predictive models for canine Lyme disease. Future studies can validate the findings of this study for other infectious and/or zoonotic diseases as well as determine if adding additional data streams, such as environmental data, can improve model performance. Overall, this study demonstrates that Google Trends search data may be useful to health departments as a tool in their toolbox that can be used alongside other surveillance data, such as tick dragging and case reports, especially for states where the models performed well.

Notes:

43 - Monitoring and predicting lyme disease trends among dogs to inform public health

Janice O'Brien[1], Courtney Sexton[1], Audrey Ruple[1]

[1]Virginia Polytechnic and State Univeristy. janiceobrien@vt.edu

Session: One Health / Public Health 1, 2024-01-21, 11:00 - 11:15

Objective: Lyme disease is the most common vector-borne disease in humans in the United States. Nearly half a million humans are diagnosed every year in the United States alone. Routine testing for Lyme disease in dogs is very common as it is part of several point-of-care testing kits that screens for multiple vector-borne diseases (e.g. heartworm, Lyme, Ehrlichia) and may be screened at annual wellness visits. Because of this testing paradigm and that dogs are more exposed to ticks, dogs may be the perfect sentinel of Lyme disease in humans, allowing us to monitor Lyme rates in dogs and alert public health officials of regional and temporal abnormalities.

Methods: Pet insurance claims from a major North American insurance provider were sorted for Lyme disease claim codes from 2008-2020. These claims were normalized by region (state or province), year, and seasonally. The seasonal trends and yearly rates were compared to publicly provided humans data from the Centers for Disease Control and Prevention (CDC) data for the same years. All graphs comparing rates among dogs and humans are graphed relative to the initial starting rate for the species, so all initial rates start at 1, and every year after is a change relative to the first year's rate.

Results: Fascinatingly, when analyzing Lyme trends within the US, the top 15 states, and within individual states, a trend appears to emerge that dog Lyme rates precede the rates for humans by 1-2 years. If there is a spike in dog Lyme cases in 2015 there will likely be a spike in humans in 2017. We do not have an explanation for a 1-2-year trend - it does correlate with the lifecycle length of the Ixodes Scapularis tick, however one would expect the same life stage ticks to bite both humans and dogs. Regardless, if dogs are contracting Lyme disease in a way that can predict the rates in humans 1-2 years prior, that leaves ample time for public health responses to occur, if only we can develop the monitoring system to detect these spikes in rates.

Conclusions: Using pet insurance data, we can monitor the Lyme disease trends of dogs to alert public health officials of emergences into new areas, and spikes in Lyme rates in endemic areas to inform public health decisions and healthcare practitioners. Additionally, using machine learning predictive models with the canine data can help to predict future trends and detect abnormal rates.

Notes:

44 - Notable multistate outbreaks of *Salmonella* infections related to animal contact — United States, 2023

Grace M. Vahey[1], G. Sean Stapleton[1], Molly M. Leeper[1], Katie N. Werner[1], Katharine M. Benedict[1]

[1]U.S. Centers for Disease Control & Prevention. pgy2@cdc.gov

Session: One Health / Public Health 1, 2024-01-21, 11:15 - 11:30

Objective: Enteric diseases linked to contact with animals or their environment cause an estimated 450,000 illnesses, 5,000 hospitalizations, and 76 deaths in the United States annually. *Salmonella* outbreaks linked to animal contact disproportionally affect young children, a population at increased risk for severe illness. We summarized notable multistate animal contact outbreaks of *Salmonella* infections from 2023.

Methods: Investigators used PulseNet, the national molecular subtyping network for enteric disease surveillance, to identify illnesses. A case was defined as *Salmonella* infection yielding an isolate highly related to an outbreak strain by whole genome sequencing. State and local public health officials interviewed people about animal exposures during the week before illness onset and collected information about animal purchase locations.

Results: As of July 20, 2023, CDC and public health officials have investigated 12 multistate outbreaks of *Salmonella* infections linked to backyard poultry, including serotypes Braenderup, Enteritidis, Indiana, Infantis, Mbandaka, and Typhimurium. A total of 690 cases were reported from 47 states and Puerto Rico; 23% were aged <5 years. Of 242 people with information on poultry purchases, 187 (77%) reported purchasing or obtaining poultry in 2023. People reported purchasing or obtaining poultry from at least 178 different locations, and 10 people reported purchasing directly from hatcheries online.

In 2022–2023, a total of 32 cases of *Salmonella* Vitkin (12 people) and *Salmonella* IIIb 61:z52:z53 (20 people) were reported from 20 states. Of 26 people interviewed, 17 (65%) reported contact with a pet bearded dragon during the week before getting sick. A total of 15 (47%) were children aged <5 years, with 11 children aged <1-year-old. In both outbreaks, 15 people reported purchasing their bearded dragons from pet stores or online. At least five breeders and suppliers were identified, including one that supplied multiple stores.

As of November 16, 2022, a total of 28 cases of *Salmonella* Stanley (25 people) and *Salmonella* Pomona (3 people) were reported from 16 states. Of the 20 people with available information, 15 (75%) reported contact with pet turtles during the week before they got sick. Six (21%) people were aged <5 years. All 14 people (100%) who reported the size of the pet turtle reported contact with small turtles (shells <4 inches long.) Despite a federal law and state regulations that ban the sale and distribution of small turtles as pets, these turtles are sometimes sold illegally online and at stores, flea markets, and roadside stands. This outbreak was reopened in 2023, with an additional 24 people infected with the outbreak strain of *Salmonella* Stanley and 2 people with *Salmonella* Pomona reported as of August 15, 2023.

Conclusions: In 2023, over 700 illnesses were identified in multistate outbreaks of *Salmonella* infections linked to backyard poultry, bearded dragons, and small turtles. These outbreaks are an ongoing problem in the United States, and disproportionately affect young children. Collaboration between federal, state, and local public health and agriculture partners is crucial in multistate enteric disease outbreaks, and increased industry engagement and education of pet owners can prevent future illnesses.

Notes:

45 - Multistate outbreaks of salmonellosis linked to contact with backyard poultry — United States, 2015-2022

G. Sean Stapleton[1], Caroline Habrun[1], Kaylea Nemechek[1], Lauren Gollarza[1], Zachary Ellison[1], Beth Tolar[1], Lia Koski[1], Joshua M. Brandenburg[1], Zainab Salah[1], Alexandra Palacios[1], Colin Basler[1], Kate Varela[1], Megin Nichols[1], Katharine Benedict[1]

[1]Division of Foodborne, Waterborne, and Environmental Diseases, Centers for Disease Control and Prevention. qbs1@cdc.gov

Session: One Health / Public Health 1, 2024-01-21, 11:30 - 11:45

Objective: Contact with backyard poultry (i.e., privately-owned, non-commercial poultry) was first associated with a multistate outbreak of salmonellosis in 1955. In recent years, backyard poultry-associated salmonellosis outbreaks have caused more illnesses in the United States than salmonellosis outbreaks linked to any other type of animal. Here, we describe the epidemiology of the most recent outbreaks from 2015-2022 with the aims of describing the epidemiology of these outbreaks, characterizing trends in demographic characteristics and behaviors of patients, and informing actions that can be taken to reduce the impact of backyard poultry-associated salmonellosis on public health.

Methods: We defined multistate backyard poultry-associated salmonellosis outbreaks as ≥2 culture-confirmed human *Salmonella enterica* infections in the United States genetically related based on pulsed field gel electrophoresis (PFGE; for 2015-2018 outbreaks) or whole genome sequencing (WGS; for 2019-2022 outbreaks) and with epidemiologic or laboratory evidence linking illnesses to backyard poultry during 2015-2022. We defined a case as a laboratory-confirmed *Salmonella enterica* infection with an isolate from an ill person, indistinguishable from the outbreak PFGE pattern or genetically related based on WGS and occurring during the outbreak investigation period (i.e., the date of first patient illness onset to the date the outbreak investigation was declared over by CDC). Data sources included the CDC Outbreak Response and Prevention Branch's outbreak management database; PulseNet, the national molecular subtyping network for enteric disease surveillance; the System for Enteric Disease Response, Investigation, and Coordination; and Epi Info™.

Results: During 2015-2022, there were 88 multistate backyard poultry-associated salmonellosis outbreaks and 7,866 outbreak-associated illnesses caused by 21 different *Salmonella* serotypes. *Salmonella* Enteritidis accounted for the most outbreaks (n=21) and illnesses (n=2,400) of any serotype. Median outbreak size was 46 cases (range: 8 to 848 cases), and median number of outbreaks per year was 11 (range: 5-17 outbreaks/year). Ill people resided in 52 United States jurisdictions (all 50 states, the District of Columbia, and Puerto Rico), with most culture-confirmed illnesses occurring in New York (n=417), Ohio (n=391), California (n=351), Pennsylvania (n=313), and Minnesota (n=311). Twenty-four percent (1,840/7,727) of patients with available information were <5 years of age. In total, 30% (1,710/5,644) of patients were hospitalized, and nine deaths were attributed to *Salmonella* infection. Throughout this period, patients reported behaviors that have a higher risk of *Salmonella* transmission, including kissing or snuggling poultry or allowing poultry inside their home. For 31 distinct outbreaks (35%), the strain of *Salmonella* associated with human illness was isolated from backyard poultry.

Conclusions: Outbreaks and illnesses linked to backyard poultry in the United States have grown in size and disproportionately affect children. Despite ongoing efforts to reduce the burden of salmonellosis associated with backyard poultry, outbreak-associated illnesses have nearly tripled and hospitalizations more than quadrupled compared with 1990-2014. Because this public health problem should be preventable, government officials, human and veterinary healthcare providers, and hatcheries and retailers should ensure that health and safety recommendations are disseminated widely to the public and should continue to collaborate to develop and implement prevention measures to reduce zoonotic transmission of *Salmonella* by backyard poultry.

Notes:

46 - Knowledge, attitudes, and practices of para-vets about ticks and tick-borne diseases in Pakistan

Abrar Hussain[1], Sabir Hussain[2, 3], Mahvish Rajput[4], Muhammad S. Sajid[4], Olivier SPARAGANO[2, 5], Rebecca L. Smith[1, 6]

[1]Department of Pathobiology, College of Veterinary Medicine, University of Illinois, [2]Department of Infectious Diseases and Public Health, Jockey Club College of Veterinary Medicine and Life Sciences, City University of Hong Kong, [3]School of Biological, Environmental, and Earth Sciences, University of Southern Mississippi, [4]Department of Parasitology University of Agriculture Faisalabad, [5]School of Agricultural Sciences and Practice, Royal Agricultural University, [6]Institute for Genomic Biology, University of Illinois. abrarh2@illinois.edu

Session: One Health / Public Health 1, 2024-01-21, 11:45 - 12:00

Objective: Global changes in climate and land use have led to a rapid escalation in the proliferation of ticks and tick-borne diseases (TBDs), significantly impacting animals and humans. Recent studies have revealed a high prevalence of tick infestation in Pakistani livestock, affecting more than 45% of the population of more than 200 million small and large ruminants. To address this issue, most livestock farmers seek assistance from para-veterinary workers (whose training level resembles that of veterinary technicians but exhibits potential variability in quality and depth). There is a dearth of information concerning the knowledge and practices of these para-veterinary workers regarding tick control and management, as well as their awareness of zoonotic risks associated with ticks. This study aims to bridge this critical knowledge gap by conducting a cross-sectional survey that evaluates the knowledge and practices of para-veterinary workers of TBDs and their perception of tick-related zoonotic risks in different regions of Pakistan.

Methods: In March 2023, we developed and administered a web-based survey using RedCap to evaluate the level of awareness and responses concerning the management of tick-borne diseases in animals and the comprehension of tick-related zoonotic risks in Pakistan. Recruitment was conducted via email, text message, and face-to-face conversation. Responses were analyzed using logistic regression to identify factors influencing the likelihood of participating in seminars or trainings about ticks and tick-borne diseases, as well as factors associated with using different protective behaviors. Poisson regression analysis was used to identify factors associated with tick-borne disease knowledge scores, including both overall and topic-specific subscores.

Results: We received 118 responses from three provinces; only 27.9% (n = 33) responded that they had attended workshops related to ticks and TBDs. Those who reported attending workshops were more likely (p = 0.013) to use personal protective equipment while handling tick-infested animals. Attending workshops was associated with a higher overall TBD score (p < 0.001), practice score (p < 0.001), and zoonotic risk score (p < 0.007) but not with knowledge and attitudes score (p = 0.056).

Conclusions: Our findings suggest that surveys are important tools for finding knowledge gaps, and workshop attendance was important in increasing overall awareness and better practices regarding TBDs. However, these workshops should be made more widely available and updated with the incorporation of more materials on common-tick species identification and tick-borne zoonosis.

Financial Support: I express my profound gratitude to Fulbright (IIE) for allowing me to pursue my Ph.D. studies in the United States. Through their sponsorship, I have been granted the means to further my academic pursuits and conduct research.

Notes:

47 - Mechanisms of susceptibility or resistance to SARS-CoV-2 variants across animal species

Jessie Lee Cunningham[1], Diego G. Diel[1], Mathias Martins[1], Salman Latif Butt[1], Mohammed Nooruzzaman[1]

[1]Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University. jlc535@cornell.edu

Session: Virology 1, 2024-01-21, 10:30 - 10:45

Objective: SARS-CoV-2 has demonstrated an incredibly broad host range that has the potential to fluctuate as critical components of the virus, such as the S protein, acquire mutations through the selective pressures applied by host immune responses. The objective of this study is to characterize how changes in the receptor binding domain of the S protein may influence species specificity at two critical interfaces: (i) the barriers at the point of virus-receptor-based entry; and (ii) the resulting post-entry events that will either limit or support replication of the virus. With this we aim to gauge the current risks that are posed to companion animals, livestock, and wildlife and to identify the potential for animal species to function as reservoirs for the virus.

Methods: Using a library of cells stably expressing ACE2 orthologues from 20 species, we were able to assess the ability of the virus to utilize the ACE2 receptor using viral entry assays, infectivity assays, and fusogenicity assays. These assays were conducted using pseudoviruses, live SARS-CoV-2 virus as well as recombinant chimeric viruses incorporating S proteins from different SARS-CoV-2 variants.

Results: Pseudoviral entry assays indicate SARS-CoV-2 exhibits a broad host-range across species that varies with the S of differing variants including B.1, Alpha, Gamma, Delta, and Omicron. Receptor usage assays demonstrated that SARS-CoV-2 variants have expanded ACE2 receptor host range via infection of cells expressing the ACE2 of pangolin, rat, and mouse. When compared to human-ACE2 cell lines, increased infection was seen for the Delta variant in cat-, dog-, and pig-ACE2 expressing lines. While the low binding predictions for dog and pig ACE2 supported the low number or lack of cases of natural infection in these species, we demonstrate that this is not due to the inability of S protein to engage with ACE2. Infection with the recombinant chimeric viruses also provided evidence that while Omicron BA.1.1 entry into target cells is faster in multiple species, at later time points pi the increased replication efficiency and overall increased fusogenicity of D614G B.1 S chimeras can compensate for an initial slower entry process.

Conclusions: There is an identifiable differential in the S-mediated susceptibility of animal species to SARS-CoV-2 infection. While some in silico predictions of the binding propensities of the S protein with the ACE2 orthologues align with the in vitro findings, several deviations from these predictions exist. It is clear that an extensive functional assessment of receptor usage and S protein interactions is necessary to fully elucidate the implications of acquired viral mutations on the susceptibility and permissiveness of target cells across species.

Financial Support: This work was supported by the USDA-NIFA grant no. 2023-70432-39463.

Notes:

48 - Detection and genetic characterisation of Canine coronavirus strains in India

Vishal Chander[1], Harsh Rajeshbhai Jogi[2], Gaurav Kumar Sharma [2], S. Nandi [2], Sonalika Mahajan[2], Mithilesh Singh[2], Mukesh Bhatt [2], K.P. Singh [2]

[1]Division of Virology, ICAR-Indian Veterinary Research Institute (IVRI), [2]ICAR-Indian Veterinary Research Institute (IVRI). drvishal1@gmail.com

Session: Virology 1, 2024-01-21, 10:45 - 11:00

Objective: Canine coronavirus (CCoV) is considered the major pathogen besides the canine parvovirus (CPV) as a leading cause of acute gastroenteritis in dogs. The samples from the dogs showing gastroenteritis symptoms including diarrhoea or vomition were screened for the presence of canine pathogens including canine parvovirus, adenovirus, and coronavirus. This study aimed to detect and characterize the CCoV strains circulating in India.

Methods: The canine viral diarrhoeal samples suspected of viral enteritis were submitted through the Veterinary Clinical Complex during 2021 which were examined, and diagnosis for these viruses was done using the polymerase chain reaction (PCR), and then they were characterized using DNA sequence analysis.

Results: Of the 200 samples screened, in the symptomatic animals, 43 were found positive for CPV strains and 7 for the CCoV strains. Two CCoV strain samples were characterized as CCoV-II. In one case the presence of both the CPV and CCoV was detected indicating mixed infection.

Conclusions: The screening and analysis of the positive cases of CCoV determine the occurrence of the CCoV-II virus in symptomatic dogs. As compared to other pathogens reports of CCoV occurrence in the country are scarce and the detection of this strain type confirms its presence. A continuous monitoring and surveillance of different canine pathogens including CCoV to rapidly detect and treat the affected animal accordingly would be beneficial for the better clinical outcome; and further evaluation of the incidence of this disease in the dog population.

Financial Support: The authors thank the Director, IVRI for providing the necessary facilities to carry out this work.

Notes:

49 - Use of canine respiratory epithelial cells to characterize viral kinetics and immunity associated with kennel cough

Swati Sharma[1], Roger Maes[1], Glorián Berríos-Vázquez[1], Gisela Soboll Hussey[1]

[1]Michigan State University. swatisha@msu.edu

Session: Virology 1, 2024-01-21, 11:00 - 11:15

Objective: Canine viral respiratory infections are of major concern when dogs are housed together in large numbers. In shelter environments, presence of different etiologic agents causes transmissible respiratory disease (or canine infectious respiratory disease complex [CIRDC], also known as "kennel cough" which is characterized by severe respiratory disease. With the widespread clinical application of molecular diagnostic assays, it has recently been discovered that the number of viruses that can infect the canine respiratory tract is much larger than it was previously thought. Canine viruses that play an important role in canine respiratory disease include canine herpesvirus-1 (CHV-1), canine adenovirus-2 (CAV-2), canine distemper virus (CDV), canine parainfluenza virus (CPiV), canine pneumovirus, canine respiratory coronavirus (CRCoV), and canine influenza virus (CIV). Viral infection initially damages the epithelium of the upper respiratory tract and induces inflammation in the upper respiratory tract. Vaccine availability and efficacy varies and is also negatively impacted by variation in viral pathogen and poor immunogenicity. Additionally, there are currently no antivirals available. Thus, the development and use of an in vitro model that represents the natural airway to study the viral growth kinetics and the host immune responses associated with canine respiratory infections is of great importance. Our hypothesis is that canine respiratory epithelial cells cultured at air-liquid interface (ALI-CRECs) can be used to characterize the viral growth kinetics and immune responses of canine viruses associated with kennel cough.

Methods: We collected epithelial cells from tracheas of 3 respiratory healthy dogs that were euthanized for unrelated reasons. Cells were isolated, cultured and characterized morphologically and immunologically before infecting them with canine distemper virus (CDV), canine herpesvirus-1(CHV-1), canine influenza virus (CIV) & canine adenovirus-2 (CAV-2) at an MOI of 0.1. Cells and supernatant were collected at 24, 48, 72- and 96-hours post-infection. DNA extraction and qPCR were done to analyze viral growth kinetics. In addition, induction of interferons, cytokines and chemokines will be described following infection with the 4 viruses associated with kennel cough.

Results: First signs of viral infection were noticed at 24 hours post-infection for infection of ALI-CRECs with CAV-2, CDV, CHV-1 and CIV. For CIV and CAV-2 peak viral titers were observed at 24 and 48 hours post infection both intracellularly (cells) and extracellularly (supernatant), whereas for CHV-1 and CDV peak viral titers were observed at 72 hours. Interferon, cytokine and chemokine responses following infection with all four viruses are currently being evaluated.

Conclusions: ALI-CRECs are ideal systems to study viral growth kinetics and induction of innate immune responses upon infection with viruses involved in kennel cough. Future experiments will use this system to test efficacy of antivirals aimed to prevent viral replication and spread.

Financial Support: This research was supported by The Morris Animal Foundation, Michigan State University, College of Veterinary medicine, and Department of Pathobiology and Diagnostic Investigation.

Notes:

50 - The role of exosomes in Marek's Disease Virus-mediated immunosuppression

Mark Parcells[1], Sohee Lee[1], Shannon Modla[2], Ken Pendarvis[3], Yaw Dwomor[4], Phaedra Tavlarides-Hontz[1], Ryan Arsenault[1]

[1]Department of Animal Sciences, University of Delaware, [2]Delaware Biotechnology Institute, [3]MZ Biolabs, LLC, [4]Department of Biological Sciences, University of Delaware. Parcells@udel.edu

Session: Virology 1, 2024-01-21, 11:15 - 11:30

Objective: Marek's disease virus (MDV) is the causative agent of Marek's disease (MD) of chickens, a disease characterized by paralysis, profound immune suppression and rapid T-cell lymphoma formation. Losses due to MD are controlled via the use of live, apathogenic vaccines, however the mechanisms mediating life-long protection from tumor formation 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 systemic anti-tumor immunity mediated by MD vaccination as well as lymphomagenesis, tumor progression, immune suppression in tumor-bearing birds. Our hypotheses are that (1) serum exosomes produced during vaccine virus replication (VEX) elicit lifelong systemic anti-viral and anti-tumor responses, and (2) serum exosomes expressed during MDV latency and from transformed T-cells (TEX) contribute to tumorigenesis and systemic immune suppression.

Methods: To address these hypotheses, we have purified exosomes from the serum of vaccinated chickens (either unchallenged and held in isolators or those surviving high pathogenicity challenge in commercial vaccine studies) as well as from tumor-bearing, as well as MDV-transformed T-cell lines of different pathogenicities via size-exclusion chromatography. These were all characterized by TEM, nanotracking analysis (NTA) and protein content, as well as whole transcriptome sequencing. To assess the effects of these in a reductionist model, we have differentiated the chicken monocyte/macrophage cell line HD11 to become activated macrophages and dendritic cells using TPA, chicken GM-CSF and IL-4. These cells have been characterized regarding their growth, changes in surface antigen expression, uptake of exosomes and the effect of exosome uptake on the proteomes of the cells. In addition, we have examined the effect of purified VEX and TEX on the vaccine responses to a virulent MDV challenge.

Results: Our results indicate that HD11 cells patterned to be activated macrophages and dendritic cells show significant and consistent differences in ribosomal biogenesis and metabolic protein expression and that the changes in metabolism-associated proteins affected cellular metabolism, as measured by Seahorse® assay. In terms of effects on exosome uptake, dendritic cell patterned HD11 cells showed the greatest change in proteomic composition following exosome uptake and these changes were not from proteins previously identified in the exosomes themselves. Our data therefore suggest that cargo delivered by the exosomes is being translated for processing by these cells, and furthermore, that the ability to affect the antigens presented by these cells can be mediated by exosome uptake.

Conclusions: Our results suggest that serum exosomes are taken up preferentially by dendritic cells, where they are processed to alter the proteomes of those cells. Our future plans are to elute peptides from dendritic cells to determine if these exosomal proteins are presented in the context of MHC-I and can stimulate cell mediated responses. Moreover, we are examining the role of VEX to enhance vaccine efficacy and TEX to impede innate immune function and MD vaccine efficacy.

Financial Support: This work was sponsored by NIFA AFRI Grants 2019-67015-29838 and 2023-67016-40112 awarded to MSP.

Notes:

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

Hafiz Sohaib Zafar[1], Nagendraprabhu Ponnuraj[1], Haji Akbar[1], Keith Jarosinski[1]

[1]University of Ilinois. hszafar2@illinois.edu

Session: Virology 1, 2024-01-21, 11:30 - 11:45

Objective: We have identified potential targets for UL13 in horizontal transmission, specifically virion protein unique short (US) 10 (US10) and cellular lymphocyte antigen 6E (LY6E), thought to be involved in enhancing virus infection. Our objective is to delineate the mechanistic importance of UL13 in transmission through US10 and LY6E

Methods: In Specific Aim 1, we used recombinant MDV expressing fluorescent proteins and epitope-tagged UL13 and US10 to study the importance of US10 in transmission using an experimental and natural infection chicken model and protein expression using western blotting.

Results: We determined that US10 expression was completely abrogated during MDV UL13-null replication, while UL13 expression was unaltered in the US10-null virus, but its localization was completely nuclear. When tested in our experimental and natural infection model in chickens, the US10-null virus transmitted like wildtype, showing that US10 is not required for transmission contrary to our original hypothesis.

Conclusions: Our results showed that expression of both UL13 and US10 are dependent on each other, with US10 protein expression abrogated in the absence of UL13, while UL13 localization is dysregulated when US10 is absent during MDV replication. However, contrary to our original hypothesis, US10 is not required for horizontal transmission of MDV. Thus, the abrogated expression of US10 in the UL13-mutant virus does not link US10 to the requirement of UL13 in horizontal transmission. Further studies are required to address the functional importance of UL13 during horizontal transmission.

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Notes:

52 - Streamlining classification of PRRSV-2 ORF5 lineages and sublineages using Nextclade

Michael Zeller[1], Jennifer Chang[2], Phillip C Gauger[3], Jianqiang Zhang[3], Giovani Trevisan[3]

[1]Iowa State University Veterinary Diagnostic Lab, [2]Fred Hutchinson Cancer Research Center, [3]Iowa State University Veterinary Diagnostic & Production Animal Medicine. mazeller@iastate.edu

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Objective: The ability to unambiguously identify PRRSV strains through a consistent nomenclature is quintessential for tracking viruses over time and space and for communication and response to viral disease outbreak(s). A standard framework for PRRSV-2 identification and classification based on genetic lineage was established and refined, but access to standard references and consistency between classification assignment methodologies still complicates the adoption of this framework. The objective of this project is to provide a standardized trivial workflow that is suitable for both swine producers and researchers for classification and comparison of their PRRSV-2 ORF5 sequences.

Methods: Yim-im et al. 2023 provided the latest refinement to the PRRSV-2 ORF5-based lineage nomenclature, extending work previously by Paploski et. al 2019 and 2021. Scaffold sequences representing this updated lineage structure, including historical and contemporary vaccines, were combined with metadata representing year, country, and RFLP of the sequence. These sequences were processed through the Nextclade pipeline using PRRSV0004437 (GenBank DQ478308), a PRRSV-2 ORF5 gene with 100% sequence homology to VR2332, as reference strains for rooting and comparison. The resultant Nextclade dataset files were staged on a GitHub repository for use with Nextclade Web or Nextclade CLI.

Results: The resultant classifier can be accessed through a link on the PRRSView homepage (PRRSView, https://prrsv.vdl.iastate.edu/). This classifier functions the same as other classifiers hosted by the Nextclade core group and can provide genetic-based PRRSV-2 ORF5 classifications on demand. Nextclade provides additional sequence metrics such as classification quality and notable mutations relative to the reference sequences. The submitted query sequences are appended to the reference tree using phylogenetic placement, allowing for comparison to nearby sequences of reference viruses and vaccine strains. The tree can display metadata about the genotype, year collected, country and state, and RFLP of the reference and vaccine sequence set for comparison. Although Nextclade is hosted as a webtool, the sequences are not uploaded to a server, and all analyses stay strictly confidential to the user.

Conclusions: The implemented PRRSV Nextclade dataset provides a transparent and consistent platform for streamlining PRRSV-2 ORF5 lineage classification. The convenient accessibility and public nature of the platform allows diagnosticians, veterinarians, producers, and researchers to assign a common nomenclature to their PRRSV-2 ORF5 sequences.

Notes:

53 - Long-distance dispersal and spread of animal and plant diseases under increased ecological complexity

C.C. Mundt[1], S. Bhattacharyya[1], S. Chatterjee[2], L. Cohnstaedt[3], D.H. Gent[4], C.M. Jones[5], R.K. Meentemeyer[5], P. Ojiambo[5], L. Santamaria[1], C. Scoglio[6], M.J. Tildesley[7]

[1]Oregon State University, [2]The City College of New York, [3]USDA/ARS, Kansas State University, [4]USDA/ARS, Oregon State University, [5]North Carolina State University, [6]Kansas State University, [7]University of Warwick. chris.mundt@oregonstate.edu

Session: Epidemiology 2, 2024-01-21, 10:30 - 10:45

Objective: The global objective of our project is to evaluate multiple outbreaks, complex host communities, and different sources of stochastic transmission during the spatiotemporal spread of epidemics caused by pathogens with long-distance dispersal (LDD). This will be done by addressing four hypotheses: 1) Locations of multiple sources of epidemic outbreak can be imputed from population dynamic models. 2) Ecological spillover effects influence the spread of LDD epidemics and the observed relationships between disease spread and taxonomic diversity. 3) Robust predictions of pathogen transmission require understanding the effects and sources of uncertainty. 4) A unifying framework of biological processes will emerge across LDD diseases incorporating diverse hosts, pathogens, and environments evolving with time.

Methods: The above hypotheses will be addressed with a set of hosts and pathogens of highly divergent taxonomy, but which share the common characteristic of potential for LDD. The six model systems are foot-and-mouth disease of livestock, West Nile Virus, sudden oak death, cucurbit downy mildew, hop powdery mildew, and wheat stripe rust. The model systems were carefully chosen based on a history of strong prior work, high quality data for parameterizing models, and extensive previous modeling efforts. Further, for five of these systems, observational tests of hypotheses from natural experiments can be made utilizing extensive data collected during actual epidemics. Two systems will also be used in manipulative experiments to test hypotheses. Several participants have long-term knowledge of their disease system that will enable them to also investigate optimization of mitigations in terms of policies, societal impacts, and epidemic spread.

Results: This presentation will describe previous work supporting commonality among plant and animal systems for LDD, plans for undertaking the described work, and preliminary results from the first year of the project.

Conclusions: Much of the theory regarding epidemic spread is understandably anchored in simplified conditions of single outbreak sites, uniform hosts, and deterministic epidemic responses. The project will add to our knowledge of disease transmission and spread by incorporating epidemic complexities that are not always considered in models and theory. The work is fundamental to our understanding of disease spread, predicting the spread of epidemic invasion, and designing disease control strategies. The work is providing a rare opportunity to test hypotheses in natural and manipulative field experiments. The applicability of a broad diversity of plant, animal, and human pathogens with fat-tailed dispersal kernels will be rigorously evaluated via the interdisciplinary modeling efforts. Conclusions should apply over a very wide range of spatial scale due to the nature of dispersal kernels of pathogens that have the potential for long-distance dispersal, and because empirical data used in the project will have been derived over varying spatial scales. The proposed work will increase our knowledge of several highly important diseases of animals and plants, which should lead to improved management strategies.

Financial Support: This work was funded by USDA-NIFA AFRI grant # 2022-67015-38059 as part of the joint USDA-NSF-NIH-UKRI-BSF-NSFC Ecology and Evolution of Infectious Diseases program.

Notes:

54 - Exploring the role of land cover on fowl cholera outbreaks in midwestern commercial poultry sites

Lingyu Ouyang[1], Ningchuan Xiao[1], Andréia G. Arruda[1]

[1]The Ohio State University. ouyang.193@osu.edu

Session: Epidemiology 2, 2024-01-21, 10:45 - 11:00

Objective: *Pasteurella multocida* (PM) is a causative pathogen for fowl cholera and is one of the most important bacteria affecting poultry. PM can be transmitted through direct contact, airborne, and fomites. However, understanding on how environmental features impact PM transmission risk remains unclear. This study aims to investigate the association between land cover and fowl cholera cases in poultry farms, focusing specifically on how land cover types and their distances to the farms affect disease outbreaks.

Methods: The case-control study used a PM incidence data set acquired from a private Midwestern poultry production company in the United States, spanning from 2013 to 2021. Landcover features were obtained from the National Land Cover Database 2016. Multivariable logistic regression models were constructed using 40 farms that experienced a fowl cholera outbreak within the study period as cases, and 40 farms that did not have a fowl cholera outbreak as controls. A total of five models were built using STATA 14, each utilizing landcover variables captured within different radii, ranging from 1 to 5 km with an interval of 1 km.

Results: Preliminary results indicate that, within a 1 km radius, wetland is the only influential land cover, increasing the odds of a farm being a case by 4.28 times compared to farms without nearby wetlands (P = 0.005). Within 2 and 3 km radii, pasture/hay emerges as the most influential land cover type. For each 0.1 km^2 increase in pasture/hay coverage, the odds of being a case rise by 1.88 (P=0.005) and 1.27 (P=0.005), respectively. Within a 4 km radius, the presence of large forest areas (over 3 km^2) increases the odds of an outbreak by 3.5 times compared to farms with smaller forest areas (P=0.009). In 5 km radius, for 1 km^2 increase in forest area, the odds of having an outbreak increase by 1.23 times (P=0.048).

Conclusions: Our preliminary results indicated that land cover can be a significant risk factor in commercial poultry fowl cholera outbreaks. Furthermore, our results suggest that the impact of land cover on disease outbreaks can vary by type and distance. Our results can inform decisions for disease prevention in the field**.** For instance, certain wildlife species, including wild birds and rodents, are susceptible to PM and could serve as carriers and vectors of PM. Moreover, proximity to certain land cover types can increase the accessibility for these wildlife species. Therefore, farms located near these identified risk land cover types could enhance their biosecurity measures by implementing fencing, which serves to limit wildlife contact. It is important to point out that the influence of land cover appeared to diminish beyond a 5 km radius, indicating that land-related risk factors for fowl cholera would likely have more impact if closer to poultry sites. Next project steps underway include collecting and accounting for confounders in the analysis, including date of outbreak, poultry density in the area, and land slope.

Notes:

55 - Disease transmission dynamics and host-pathogen coevolution under varying temperatures

Bret Elderd[1], Scott Grimmell[1], Kale Costanza[1], Nathaniel Haulk[1]

[1]Louisiana State University. elderd@lsu.edu

Session: Epidemiology 2, 2024-01-21, 11:00 - 11:15

Objective: As the climate continues to warm, we are seeing a variety of responses including species range shifts, changes in population demography, and altered species interactions. For host-pathogen interactions, climate change may either increase or decrease disease transmission depending on the ecological context. However, little is known about the evolutionary and coevolutionary effects of climate change on disease transmission.

Methods: Using an easily manipulated insect host-pathogen system -- the fall armyworm (*Spodoptera frugiperda*), an agricultural pest, and its lethal baculovirus -- we examined host-pathogen interactions across multiple generations and under multiple temperature regimes. The host is an agricultural pest and, like other outbreaking insects, fall armyworm population dynamics can be pathogen regulated. Larvae become infected while feeding on leaf tissue that has been contaminated with the lethal virus. To examine disease transmission, we exposed hosts that had been coevolved with the virus to varying amounts of the coevolved virus while the host fed on leaf tissue. In a separate set of experiments, we quantified hemocyte production and pathogen production in exposed hosts. We conducted both experiments using multiple generations of the host and the virus.

Results: Our transmission experiments show how transmission dynamics vary across multiple generations according to temperature. Our experiments on host and pathogen responses, show how hosts and pathogens respond to varying temperatures via changes in hemocyte and pathogen production, respectively.

Conclusions: The conclusions drawn from this research are not only applicable to this system but also other silvicultural and agricultural pathogen-susceptible pest species of which there are many. This research will, in turn, improve our ability to determine how best to use these pathogens as bioinsecticides. In future research, we will examine the differences in transmission dynamics between evolved and coevolved pathogen lines under differing temperature regimes.

Financial Support: This work was supported by USDA grant 2019-67014-29919 as part of the joint NSF-NIH-USDA Ecology and Evolution of Infectious Diseases program.

Notes:

56 - Importance of waterfowl migration timing on the transmission dynamics of HPAI at the waterfowl-poultry interface

Sebastian G. Llanos-Soto[1], Dylan Yaffy[2], Marina Pavlak[3], Renata Ivanek[1]

[1]Department of Population Medicine and Diagnostic Sciences, Cornell University, [2]Department of Pathobiology and Population Sciences, Royal Veterinary College, [3]Department of Veterinary Economics and Epidemiology, Faculty of Veterinary Medicine, University of Zagreb. sgl67@cornell.edu

Session: Epidemiology 2, 2024-01-21, 11:15 - 11:30

Objective: Evaluate the impact of waterfowl migration timing on the dynamics of highly pathogenic avian influenza (HPAI) at the waterfowl-poultry interface and the risk of HPAI introduction to backyard poultry farms.

Methods: We developed a modified Susceptible-Infected-Recovered model of HPAI spread among key waterfowl in a natural wetland area and the HPAI introduction to nearby backyard poultry farms. An agricultural region in Croatia that includes the Crna Mlaka Ornithological Reserve was used as a model system due to historical data on HPAI in backyard poultry farms. Waterfowl were represented by mute swans (*Cygnus olor*), as a relevant HPAI sentinel species, and mallards (*Anas platyrhynchos*), both resident and migratory populations, as a key species responsible for the initial introduction and spread of HPAI into waterfowl-inhabited areas. The baseline model accounted for direct intraspecies transmission of HPAI in wild birds and indirect transmission through the environment. Stochasticity was incorporated via Monte Carlo simulations. The transmission of HPAI from mallards to backyard poultry farms was calibrated with data on waterfowl demographics and HPAI outbreaks in backyard poultry in the region. Partial rank correlation coefficient (PRCC) analysis was performed to determine how the timing of waterfowl migration, HPAI viral shedding to the environment, and the rates of recovery and mortality from HPAI infection in wild birds influences the probability of HPAI introduction to backyard poultry farms. The importance of the intraspecies HPAI transmission rate in mallards and mute swans was assessed in a scenario analysis.

Results: Model predictions indicate a median probability of HPAI introduction to backyard poultry farms of 0.14% (5^{th}-95^{th} percentiles (P_5-P_{95}): 0.06%-0.24%) and a median mute swan mortality of 0.17% (P_5-P_{95}: 0.02%-0.77%) during a one-year period starting in September (prior to mallard fall migration). The overlap period between migratory mute swans and mallards was positively correlated with the infection probability in backyard poultry farms (ρ=0.74) and mute swan mortality (ρ=0.50). Meanwhile, HPAI virulence in mallards (i.e., mortality rate) showed a strong negative correlation with the infection probability in backyard poultry farms (ρ=-0.90) and mute swan mortality (ρ=-0.72). Scenario analysis revealed the sensitivity of model predictions to the uncertainty in the intraspecific HPAI transmission rate (per mallard per day).

Conclusions: Insights from this study highlight the importance of monitoring waterfowl migratory movements in densely populated wetland areas to prevent outbreaks in backyard poultry farms. As migration times can change from year to year in waterfowl, this information will assist in identifying areas during the winter season where HPAI surveillance efforts and biosecurity measures should be prioritized. Future studies are needed to improve understanding of HPAI virulence in reservoir species, such as mallards, to enhance outbreak forecasting in backyard poultry farms. Furthermore, improving the knowledge of intra- and interspecies transmission of HPAI in waterfowl species will assist in better representing HPAI dynamics among wild bird populations and the risk of transmission to poultry farms, as well as the role of other coexisting waterfowl species in the dynamics of HPAI.

Notes:

57 -- Japanese encephalitis virus transmission in swine populations: A rapid systematic review of the literature

Vanessa Horton[1], Christy J. Hanthorn[1], Ashley Thackrah[1], Stephen Edache[1], Andrea L. Dixon[1], Leela Noronha[2], Dana Mitzel[2], Vienna R. Brown[3], Lee Cohnstaedt[2], Chad Mire[2], Natalia Cernicchiaro[1]

[1]Kansas State University, Center for Outcomes Research and Epidemiology, [2]United States Department of Agriculture, Agricultural Research Services, National Bio and Agro-Defense Facility, Foreign Arthropod-Borne Animal Diseases Research Unit, [3]United States Department of Agriculture, Animal and Plant Health Services, Wildlife Services, National Feral Swine Damage Management Program. veloso@ksu.edu

Session: Epidemiology 2, 2024-01-21, 11:30 - 11:45

Objective: The United States (US) is considered a susceptible region for introduction of Japanese encephalitis virus (JEV) given ample competent mosquito vectors, susceptible hosts, and environmental conditions. As JEV incursion represents a threat to the US, provision of up-to-date information on mechanisms of disease, control, detection, and treatment is crucial to ensure preparedness. As pigs are considered the main amplifying host for JEV, a rapid systematic review (SR) of the literature was conducted to investigate the role of swine (domestic and feral) in JEV transmission and identify knowledge gaps that may guide future research efforts.

Methods: From an initial screen of 3,163 records from reference databases and 497 records from governmental websites and hand searches, a total of 222 articles were deemed relevant, and included for data extraction and synthesis. To expedite the SR process, recommended strategies were implemented, including limiting the number of databases searched, restricting secondary screening only to abstracts of uncertain relevance, not assessing risk of bias, and others. Outcomes of interest, such as mechanisms of transmission, clinical and pathological signs, risk factors, diagnostics performance, vaccine efficacy, and control measures were extracted from included articles. A framework that has been previously piloted and published was used to systematically identify the gaps in knowledge.

Results: Transmission of JEV via infected mosquito bites has been widely documented and is well established; however, some evidence of JEV oronasal transmission has been also reported. Despite pigs exhibiting a short-lived viremia of 4 to 5 days, JEV has been demonstrated to persist in their tonsils 25 days post infection. The inability of swine to efficiently clear JEV from this site may explain their role as a reservoir and amplifying host. In adult swine, JEV infection may cause reproductive disorders, including abortion, stillborn and (or) mummified fetuses in sows, and orchitis in boars. Although maternal antibodies confer protection under field conditions during the first months of life, naïve piglets can manifest neurologic signs including ataxia and tremors, which may progress to wasting disease and death. To this date, no specific therapy is available for mitigating JEV infection in pigs. Application of biosecurity practices, vector control, and vaccination are recommended as preventive measures to combat introduction and spread of JEV in piggeries, yet, literature supporting the effectiveness of biosecurity and vector control strategies were limited. Although there is no JEV vaccine licensed for pigs in the US, attenuated vaccines are reported to elicit superior immunogenicity compared to inactivated vaccines. Various diagnostic tests can be utilized to detect JEV in swine, with titration methods of neutralizing anti-JEV antibodies being regarded as the gold standard.

Conclusions: Although current conditions have not resulted in a JEV incursion in the US, the recent expansion of JEV in Australia highlights its potential to expand under increasing globalization and climatic change. This rapid SR summarizes the available, up-to-date literature on epidemiology, risk factors, pathophysiology, diagnostics, and control measures of JEV in swine, to provide a better understanding of this virus and support US preparedness efforts.

Financial Support: Swine Health Information Center (SHIC); Kansas State University, College of Veterinary Medicine

Notes:

58 - Insights into Japanese Encephalitis Virus transmission: A systematic review update on vector and host competence

Stephen Edache [1], Ana Oliveira[2], Vanessa Horton[1], Andrea Dixon[1], Natalia Cernicchiaro[1]

[1]College of Veterinary Medicine, Kansas State University, [2]School of Veterinary Medicine, University of California – Davis.
edache@ksu.edu

Session: Epidemiology 2, 2024-01-21, 11:45 - 12:00

Objective: Japanese Encephalitis Virus (JEV), transmitted primarily by mosquitoes, causes encephalitis in multiple host species including humans. It remains a zoonotic pathogen across Asia and the Western Pacific region, and its potential introduction into the United States (US) could result in major disease outbreaks and economic consequences. In light of JEV's geographic expansion and its potential imminent incursion in the US, assessing transmission efficiency and host susceptibility is critical to understanding the epidemiology of JEV and its epidemic potential. This study updates the previous systematic literature review by Oliveira et al. (2018) by appraising scientific literature published from 2016 to 2022 on host and vector competence for JEV transmission.

Methods: Following the protocol designed *a priori*, a comprehensive literature search was carried out using Web of Science, PubMed, Scopus, and Armed Forces Pest Management Board databases. Using a systematic review software and a set of eligibility criteria, the title and abstracts of the searched articles were screened for relevance. All relevant articles were assessed for risk of bias, to evaluate both internal and external validity, and data extraction to collect information pertinent to outcomes of interest, such as transmission efficiency (infection, dissemination, and transmission rates), host preference, and susceptibility to infection.

Results: Data were extracted from a total of 62 relevant articles, with the majority being observational studies (66.1%, 41/62). In terms of vector competence, the reported percentages of JEV positive mosquitoes/mosquito pools ranged between 0.0% (several mosquito species) and 63.6% (*Culex tritaeniorhynchus*). The infection, dissemination, and transmission rates among mosquito vectors showed considerable variation, with values ranging from 0 to 100%, 0 to 96%, and 0 to 100%, respectively. *Culex* species were the most commonly identified vectors across all studies and showed a preference for feeding on pigs, birds, and cattle blood. Regarding host competence, the reported percentages of JEV positive hosts varied widely, ranging from 0.0% to 96.7%, with the highest percentage reported in pigs. In addition, pigs also had a higher reported duration of viremia (14 days) and infectiousness (10 days), compared to other host species, such as birds, where viremia lasted for 2 to 3.5 days. Notably, vector-free transmission was also reported in pigs, which resulted in viremia lasting for 1 to 4 days.

Conclusions: Obtaining current and in-depth information on vector and host competence is vital to understand JEV transmission dynamics, which are necessary to establish robust surveillance programs and implement effective preventive and control measures at both local and global levels.

Financial Support: Swine Health Information Center (SHIC), and College of Veterinary Medicine, Kansas State University.

Notes:

59 - Effect of benchmarking reports on the transfer of passive immunity, navel health, and hydration in surplus dairy calves

Ting-Yu Cheng[1], Jessica Pempek[2], David Renaud[3], Kathryn Proudfoot[4], Zachary England[5], Devon Wilson[3], Nora Bello[5], Gregory Habing[1]

[1]Department of Preventive Veterinary Medicine, College of Veterinary Medicine, the Ohio State University, [2]Livestock Behavior Research Unit, USDA-ARS, [3]Department of Population Medicine, Ontario Veterinary College, University of Guelph, [4]Department of Health Management, Atlantic Veterinary College, University of Prince Edward Island, [5]Department of Animal Sciences, College of Food, Agricultural, and Environmental Sciences, the Ohio State University. cheng.1784@osu.edu

Session: General Health & Physiology 1, 2024-01-21, 10:30 - 10:45

Objective: Surplus dairy calves, or calves that cannot be used to replace the milking cows, are often marketed within the first week of life and receive suboptimal early-life care. This study aimed to test the hypothesis that delivering benchmarking reports containing the health data of calves at calf dealers back to the source dairy farms would improve the metrics for the transfer of passive immunity (TPI), navel health, and hydration of calves delivered to calf dealers.

Methods: Overall, 13 dairy farms were recruited and randomly assigned to intervention (n = 6) and control (n = 7) groups. Two calf dealers were visited in May 2021 - June 2022 for health assessment in calves recruited farms. Six months after the study initiation, farm-wise benchmarking reports were generated for intervention farms containing metrics for passive transfer (total serum protein ≥ 5.1 g/dL), navel health, and hydration. De-identified results from other farms were included in the reports. Changes in these metrics by the effect of benchmarking report reception were investigated using 3 calf-level logistic mixed models with "farm" as the experimental unit.

Results: A total of 653 calves were sampled and assessed from 6 intervention (n = 282) and 7 control (n = 371) farms. Model estimates for the overall probability of failure of TPI and navel infection were 21.4% (95% CI = [13.9, 31.6]) and 20.5% [15.5, 26.5], respectively. Dehydration was marginally less likely from intervention farms compared to control farms after receiving the benchmarking reports (OR = 0.29, [0.07, 1.13], p = 0.07).

Conclusions: The results suggest the incidence of calf dehydration may decrease by delivering benchmarking reports back to dairy producers. This demonstrates the positive effect of feedback on health status of off-farm calves to motivate dairy producers to improve care practices in surplus calves.

Financial Support: This project was supported by Agriculture & Food Research Initiative Competitive Grant no. 2019-67015-29574 from the USDA National Institute of Food and Agriculture.

Notes:

60 - Exposure to wildfire smoke PM2.5 causes pulmonary inflammation and behavioral alterations in dairy heifer calves

Alexandra Pace[1], Kaitlin M. Mirkin[1], Mallery Larson[1], Denise E. Konetchy[1], Pedram Rezamand[1], Amy L. Skibiel[1]

[1]University of Idaho. pace4745@vandals.uidaho.edu

Session: General Health & Physiology 1, 2024-01-21, 10:45 - 11:00

Objective: Wildfires are becoming more severe with climate change, producing toxic air pollutants that can be transported extensive distances. One of these pollutants, fine particulate matter ($PM_{2.5}$), can reach deep within the pulmonary tract following inhalation, which adversely impacts respiratory health in several species. We previously established wildfire-$PM_{2.5}$ exposure causes a systemic inflammatory response and shifts in energy metabolism in pre-weaned dairy heifers, paralleled with indications of reduced health. However, the effects of natural exposure to wildfire-$PM_{2.5}$ on the inflammatory response within the respiratory tract, or how these responses may be reflected in the behavior of calves, has yet to be described.

Methods: Holstein heifers (n=17) born in July 2022 were monitored from birth to 90-d of age to assess responses to wildfire-$PM_{2.5}$. Respiratory rates (RR), rectal temperatures (RT), heart rates (HR), and health scores were documented 3 times weekly, while thoracic ultrasounds (TUS) were conducted 1 time per week. Additionally, blood was collected 5 times for hematology analysis. Trans-tracheal washes (TTW) were performed 2 times over the course of the study on a subset of heifers (n=13) to assess differential leukocyte proportions within the respiratory tract before and during smoke exposure. Daily and hourly standing and lying behavior of this subset was recorded continuously using accelerometers. $PM_{2.5}$, temperature, and humidity were recorded hourly. Temperature-humidity index (THI) was calculated using temperature and humidity data, and elevated $PM_{2.5}$ concentrations were confirmed to be derived from wildfires using HYSPLIT modeling. Hourly behavioral and TTW data were analyzed with generalized linear mixed models. All other data were analyzed using general linear mixed models.

Results: During this study, $PM_{2.5}$ concentrations reached a daily average of 113.5 $\mu g/m^3$ and maximum hourly average of 150 $\mu g/m^3$. Higher combined $PM_{2.5}$ and THI resulted in greater RT and HR, while reducing RR (all P<0.04). Several white blood cell populations were initially reduced when $PM_{2.5}$ and THI were increased at the same time, then increased in the following days (all P<0.05). Basophils and eosinophils were increased with higher $PM_{2.5}$ and THI together (both P<0.05). With higher $PM_{2.5}$ and THI, eye scores increased, while cough scores decreased (all P<0.05). TUS scores increased for several days following exposure to higher $PM_{2.5}$ and THI together (P<0.05). During wildfire-$PM_{2.5}$ exposure as compared to pre-exposure, TTW macrophage proportions increased (22.9 ± 7.8% vs 5.7 ±0.7%; P<0.001). Lastly, when $PM_{2.5}$ and THI were greater, daily and hourly standing times were increased, while lying times were decreased (all P<0.0001).

Conclusions: Alterations in pulmonary leukocytes and greater TUS scores indicate an inflammatory response occurred within the pulmonary tract of heifers exposed to wildfire-$PM_{2.5}$. This may indicate risk of reduced respiratory health in calves exposed to wildfire-$PM_{2.5}$, and heightened THI may exacerbate these effects. The behavioral responses of these calves indicate discomfort or stress, which is a welfare concern. Further investigation is necessary to understand the interconnections between pulmonary and circulating leukocyte alterations of heifers that inhale wildfire-$PM_{2.5}$, and their relationship with behavioral changes, to maximize health and welfare of calves exposed to wildfire-$PM_{2.5}$.

Financial Support: This project was supported by the University of Idaho and the College of Agricultural and Life Sciences.

Notes:

61 - Pre-partum acetylsalicylic acid in dairy cattle: Effects on metabolism, health, and performance

A. A. Barragan[1], E. Jimenez[1], P. Zarei[1], J. Spring[1], J. Lection[1], M. C. Martinez[1], E. Hovingh[1]

[1]Penn State Unviersity. axb779@psu.edu

Session: General Health & Physiology 1, 2024-01-21, 11:00 - 11:15

Objective: The objective of this study was to assess the effects of prepartum administration of acetylsalicylic acid on: 1) metabolic status and systemic inflammation, 2) daily milk yield in the first 50 days in milk (DIM), 3) incidence of diseases in the first 60 DIM, and 4) reproductive performance in the first 150 DIM in dairy cattle.

Methods: At 14 days before the expected calving date, cows (n=404) and heifers (n=160) were blocked by body condition score (optimal=3-3.5; high ≥3.75) and parity (nulliparous; parous), and randomly allocated to one of two treatment groups: 1) ASA (n=286): receive one oral administration of acetylsalicylic acid (4 boluses; 480 grain/bolus; 125 g/d); or 2) PLC (n=278): receive one oral treatment with gelatin capsules filled with water. Body condition score (BCS) was assessed, and blood samples were collected (i.e., β-hydroxybutyrate [BHB] and haptoglobin [HP] concentration assessment), weekly starting 1 week before treatment until 3 weeks after calving. Daily milk yields were collected for the first 100 DIM from on-farm computer records. Subclinical ketosis and clinical metritis were assessed weekly for the first 21±3 DIM. Clinical disease events in the first 60 DIM and reproductive performance by 150 DIM were collected from on-farm computer records. The data were analyzed using MIXED, GLIMMIX and LIFETEST procedures of SAS as a randomized complete block design.

Results: There was no difference in concentration of BHB between cows treated with ASA and PLC in the first 21 DIM. Multiparous cows treated with ASA tended to have lower BCS at calving, 7±3, 14±3 and 21±3 DIM compared to multiparous cows treated with PLC. With regard to daily milk yield, there was an interaction between treatment, day and parity (p = 0.02). Multiparous cows treated with ASA produced, on average, 1.6 kg less milk (days 69, 74, 82, 85, 90, and 94) compared to PLC multiparous cows, while primiparous cows treated with ASA produced, on average, 3.9 kg more milk (days 9, 12, 48, 54 and 74) compared to PLC primiparous cows. Cows and heifers treated with ASA had lower HP concentrations at 7±3 DIM compared with cows and heifers treated with PLC (ASA=58.78 µg/mL,95% CI=34.94-88.77; PLC=128.50 µg/mL, 95% CI=93.56-168.98; p=0.03). Over-conditioned cows treated with ASA had a lower incidence of subclinical ketosis at 7±3 DIM compared to ASA optimal body condition cows (Over conditioned ASA=32.28±5.26%; Optimal body conditioned ASA=56.48±9.08%; *p*=0.04). Primiparous cows treated with ASA tended to have higher subclinical ketosis incidence at 21±3 DIM compared to primiparous cows treated with PLC. A larger percentage of primiparous cows treated with ASA became pregnant in the first service compared to PLC primiparous cows (ASA= 56.14±7.75%; PLC=32.1±4.93%; *p*=0.008). Similarly, primiparous cows treated with ASA required fewer days to become pregnant compared to primiparous PLC cows (ASA=106.45±3.48 d; PLC=124.12±3.81 d; *p*=0.004).

Conclusions: These findings suggest that treatment with prepartum acetylsalicylic acid may have positive effects on primiparous cows' performance, while it may negatively affect multiparous cows. In addition, this treatment may have positive effects on post-partum cow health and reproductive performance.

Financial Support: This work was funded by the USDA National Institute of Food and Agriculture (grant # 2022-67015-36353).

Notes:

62 - Endotoxemia triggers lipolysis and modifies dairy cows' adipose tissue function

Miguel Chirivi[1], Lynn Worden[2], Jair Parales-Giron[2], Adam L. Lock[2], G. Andres Contreras[1]

[1]Department of Large Animal Clinical Sciences, Michigan State University, [2]Department of Animal Science, Michigan State University. chirivim@msu.edu

Session: General Health & Physiology 1, 2024-01-21, 11:15 - 11:30

Objective: The periparturient period is characterized by intense lipolysis in adipose tissues (AT) and systemic inflammation that together increase the risk of disease. Systemic inflammation is often associated with endotoxemia and a higher incidence of periparturient diseases. In vitro, endotoxins increase lipolysis in adipocytes, however, the systemic effect of endotoxemia on AT lipolytic activity and function is unknown. The goal of this study was to characterize the adipose tissue responses of dairy cows with endotoxemia.

Methods: Multiparous Holstein dairy cows [204 (SD=21.7) DIM] were infused IV with **LPS** from E. coli O55:B5 (n=4)] at 1 µg/kg BW or 100 mL of saline infusion control (**CON** n=4). Plasma samples collected at -24, 0, 2, 6, 12, 24, 48, 72, 96, and 120 h relative to infusion were used to quantify non-esterified fatty acids (NEFA; mmol/L), β-hydroxybutyrate (BHB; mmol/L), calcium (Ca^{+}; mg/dL), LBP (mg/mL), and haptoglobin (Hp; mg/mL). Subcutaneous AT (SCAT) biopsies were collected (right flank) 24 h after infusion. SCAT were incubated in the presence of the lipolytic agent isoproterenol (ISO=1 µM, BASAL=0 µM) for 3 hours. Lipolysis was assessed by glycerol release (nmol glycerol/ mg of AT). A linear mixed model was used to evaluate mean differences.

Results: Compared to CON, LPS increased NEFA at 24 h with a peak at 48 h (1.44 vs 0.78±0.30; $P<0.05$). Compared to CON, LPS elevated LBP and Hp at 12 and 24 h with LPB peaking at 24 h (15.19 vs 1.9±0.60) and Hp at 96 h (1.86 vs 0.66±0.18; $P<0.001$). Compared to CON, LPS reduced BHB at 2 h (0.16 vs 0.62±0.04), and calcium by 6h (6.92 9.75±0.48) returning to pre-infusion levels at 24 h and 48 h, respectively ($P<0.001$). Compared to CON, LPS lowered BASAL lipolysis in AT (0.32 vs 2.34±0.60; $P<0.05$). ISO-induced lipolysis did not differ between LPS and CON.

Conclusions: These data suggest that endotoxemia activates lipolysis and systemic inflammation. The reduced BASAL lipolysis may be associated with the depletion of triglyceride reserves in AT after lipolysis. Future studies will explore the effects of endotoxemia on AT inflammation and macrophage trafficking and its association with metabolic disease in dairy cows.

Financial Support: This research was supported by the USDA-National Institute of Food and Agriculture (Washington, DC); competitive grant 2021-67015-34563.

Notes:

63 - Endocannabinoid synthesis is modulated by lipolysis pathways in dairy cows' adipose tissue

Madison Myers[1], Miguel Chirivi[1], Jeff Gandy[1], Joseph Tam[2], Maya Zachut[3], G. Andres Contreras[1]

[1]Department of Large Animal Clinical Sciences, College of Veterinary Medicine, Michigan State University, [2]Institute for Drug Research, School of Pharmacy, Faculty of Medicine, Hebrew University of Jerusalem, [3]Department of Ruminant Sciences, Agriculture Research Organization, Volcani Institute, Israel. smit2477@msu.edu

Session: General Health & Physiology 1, 2024-01-21, 11:30 - 11:45

Objective: In dairy cows, intense lipolysis (release of fatty acids, FAs) and inflammation in adipose tissue (AT) characterize the periparturient period. When lipolysis is excessive and prolonged, cows' susceptibility to disease, milk yield losses, reproductive failures, and culling are amplified. Endocannabinoids (eCBs), bioactive molecules derived from FAs, modulate lipolysis and inflammation in the AT of monogastric species. While currently unknown, the objective of this study was to determine if eCB quantity and profile are mediated by 1) the abundance of FAs released, and 2) pathways activated during lipolysis in bovine AT.

Methods: We compared the effects of basal (BAS; untreated) with stimulated canonical (isoproterenol, ISO) and inflammatory (lipopolysaccharide, LPS) lipolysis pathways in adipocytes cultured from non-lactating, non-gestating dairy cows and evaluated their effects on lipolysis, eCB production (LC-MS/MS), and gene expression (bulk RNA-seq). Statistical calculations included one-way ANOVA with pairwise comparisons using Tukey's HSD and Pearson's correlations for LC-MS/MS and glycerol data in JMP and, for sequencing data, DESeq2R-normalized counts were log-transformed and P-values adjusted using Benjamini and Hochberg's approach for controlling FDR. Genes were considered differentially expressed when fold-changes >1 and adjusted P-values <0.05.

Results: Induction of canonical lipolysis (ISO) resulted in the greatest FA release, which corresponded with increased eCB abundance, enhanced expression of the eCB receptor *CNR1* and eCB-synthesizing enzymes *PTPN22* and *PLCB1*, and reduced expression of the receptor *TRPV1*. Inflammatory lipolysis (LPS) promoted FA release and enhanced eCB content vs. BAS - although not to the extent of ISO - and promoted expression of *TRPV1*, eCB-synthesizing *NAPEPLD*, eCB-degrading *PTGS2* and *MGLL*, and reduced expression of *CNR1*.

Conclusions: Lipolysis intensity, which differs upon stimulation of canonical and inflammatory lipolysis pathways, is paralleled by eCB abundance, changes in eCB profile, and promotes differential expression of eCB system components in dairy cows' AT. Collectively, these findings indicate that a complex relationship between the eCB system, inflammation, and lipolysis exists and, to promote the health and maximize the productivity of dairy cattle, these pathways must be explored extensively in future studies.

Financial Support: We extend our sincere gratitude to the following funding agencies: USDA-NIFA #2019-67015-29443, #2021-67015-34563, and #2021-67037-34657; MAAA #AA-23-0014; US-Israel BARD #IS-5167-19.

Notes:

64 - Genetics of stress hormone concentrations in hair of healthy nursery pigs and their relationship with back test responses

Fazhir Kayondo[1], Hayder Al-Shanoon[2], Dylan Carette[2], Carmen Cole[2], David Janz[2], Frederic Fortin[3], John Harding[2], Michael Dyck[4], Graham Plastow[4], Pig Gen[5], Jack Dekkers[1]

[1]Iowa State University, [2]University of Saskatchewan, [3]Centre de Devéloppement du Porc du Québec, [4]University of Alberta, [5]Canada. jdekkers@iastate.edu

Session: General Health & Physiology 1, 2024-01-21, 11:45 - 12:00

Objective: Cortisol and Dehydroepiandrosterone (DHEA) are circulating adrenal hormones whose concentration in the blood varies in the face of infectious and non-infectious stressors. Their incorporation into hair makes their concentration in hair a robust retrospective assessment of HPA-axis activity as a measure of an animal's stress response over the period of hair growth. Our objective was to estimate the genetic parameters of hormone concentrations in the hair of healthy pigs and their genetic correlation with responses to the standard back-test.

Methods: Hair shaved from the rump area of 871 Large White X Landrace barrows (~40 d of age) from 6 breeding companies, maintained in a high-health nursery, was analyzed for cortisol and DHEA concentrations. Behavioral responses to a 60 s standard back-test were obtained at ~26 d of age, including the number of vocalizations (VN) and struggles (SN) and the intensity of the vocalizations (VI) and struggles (SI). All pigs were genotyped with a 50K SNP panel and imputed up to 650K.

Results: Estimates of heritability of log-transformed levels of cortisol and DHEA using univariate animal models were 0.26 ± 0.08 and 0.00, respectively, while litter effects explained 0.19 ± 0.05 and 0.09 ± 0.04 of the phenotypic variance, respectively. Back-test responses were more heritable, ranging from 0.34 ± 0.08 (VI) to 0.58 ± 0.08 (VN), with small litter effects (0.00 to 0.06). Estimates of genetic correlations using bivariate animal models were high among the back-test responses, ranging from 0.58 ± 0.16 for SN-VN to 0.96 ± 0.04 for VN-VI and 0.99 ± 0.10 for SN-SI. Estimates of genetic correlations of cortisol with back-test responses ranged from low (0.08 ± 0.23 with SI) to moderate (0.66 ± 0.27 with SN).

Conclusions: The concentration of cortisol in the hair of healthy nursery pigs is a heritable molecular phenotype and is genetically correlated with responses to the back-test. In subsequent research, their potential as indicator traits to select for disease resilience will be investigated.

Financial Support: This study was funded by Genome Canada, PigGen Canada, and Agriculture and Food Research Initiative Competitive Grant # 2021-67015-34562 from the USDA National Institute of Food and Agriculture.

Notes:

65 - One Health interventions to better understand transmission and prevent vector-borne zoonoses spread

Christine Petersen[1]

[1]Department of Epidemiology, University of Iowa. christine-petersen@uiowa.edu

Session: CRWAD Council Keynote and Special Symposium, 2024-01-21, 2:00 - 3:00

Still in the shadow of the SARS Co-V2 pandemic, there is a much greater scientific and public recognition of the impact of zoonotic diseases, which are responsible for 75-85% of emerging infectious outbreaks (in people). Despite this increased recognition, there are still many limitations to how animal vs human infectious diseases are considered. Our work focused on zoonotic vector-borne diseases has led to a recognition that interventions that limit transmission from a domestic reservoir, focusing on dogs, can lead to improvement of both animal and human health both within the United States and globally. These One Health approaches are gaining in recognition for the ability to decrease morbidity and mortality against infectious agents like the obligate intracellular protozoan parasite *Leishmania infantum* and the most common human vector-borne disease in the US, *Borrelia burgdorferi.* I will discuss the findings from both vaccine and insecticide field trials from my collaborative group and lessons to take forward from our findings.

Notes:

66 - Genomic solutions for sustainable beef production: Climate-smart cattle breeding

Raluca G. Mateescu[1]

[1]Department of Animal Sciences, University of Florida. raluca@ufl.edu

Session: CRWAD Council Keynote and Special Symposium, 2024-01-21, 3:00 - 3:45

In tropical and subtropical regions where more than half of the world cattle are maintained, climatic stress is a major limiting factors of production efficiency. This stress is expected to increase due to predicted changes in climate. Beef cattle when exposed to environmental high temperature and humidity, exhibit significant declines in feed intake, growth, fertility and welfare. Selection to increase productivity disregarding the genotype x environment interaction is likely to increase susceptibility to climatic stress. This makes the quest for heat-tolerant cattle with increased efficiency of production and reproduction increasingly important. Bos indicus cattle exhibit increased resistance to environmental stressors but they also have slower growth, lower fertility and meat quality relative to Bos taurus cattle. Beef producers in tropical and sub-tropical environments are incorporating a certain proportion of 'indicus' genes in their herds but, without knowledge of genes associated with thermotolerance, this also brings along negative aspects of indicus cattle. My research strives to uncover the phenotypic and genetic relationships underlying this thermotolerance-production complex and subsequently identify the functional variants for thermotolerance without an antagonistic pleiotropy on production and reproduction. This will allow the incorporation of the GxE interaction in genomic selection programs for improvement of economically important traits in a predicted hotter world.

The values of heritability estimated by my group indicate a large, exploitable genetic variance which can be used in selection programs to improve heat tolerance in cattle. Novel traits describing the thermotolerance phenotype such as sweat gland area, short hair length and body temperature under high THI conditions had medium to high heritabilities. More importantly, the genetic correlations estimated in this population are encouraging, indicating favorable relationships between the thermotolerance phenotypes and the production traits. This would suggest that genetic programs to improve resilience to environmental stress could be successful and opportunities exists for simultaneous improvement of production related traits.

Notes:

67 - R&D needs for U.S. agriculture in a changing climate

Ariel Ortiz-Bobea[1]

[1]Charles H. Dyson School of Applied Economics and Management and Jeb E. Brooks School of Public Policy, Cornell University. ao332@cornell.edu

Session: CRWAD Council Keynote and Special Symposium, 2024-01-22, 4:15 - 5:00

Recent evidence indicates anthropogenic climate change has reduced global agricultural productivity (Ortiz-Bobea et al., 2021). There are also indications that US productivity growth is slowing (Ball et al. 2013; Andersen et al. 2018). But US agriculture remains among the most productive in the world due relatively high historical investments in research and development (R&D). However, the adequacy of current R&D spending in the face of rapid climate change is not well understood.

We quantify the R&D needs necessary to offset the emerging impacts of climate change on US agricultural productivity. Our approach is based on an econometric model estimating the effect of R&D and weather fluctuations on Total Factor Productivity (TFP) coupled with counterfactual climate simulations from the Coupled Model Intercomparison Project Phase 6 (CMIP6). TFP is a common aggregate measure of productivity used in economics to jointly track the productivity of all agricultural inputs (as opposed to partial measures of productivity like crop yield or labor productivity).

Our preliminary findings indicate that under a rapid warming scenario (SSP5-8.5), climate change would cause about a 20% reduction in US agricultural productivity relative to a scenario without climate change. This means US agriculture would produce 20% less for the same level of inputs. This effect is equivalent to reducing the TFP growth rate from the historical 1.5%/year to about 1.2%/year. Compensating for this slowdown require more rapid growth in R&D. We estimate a preliminary elasticity of the research stock on TFP of 0.4 which is in line with the literature. This implies that to sustain the historical TFP growth rate of 1.5%/year would require a 3.75%/year growth in the research stock in the absence of climate change. With climate change that rate would need to increase to 4.5%/year. We explore the sensitivity of these results to numerous variations of the econometric model.

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Notes:

68 - Food's journey in the last mile - managing food waste as untapped resources

Zhengxia Dou[1]

[1]Department of Clinical Studies, School of Veterinary Medicine, University of Pennsylvania. douzheng@vet.upenn.edu

Session: CRWAD Council Keynote and Special Symposium, 2024-01-21, 5:00 - 5:45

Food comes a long way literally and figuratively considering the natural as well as manufactured resources used to produce it. Unfortunately, about 1/3 of food produced for human consumption is lost and wasted from harvest to table. Food loss and waste (FLW) has profound impact. It undermines our ability to combat hunger. The production of the uneaten food contributes significantly to climate and resource burdens. For example, roughly 30% of arable land, 20% of freshwater withdrawals, and 38% of total energy consumption are used in vain for producing the food that is lost and wasted globally. FLW has climate footprint accounting for 15% of the anthropogenic greenhouse gas emissions. To mitigate the unwanted consequences, the best approach is to reduce food loss and waste at the source. Indeed, waste prevention has been the highest priority in food waste reduction and recovery hierarchy. However, despite tremendous effort and some success locally, progress has been limited and the scale and magnitude of FLW have not abated. The latest assessments based on updated loss and waste parameters reported global FLW to approximate 1.6 billion tonnes, compared to the 1.3 billion tonnes estimates over a decade ago. Clearly, societies must not rely on waste prevention alone but must also accelerate the progress in tandem with improving the management of food waste in the last mile, that is, the handling and end-of-life disposal of the biomass materials in order to mitigate climate, resource, and various sustainability burdens.

Embedded in food waste materials are hundreds of million tonnes of biogenic carbon, which exists as carbohydrates, along with other nutrients such as proteins, lipids, and minerals. At the University of Pennsylvania School of Veterinary Medicine, we are developing ways and means for upcycling food waste and its nutrients to animal feed to support meat, milk, and egg production while addressing climate and resource challenges. My talk will present quantitative data from interdisciplinary studies on GHG emission reduction and land, water, agri-chemical sparing capacities when suitable food waste is upcycled to animal feed. Collective evidence from feeding studies on animal performance in response to diet incorporated with many types of food waste sources will be provided. I will also discuss de-risking strategies for disease transmission mitigation and overcoming policy bottlenecks to support more beneficial and sustainable use of the untapped resources.

Notes:

69 - What question are we trying to answer? Embracing causal inference

Jan M. Sargeant[1]

[1]Ontario Veterinary College, University of Guelph. sargeanj@uoguelph.ca

Session: AVEPM - Schwabe Symposium, 2024-01-22, 8:30 - 9:15

The theme for the 2024 Schwabe Symposium is *"Remaining relevant: Conceptual advances in research in veterinary populations"*. This abstract describes the keynote address. As epidemiologists, we are taught from the start of our training that "correlation does not imply causation". While this is true, it is also true that identifying causes is a key objective for much of the research that we conduct. Random allocation to intervention groups addresses concerns with the temporal sequence of the exposure and outcome and with confounding, and therefore is a strong design for determining causation. However, an experimental approach is not always ethical or feasible. Therefore, observational studies are common in research in animal populations. Analytical observational studies may be used to predict an outcome, to identify potential associations for further study, or to identify causal associations. There is empirical evidence that veterinary epidemiologists are conducting observational research with the intent to identify causes; many studies include control for confounding (a causal construct) and causal language often is used when interpreting study results. Conceptual frameworks for causal studies are available and include a progression from development of specific hypotheses to be tested, approaches for selection of variables, methods for statistical estimation of the relationship between the exposure and the outcome, and interpretation of that relationship as causal. When comparing observational studies in veterinary populations to those conducted in human populations, the application of each of these steps is quite different. For instance, prior knowledge is used to selection confounding variables in the majority of observational studies in human populations, whereas data driven (algorithm-based) approaches are the norm in observational studies in veterinary populations. The implications of differences in approaches between observational studies in human and animal populations will be discussed and potential solutions will be presented.

Notes:

70 - Rethinking variable selection and study design approaches

Annette O´Connor[1]

[1]Dept. Large Animal Clinical Sciences, Michigan State University. oconn445@msu.edu

Session: AVEPM - Schwabe Symposium, 2024-01-22, 9:15 - 10:00

In keeping with the theme 2024 Schwabe Symposium, honoring the lifetime achievements of Dr. Jan Sargeant, “Remaining relevant: Conceptual advances in research in veterinary populations,” this talk will explore the complexities of estimating causal parameters. In the pursuit of understanding the causes of disease, researchers often conduct observational studies seeking to obtain unbiased estimates in causal exposure-disease relationships. However, estimating causal effects from observational studies presents a complex challenge when contending with confounders and intersecting causal pathways, which can obscure estimation. This presentation aims to unravel these complexities by spotlighting the use of directed acyclic graphs (DAGs) from the conceptualization phase of study design to understand how we should select variables to control. Directed acyclic graphs are not merely tools for modeling; they are fundamental in the study design process, enabling researchers to visually and logically map out potential causal relationships and confounding factors. This approach ensures a more rigorous and nuanced control of variables, using either design or analysis or both- enhancing the integrity of the study's findings. This presentation will underscore the importance of DAGs in identifying the multiple causal pathways and biasing pathways when deciding what to control using either the design or the analysis. To illustrate the practical application and significance of this methodology, we will use a case study within the One Health framework. This example will focus on the impact of emissions from animal feeding operations on the health of nearby community members. By employing DAGs in this context, we aim to demonstrate how they can effectively disentangle complex environmental and health interactions, thereby providing more accurate and actionable insights. Moreover, we will discuss the repercussions of neglecting DAGs in study conceptualization, highlighting instances of potential research wastage due to inadequate variable control. Through this presentation, we aim to advocate for the broader adoption of DAGs in observational study designs, promoting a more systematic and evidence-based approach to understanding exposure-disease relationships.

Notes:

71 - Investigating the effects of dietary and management modifications on *Salmonella enterica* population in harvest-ready beef cattle

Yesica Botero[1], Kasi Schneid [2], Kendall L. Samuelson [2], John T. Richeson[2], Ty E. Lawrence[2], Gizem Levent [1]

[1]Texas Tech University School of Veterinary Medicine, [2]West Texas A&M University Department of Agricultural Sciences. ybotero@ttu.edu

Session: Food Safety, 2024-01-22, 8:30 - 8:45

Objective: *Salmonella enterica* is one of the leading foodborne pathogens in the USA, found in beef products, cattle, and their environment. *Salmonella* prevalence and serotype distribution in cattle and feedlots can be impacted by dietary interventions leading to liver abscesses in cattle, an area that remains largely unexplored. Our randomized controlled feedlot trial investigates the impact of high-starch (H-STARCH) or control (CONT) diets with regular (REG) or erratic (ERRA) feeding schedules on *Salmonella* populations found in cattle (feces and lymph nodes), and their environment (hide and pen composite).

Methods: Cattle (n=720) were weight-blocked and randomized into 12 blocks with 4 pens each (15 cattle/pen). Each pen received one of the 2x2 treatments for an average of 206 days. From a subset of cattle, fecal samples were collected on days 56, 112, and immediately before slaughter, along with 2 pen composite samples. Before shipping, hide swabs were obtained and at slaughter, subiliac lymph nodes were collected. *Salmonella* was isolated using standard methods. A multi-level logistic regression model incorporating fixed effects of treatment and day as well as the random effect of pen and animal was used to estimate treatment effects on *Salmonella* prevalence within each sample type.

Results: A total of 863 fecal samples, 134 lymph nodes, 309 hide swabs, and 288 soil samples were collected and processed. Overall, fecal, hide, lymph node, and pen-soil *Salmonella* prevalence and their 95% CIs were 0.33 (0.30-0.36), 0.55 (0.49-0.61), 0.26 (0.19-0.36), and 0.59 (0.54-0.65), respectively. Day 56 fecal *Salmonella* prevalence was 0.22 (0.17-0.27), day 112 was 0.23 (0.18-0.29) and before slaughter was 0.51 (0.45-0.57). The marginal predicted *Salmonella* prevalence in cattle feces was lowest across all days in H-STARCH-fed cattle and highest in CONT-fed cattle across 3 sampling days. Day influenced overall *Salmonella* prevalence (P= 0.0002) in feces as summer approached, increasing from 0.18 (0.14-0.23) to 0.48 (0.41-0.54). Similar patterns were observed for *Salmonella* prevalence in soil samples, with the effect (P=0.005) of day increasing prevalence from 0.38 (0.29-0.48) to 0.80 (0.72-0.87). *Salmonella* lymph node prevalence was lower in the H-STARCH-fed cattle for both ERRA (0.15 [0.02-0.29]) and REG (0.15 [0.02-0.29]) feeding compared to CONT fed cattle with REG (0.35[0.17-0.53]) and ERRA (0.37 [0.18-0.55]) feeding schedules. The effects of diet (P=0.1043) and feeding schedule (P=0.9107) were not significant on *Salmonella* prevalence in lymph nodes; however, the H-STARCH diet lowered prevalence by 0.20 (-0.02-0.43). Results were similar for hide swabs and soil samples.

Conclusions: Results did not show significant treatment effect on *Salmonella* prevalence across different sample types. However, a high starch diet reduced *Salmonella* prevalence in cattle lymph nodes. Further analysis will explore the clonal distribution of *Salmonella* across treatments and sample types using whole-genome sequencing and bioinformatics.

Financial Support: Texas Tech University School of Veterinary Medicine, start-up fund

Notes:

72 - In-water supplementation of Trans-cinnamaldehyde nanoemulsion reduces *Salmonella* colonization in broiler chickens

Trushenkumar Shah[1], Chetna Shah[1], Chen Zhu[1], Atul Walunj[1], Jodie Allen[1], Brindhalakshmi Balasubramanian[1], Ana Leticia[1], Kimberly Rankin[1], Neha Mishra[2], Indu Upadhyaya[3], Kumar Venkitanarayanan[1], Abhinav Upadhyay[1]

[1]Department of Animal Science, University of Connecticut, [2]Department of Pathobiology, University of Connecticut, [3]Department of Extension, University of Connecticut. trushenkumar.shah@uconn.edu

Session: Food Safety, 2024-01-22, 8:45 - 9:00

Objective: *Salmonella* Enteritidis (SE) is a major foodborne pathogen that causes severe diarrhea in humans. Chickens act as a reservoir host for SE, wherein the pathogen colonizes the ceca leading to carcass contamination during slaughter and subsequent human infections. Several multi-drug resistant SE strains have been isolated from poultry and poultry products fuelling the research for developing antibiotic alternatives for controlling SE in chickens. Trans-cinnamaldehyde (TC) is a Generally Recognized as Safe status phytochemical that exerts significant anti-*Salmonella* efficacy in chickens. However, the challenge working with TC oil is its low solubility in water. To overcome this challenge and enhance dispersion of hydrophobic TC oil in water, TC nanoemulsion (TCNE) was prepared with food grade Gum Arabic and lecithin emulsifiers. Thereafter, the efficacy of in-water supplementation of TCNE in reducing SE cecal colonization in 28-day-old broiler chickens was studied.

Methods: In two separate trials, 192 day of hatch broiler chickens (Cornish cross; 16 birds/treatment/trial) were purchased and randomly allocated to 12 groups. The groups were Control, emulsifier control, TC 0.03, TC 0.06, TCNE 0.03, TCNE 0.06 for challenge (to be infected with SE) and non-challenge (no infection), respectively. Starting day 8, birds in groups TC 0.03, 0.06 and TCNE 0.03, 0.06 were supplemented with TC oil and nanoemulsion, respectively, in drinking water till day 28. On day 14, the birds in the challenge groups were inoculated with a four-strain cocktail of SE (~9 log CFU/bird) by oral gavage. On day 21 and 28, 8 birds/group were sacrificed followed by SE enumeration in cecal contents and liver tissue. In addition, cecal contents and tissue were processed for microbiome analysis and histopathology. Weekly body weight gain, feed and water intake was measured. Data were analysed using one-way ANOVA. Differences between the means were considered significant at $P<0.05$.

Results: TCNE had a size of ~ 100 nm, PDI of <0.3 and Zeta potential of -30 mV. Administration of TC nanoemulsion in drinking water at 0.03 and 0.06 % reduced SE colonization by ~ 2 logs CFU/g of cecal content in both trial 1 and trial 2 as compared to respective controls ($P<0.05$). No reduction in feed consumption, water intake or body weight gain was observed in any treatment groups as compared to controls ($P>0.05$). The alpha and beta diversity analysis did not show any significant changes in diversity and evenness of the cecal microbiome profile of TCNE treated broiler chickens on day 28 as compared to control. Histopathology evaluation of liver and jejunum tissues from TCNE treated chickens did not reveal any adverse changes as compared to control.

Conclusions: Results suggest that TC nanoemulsion could potentially be used to control SE colonization in broiler chickens without affecting cecal microbiome profile and growth performance parameters.

Financial Support: Funded by USDA-NIFA-SAS grant (2020-69012-31823).

Notes:

73 - Antimicrobial resistance in *Escherichia coli* and *Salmonella* isolated from a broiler supply chain and its environment

Mohammad Nasim Sohail[1, 2], Doddamane Rathnamma[2], Csaba Varga[1, 3], Shrikrishna Isloor[2], Wilfred S. Ruban[4], Nagendra R. Hegde[5], Nicola J. Williams[6]

[1]Department of Pathobiology, College of Veterinary Medicine, University of Illinois., [2]Department of Veterinary Microbiology, Veterinary College, [3]Carl R. Woese Institute for Genomic Biology, University of Illinois Urbana-Champaign, [4]Department of Livestock Products and Technology, Veterinary College, [5]National Institute of Animal Biotechnology, Hyderabad, [6]Department of Livestock and Population Health, University of Liverpool. mnsohail@illinois.edu

Session: Food Safety, 2024-01-22, 9:00 - 9:15

Objective: The emergence of AMR has significant global health effects. Indiscriminate use of antimicrobials results in the selection of antimicrobial resistance (AMR) in enteric bacteria of the broiler chicken supply chain. This study aimed to evaluate AMR in two important enteric bacteria *Salmonella* and *Escherichia coli* (*E. coli)* isolated from a broiler supply chain and its environment in Bengaluru, India.

Methods: Samples were collected from broiler breeder farms (BBFs), hatcheries, commercial broiler farms (CBFs), and retail meat shops (RMSs) from three different broiler chicken integrators, and in each integration, the sample batch was tracked from BBF until it reached RMS. Broiler farms were randomly selected from all healthy farms in the area where no outbreaks of any bacterial infections were recorded. The samples were cultured to identify *E. coli* and *Salmonella*, and disk diffusion and minimum inhibitory concentration (colistin only) methods were used to investigate the presence of AMR phenotypes. Pairwise correlation coefficients among antimicrobial resistances were calculated. Clustering dendrograms were constructed using the single-linkage clustering method and were illustrated in heatmaps.

Results: A total of 106 *Salmonella* and 219 *E. coli* were isolated. The overall prevalence of *Salmonella* and *E. coli* in the complete poultry supply chain was 20% and 71.55%, respectively. A significantly higher ($P<0.05$) presence of *Salmonella* was observed in RMS (46%), followed by CBF (19%) and hatcheries (10%). *Salmonella* and *E. coli* isolates were resistant to at least one antibiotic in the study. Seventy-six and seventy-five percent of *Salmonella* and *E. coli* isolates were multidrug-resistant (MDR) and 17 and 41% were Extended Spectrum Beta-Lactamase (ESBL) producers, respectively. Among *Salmonella* isolates from the entire broiler supply chain, highest resistance was observed to doxycycline (94.34%) followed cefpodoxime (85%), ciprofloxacin (73%), gentamicin (65%), amikacin (35%), ampicillin (34%), neomycin (33), colistin (32%), cefotaxime (30%), ceftazidime (29%), trimethoprim-sulfamethoxazole (24%), amoxicillin + clavulanic acid (22%), and chloramphenicol (12%). In *E. coli* isolates the highest resistance was observed to doxycycline (97%) followed by, cefpodoxime (86%), ciprofloxacin (79%), ampicillin (69%), cefotaxime (56%) gentamicin (56%), ceftazidime (54%), trimethoprim-sulfamethoxazole (47%), amoxicillin + clavulanic acid (40%), amikacin (35%), chloramphenicol (23%), neomycin (22%) and colistin (21%). Significantly high pairwise correlation between resistances to several antimicrobials among both the *E. coli* and *Salmonella* isolates were observed. The clustering dendrogram for AMR in *Salmonella* isolates revealed three main cluster patterns i) resistance to gentamicin, ciprofloxacin, and doxycycline, ii) resistance to ceftazidime, cefotaxime, neomycin, and amikacin iii) resistance to chloramphenicol, colistin, trimethoprim-sulfamethoxazole, amoxicillin-clavulanic acid, and ampicillin. The *E. coli* isolates showed two main cluster patterns of resistance i) resistance to ciprofloxacin, doxycycline, gentamicin, ampicillin, ceftazidime, and cefotaxime ii) resistance to neomycin, chloramphenicol, colistin, amikacin, amoxicillin-clavulanic acid, and trimethoprim-sulfamethoxazole.

Conclusions: Antimicrobial-resistant *E. coli* and *Salmonella* were present throughout the poultry supply chain; however, CBF and RMS were the major focal points of AMR. The presence of resistant isolates and coresistance among several antimicrobials in all parts of the supply chain indicates the need for efficient monitoring and control strategies for the effective prevention of AMR in the complete broiler supply chain.

Financial Support: The authors acknowledge research sponsorship from the Indo-UK project (BT/IN/Indo-UK/AMR/05/NH/2018-19), Chicken or egg: Drivers of antimicrobial resistance in poultry in India, by the Department of Biotechnology, Ministry of Science and Technology, Government of India.

Notes:

74 - Factors associated with the dynamics of antimicrobial resistance in the environment of backyard poultry

P Gaonkar[1], R Golden[1], K Bhimavarapu[1], B Mccrea[2], K Macklin[3], L Huber[1]

[1]Department of Pathobiology, Auburn University, [2]Alabama Cooperative Extension System, Auburn Univeristy, [3]Department of Poultry Science, Mississippi State University. ppg0001@auburn.edu

Session: Food Safety, 2024-01-22, 9:15 - 9:30

Objective: Over the past two decades, the population of backyard poultry in the United States has risen significantly. Due to less stringent biosecurity measures, these farms can pose higher risk for spread of antimicrobial resistance (AMR). Our study aimed to: i) assess AMR spread between inside the chicken coop and outside environment; ii) detect antimicrobial residues in their environment; and iii) investigate the impact of farm management practices on the AMR in backyard poultry farms raised to produce eggs for consumption and local commercialization.

Methods: Information related to the farm's managemental practices, focusing on biosecurity and antimicrobial use, was collected by questionnaire administration. Litter, chicken fecal, and soil samples were collected from inside the coop. In outside environment, we collected soil and fecal samples from domestic and wild animals, spanning at different distances from the chicken coop. The collected samples are currently undergoing processing and analysis. The frequency of 3 mobile genetic elements (MGE) and 14 antimicrobial resistance genes (ARGs) that confer AMR to 8 antimicrobial classes was assessed using qPCR. Data analysis was performed using RStudio. Liquid Chromatography-Mass Spectrometry (LC-MS) will be employed to detect antimicrobial residue in the samples.

Results: We found that antimicrobials were not used to treat or prevent diseases, and coop disinfection was not practiced in all enrolled farms. Veterinary care was lacking in the surveyed farms. The detection of ARGs was higher inside the chicken coop compared with the outside environment. Among all the samples, the frequency of ARGs associated with the aminoglycoside, antiseptic, beta-lactam, and tetracycline resistance was higher in chicken fecal samples followed by the litter samples. Whereas macrolide-lincosamide-streptogramin B and sulfonamide ARGs were most frequent in the litter sample followed by chicken fecal samples. Most of the backyard poultry flocks had contact with other domestic and wild animals. The frequency of ARGs related to beta-lactam resistance in the fecal samples from outside environment was comparable with samples from inside the chicken coop. Moreover, ARG associated with the quaternary ammonium compounds was absent in outside soil. MGEs were present in all the samples but were more frequent inside the chicken coop. In addition to these results, we will be presenting antimicrobial residue detection and its association with AMR.

Conclusions: Our study highlights the dynamics of AMR between inside the chicken coop and outside environments in backyard poultry farms. Gaining insights about factors associated with the dissemination of ARGs will inform the development of control points to limit the spread of AMR in backyard poultry farms. Future studies will explore the effect of antimicrobial residues and farm practices on AMR in backyard poultry.

Notes:

75 - Assessing the risk of antimicrobial resistant enterococcal infections in humans due to bacitracin usage in poultry

Randall Singer[1], Timothy Johnson[1]

[1]University of Minnesota. rsinger@umn.edu

Session: Food Safety, 2024-01-22, 9:30 - 9:45

Objective: The objective of the present study was to perform a quantitative risk assessment (QRA) to estimate the potential risk in the U.S. of human infection with antimicrobial resistant bacteria, namely *Enterococcus faecalis* and *Enterococcus faecium* derived from chicken and turkey products, as a result of bacitracin usage in U.S. poultry.

Methods: The modeling approach begins with the annual number of healthcare-associated enterococcal infections in the U.S. and then estimates the number of these infections that would be resistant to antimicrobial therapy and that would be derived from poultry sources because of bacitracin use in poultry. Attribution estimates were generated by analyzing whole genome sequencing data and then comparing the resistance gene overlap among host species with random forest classification models. Because bacitracin is not used in human medicine to treat enterococcal infections, we assessed the prevalence of co-resistance between bacitracin and other resistances that might compromise antimicrobial therapy by using phenotypic and genotypic data.

Results: While approximately 60% of *E. faecalis* and *E. faecium* derived from poultry were predicted to possess bacitracin resistance based on the presence of the *bcrABDR* gene locus, very few human-derived isolates possessed this trait. Furthermore, no vancomycin or linezolid resistant strains of *E. faecalis* or *E. faecium* were detected in poultry sources between the years 2002 and 2019. The model estimated the number of antimicrobial resistant *E. faecalis* and *E. faecium* cases per year that might resist therapy due to bacitracin use in poultry as 0.23 and 0.18, respectively. This translates to an annual risk estimate of less than 1 in 1 billion for members of the U.S. population for each bacterial species, representing a negligible risk.

Conclusions: Even with the use of risk maximizing assumptions and parameter estimates, the results indicate that there is a high probability that the use of bacitracin according to label instructions in U.S. poultry presents a negligible risk to human health.

Financial Support: Funding for this risk assessment was provided by Zoetis, Kalamazoo, MI. The project was conducted independently by the authors, who attest that the opinions and work contained herein accurately reflect their opinions and not necessarily those of Zoetis.

Notes:

76 - Identification of multi-serotype *Salmonella* populations in beef cattle samples using CRISPR-SeroSeq

S.M. Bradley[1], A.T. Siceloff[2], N.W. Shariat[2], G. Levent[3], H.M. Scott[1]

[1]Department of Veterinary Pathobiology, Texas A&M University, [2]Department of Population Health, University of Georgia, [3]School of Veterinary Medicine, Texas Tech University. sbradley2@tamu.edu

Session: Food Safety, 2024-01-22, 9:45 - 10:00

Objective: Traditionally *Salmonella* is isolated using culture-based methods to identify 1-3 dominate serotypes per sample. This approach fails to fully define multi-serotype populations. In the food safety setting culture-based methods may fail to identify vital serotypes of public health importance and never define the complete risk of the food product to the consumer. The objective of this project was to utilize CRISPR-SeroSeq (CSS) technology to analyze and define multi-serotype populations of *Salmonella* in bovine feces, hide swabs, and lymph node tissues and compare those results to traditional culture-based methods. The importance of fully defining the *Salmonella* population in beef lymph nodes cannot be understated. Recent studies have shown that inclusion of lymph node tissue in ground beef results in *Salmonella* contamination of the finished consumer ready product. CSS is an amplicon-based sequencing approach that uses the native CRISPR spacer sequences in *Salmonella* to quantify multiple serotypes in a sample.

Methods: Levent et al. (2019) collected fecal, hide swabs, and lymph node samples from cohorts of cattle in a Texas feedlot and used traditional culture methods to identify the dominant *Salmonella* serotype. Thirty culture positive samples from this study were selected for enrichment and analysis with CSS. We isolated the total genomic DNA from the enriched cultures and performed CSS in 2 sequential PCR steps. The first PCR targeted the CRISPR regions, and the second added dual index barcodes to facilitate multiplexed sequencing. DNA libraries were pooled in equimolar amounts and sequenced on an Illumina NextSeq.

Results: In the original study a single isolate of 5 different serotypes was identified per sample: Cerro (15/30), Anatum (7/30), Lubbock (4/30), Montevideo (3/30), and Newport (1/30). CSS identified an average of 1.5 serotypes per sample with 5 serotypes total (Anatum, Mbdanka, Montevideo I, Newport II, Cerro). Three serotypes were identified in two of the samples and two serotypes were identified in 11 of the samples. Anatum was the most identified serotype in 67% (20/30) of samples and Cerro was the second most identified serotype in 37% (11/30) of samples. Concordance was found in 70% of samples with complete concordance (culture and CSS identified the same single serotype) in 47% (14/30) and some concordance (CSS identified the cultured serotype as dominant along with other serotypes) in 23.3% (7/30) of samples. Interestingly, CSS identified multiple serotypes in 50% (6/12) of the lymph node samples.

Conclusions: CSS is an effective method to define and quantify multi-serotype *Salmonella* populations within bovine lymph node, fecal, and hide samples when compared with traditional culture methods. In this study only 1 sample had a *Salmonella* serotype known to cause human illness (S. Newport) which was identified by both culture-based methods and CSS. Comparing the prevalence of specific serovars with the total *Salmonella* CFUs of a sample, CSS could further define the risk of individual animals, pens, or farms to foodborne illness and contamination of the processing environment.

Financial Support: Texas A&M Agri-Life Research; USDA NIFA.

Notes:

77 - Identification of crucial factors for seawater adaptation and pathogenesis activation in *A. salmonicida*

Ignacio Vasquez[1], Vimbai Machimbirike[1], Trung Cao[1], Javier Santander[1]

[1]Memorial University of Newfoundland and Labrador. ivasquezsoli@mun.ca

Session: Aquaculture, 2024-01-22, 8:30 - 8:45

Objective: *A. salmonicida* is one of the most frequent pathogens worldwide, with a high social-economic impact. This Gram-negative bacterium has a high genome plasticity and a wide host range, causing a devasting impact on wild and farm fish species. However, how *A. salmonicida* modulates its gene repertory to adapt and survive in a marine environment, and which virulence factors activate in the presence of the host, remains unknown. Here, we aim to identify new insights into genotypical adaptation to seawater and early virulence-associated gene expression in *A. salmonicida.*

Methods: Here, we performed an RNA-sequencing analysis of *A. salmonicida* J223 strain grown under laboratory conditions Trypsin soybean broth (TSB), incubated for 40 min in seawater (SW) at 10° C, and incubated in the presence of naïve juvenile lumpfish (*C. lumpus*) for 40 min at 10° C determined as seawater-fish (SWF). Variance and gene expression were evaluated by principal component analysis (PCA) and heat map analysis. Differential expressed genes (DEGs) analysis was performed using a Log2 Fold-change (absolute values >1) and a False Discovery Rate ($p \geq 0.05$). Analyses were performed using the RNA-sequencing tool provided by CLC workbench (CLCBio).

Results: Gene expression analysis within the three conditions showed a total of 1,355, 1,186, and 401 DEGs observed in SW-TSB, SWF-TSB, and SW-SWF comparisons, respectively. Gene Ontology enrichment of identified DEGs showed an association with biosynthetic processes, organic compound metabolic processes, cytoplasm, intracellular and anatomical entity, binding, gene expression, translation, and protein biosynthesis. However, among DEGs observed in the presence of a host, we identified up-regulated genes associated to virulence factors that encoded for porins, riboflavin synthase, immune inhibitors, effectors, chaperones, and structural genes of T3SS, T4SS, and T6SS secretion system.

Conclusions: These results suggest that *A. salmonicida* in seawater does not get into starvation or a non-viable cultivable state, shuts down its metabolism as energy reservoir and minimizes cell duplication, while in the presence of the host activates iron-regulated genes, T3SS virulence factors as primary response to initiate virulence.

Financial Support: Sponsored by the Natural Science and Engineering Research Council of Canada (NSERC).

Notes:

78 - Virulence associated proteins of Flavobacterium covae, an important channel catfish pathogen

H. Tekedar[1], S. Kumru[2], C. Older[3], C. Ware[1], Fenny Patel[1], Lorelei Ford[1], Harun Pirim[4], Mohammad Marufuzzaman[5], Thomas Loch[6], Benjamin LaFrentz[7], Esteban Soto Martinez[8], Geoffrey Waldbieser[3], Matt Griffin[1], Larry Hanson[1]

[1]College of Veterinary Medicine, Mississipi State University, [2]Recep Tayyip Erdogan University, Rize Turkey, [3]USDA ARS Warmwater Aquaculture Research Unit, [4]Department of Industrial and Manufacturing Engineering, North Dakota State University, [5] Industrial and Systems Engineering, Mississippi State University, [6]Michigan State University, [7]USDA ARS Aquatic Animal Health Research, [8]University of California Davis. hanson@cvm.msstate.edu

Session: Aquaculture, 2024-01-22, 8:45 - 9:00

Objective: *Flavobacterium covae* is an important Gram-negative bacterial pathogen of channel catfish and channel X blue hybrid catfish. It infects the skin and gills causing columnaris disease which results in high mortality. The goals of this study are to identify *F. covae* virulence mechanisms and evaluate vaccine candidates through sequence analysis and mutagenesis studies.

Methods: The genomes of **r**epresentative *Flavobacterium* isolates from different geographical locations and genetic groups that cause columnaris disease were sequenced using Nanopore methods. Then comparative genomics and computational methods were used to identify virulence factors, and critical genes of virulence-associated pathways are to be deleted.

Results: We sequenced 62 different whole genomes of *Flavobacterium species* that cause columnaris disease, including 15 *F. covae* isolates. Each sequence has over 100x coverage and represents 4-5 contigs. The putative functions of hypothetical proteins were predicted through computer simulations and machine learning.

Comparative genomics and network analysis revealed five gene pathways are unique to virulent *F. covae* 94-081. To target these pathways for deletion production, we established suicide plasmids constructs that target the 23 genes representing these pathways. Conjugation transfers of these constructs have demonstrated initial production of recombinant *F. covae,* but the recombinants are challenging to purify.

Conclusions: The sequenced genomes are being evaluated using artificial intelligence to help functionally identify putative genes and evaluate unique and shared sequences among the isolates. The virulence factor knock-out mutants will be evaluated for attenuation and ability to induce protection. Further, putative genes will be evaluated to identify potential protective antigens using reverse vaccinology and tested by cloning the genes into expression systems and evaluated for their ability to induce protection in vaccinates. These data will help us identify how *F. covae* causes disease in catfish and will help in vaccine development.

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

Notes:

79 - Role of chitinase and RTX toxin in the pathogenesis of virulent *Aeromonas hydrophila*

Saida Zinnurine[1], Basant Gomaa[1], Q M Monzur Kader Chowdhury[1], Hasan C. Tekedar[1], Mark L. Lawrence[1]

[1]Department of Comparative Biomedical Sciences, College of Veterinary Medicine, Mississippi State University. sz281@msstate.edu

Session: Aquaculture, 2024-01-22, 9:00 - 9:15

Objective: *Aeromonas hydrophila* is a Gram-negative motile, mesophilic species that causes Motile Aeromonad Septicemia (MAS), which is characterized by a destructive systemic infection in channel catfish. In 2009, an emergent clonal group of *A. hydrophila* strains referred to as virulent *A. hydrophila* (v*Ah*) was responsible for the loss of 5.5 million pounds of market-size fish in the US, and it remains a major threat to the catfish aquaculture industry. Our comparative genomic analysis indicated that genes encoding several putative secreted enzymes, including chitinase and RTX (repeats in toxin) family proteins, are unique to v*Ah*. Chitinase is a glycosyl hydrolase encoded by *chiA* that hydrolyzes chitin. The v*Ah* RTX toxin is encoded in an operon consisting of two genes (*rtxC* and *rtxA*) that encode cytolysin-activating lysine-acyltransferase and membrane-damaging MARTX multifunctional-autoprocessing repeats-in-toxin holotoxin, respectively. The objective of this study was to decipher the role of chitinase and RTX toxin in the virulence of v*Ah* in catfish.

Methods: In this study, construction of the v*Ah*Δ*chiA*, v*Ah*Δ*rtxC*, v*Ah*Δ*rtxA*, and double deletion v*Ah*Δ*rtxC*Δ*rtxA* mutants was accomplished by in-frame deletion method. Growth kinetics, hemolysis of sheep red blood cells, and biofilm formation for each of the mutants were compared to parent wild type strain. Virulence and live attenuated vaccine potential of mutants were evaluated by intraperitoneal injection in channel catfish.

Results: There was no difference in growth kinetics between the wild type v*Ah* and mutants. v*AhΔchiA*, v*Ah*Δ*rtxA*, and v*Ah*Δ*rtxC*Δ*rtxA* mutants showed significantly higher (p<0.05) hemolytic activity compared to parent strain v*Ah.* Our findings indicated that RTX toxin cytolysin-activating lysine-acyltransferase played a significant role after 24 and 72 hours in biofilm formation by v*Ah*. Chitinase played a significant role only after 72 hours. RTX toxin demonstrated a highly significant (p<0.001) role in the virulence of v*Ah* in channel catfish, but the role of chitinase was not significant. Furthermore, vaccination of catfish with three attenuated mutants v*Ah*Δ*rtxC*, v*Ah*Δ*rtxA*, and v*Ah*Δ*rtxC*Δ*rtxA* provided significant (p<0.001) protection against experimental infection with the virulent wild type strain at 21 days post-vaccination. The percent mortality of catfish after challenging with 5*10^6 CFU v*Ah* was observed to be 0% in v*Ah*Δ*rtxC*, v*Ah*Δ*rtxA*, v*Ah*Δ*rtxC*Δ*rtxA*, and 47.5% in sham vaccinated group.

Conclusions: These findings demonstrated that chitinase and RTX toxin contribute to hemolytic activity and biofilm formation of v*Ah*. In addition, RTX toxin plays a significant role in v*Ah* virulence in catfish, and the deletion mutant has vaccine potential for protection against parent strain v*Ah* infection. Chitinase has no significant role in the virulence of v*Ah*.

Financial Support: This project was supported by the Mississippi State University College of Veterinary Medicine and the Mississippi State University Global Center for Aquatic Health and Food Security.

Notes:

80 - Development of a PCR-based diagnostic assay for white spot syndrome virus (WSSV) in formulated aquafeed

Hung Mai[1], Arun Dhar[1], Paul Schofield[1], Wendy Sealey[2]

[1]The University of Arizona, [2]United States Department of Agriculture. hungmai@arizona.edu

Session: Aquaculture, 2024-01-22, 9:15 - 9:30

Objective: Formulated aquafeed holds promise as a safer alternative to fresh feed, offering numerous advantages in large-scale farming of crustacean and fin fish. However, the current limitations of PCR-based detection methods hinder their broad application for testing a diverse range of aquafeed and feed ingredients. In response to this challenge, our objective is to develop a robust and validated PCR-based diagnostic assay for detecting WSSV in aquafeed. This assay will play a pivotal role in facilitating disease-free certifications of formulated aquafeeds, thereby ensuring the health and well-being of farmed aquatic animals.

Methods: Experimental shrimp feeds were produced by incorporating WSSV-infected tissue containing a viral load of 3.07 x 10^7 copies/mg of tissue into a commercial-type shrimp feed formulation prior to extrusion. In order to find an optimal method of DNA isolation from aquafeed, total genomic DNA was isolated using four different methods. The viral polymerase gene was selected as a target gene to develop a PCR protocol for WSSV detections since the polymerase gene plays an important role in WSSV replication. Four sets of primers/probes (corresponding to three conventional PCR protocols and one real-time PCR protocol) targeting the polymerase gene of WSSV were developed. The specificity and limit of detection of the newly developed PCR protocols were determined. Diagnosis sensitivity (Dse) and Diagnosis specificity (Dsp) of the PCR methods were determined using known WSSV-spiked feed.

Results: The newly developed primers and probes detected only WSSV in the specificity test. To determine the limit of detection (LOD), three independent assays were conducted for each primer pair. The LOD was 100 copies/reaction and 10 copies/reaction for conventional PCR and real-time PCR assays, respectively.

Conclusions: The PCR assay using WSSV polymerase as a target gene for PCR amplification has a high sensitivity and specificity in detecting WSSV in formulated aquafeed. Since polymerase is a critical gene in viral replication, a lack of amplification of polymerase would indicate that aquafeed does not contain infectious WSSV. The WSSV detection method described here can provide an effective means of determining the infectivity of aquafeed products. By providing a means to evaluate the infectivity of aquafeed products, we empower industry stakeholders to make more informed choices, reduce waste, and optimize the utilization of resources, ultimately benefiting both the environment and economic sustainability.

Financial Support: This project has been funded by USDA.

Notes:

81 - A replication-deficient shrimp viral vector engineered for the delivery of therapeutic RNA

Rod Russel R. Alenton[1], Hung N. Mai[1], Arun Dhar[1]

[1]University of Arizona. adhar@arizona.edu

Session: Aquaculture, 2024-01-22, 9:30 - 9:45

Objective: Delivery of synthetic RNA into crustaceans initiates and directs the antiviral mechanism by RNA interference (RNAi) and has been proven to reduce mortality during infection. The lack of an effective oral delivery platform, however, precludes the advancement of RNAi to field application. In this study, we aim to develop and use an oral delivery platform of RNA-based antiviral therapy in shrimp.

Methods: To fulfill the therapeutic promise of RNAi, we developed a viral vector-based platform capable of simultaneously producing large-scale therapeutic RNA and packaging it into the replication-deficient *Machrobrachium rosenbergii* nodavirus ($MrNV^{DRdRp}$). As a proof of concept, we successfully expressed the MrNV capsid and GFP RNA serving as the RNA payload into a bac-to-bac® baculovirus expression system in Sf9 cells.

Results: Efficient vector-cargo complex ($MrNV^{DRdRp}$-GFP) by baculovirus expression system in Sf9 cells was evident in fluorescence microscopy and flow cytometric analysis. Evidence of assembled $MrNV^{DRdRp}$ particles was also seen in ultrathin sections of Sf9 cells and negatively stained purified viral particles using transmission electron microscopy (TEM). Successful oral delivery of the viral vector was confirmed by the detection and quantification of the MrNV RNA2 and GFP RNA in strategic shrimp tissues demonstrating the spread of $MrNV^{DRdRp}$-GFP. Viral particles were also observed in ultrathin sections of hemocyte cells by TEM analysis. Histopathology was also used to assess biosecurity in using $MrNV^{DRdRp}$, where treated animals were confirmed to be free from any pathological signs of infection typical of the wild-type MrNV. This is also corroborated by the gradual decrease in MrNV-RNA2 copy number confirming $MrNV^{DRdRp}$ is indeed replication-incompetent and therefore non-infectious.

To test the platform's therapeutic capability, we replaced the GFP payload with a hairpin RNA targeting white spot syndrome virus (WSSV) viral protein 28 (VP28). Production of the $MrNV^{DRdRp}$ -hRNA-WSSV-VP28 was optimized and confirmed to produce the intact therapeutic hRNA. To test the efficacy and optimize the treatment routine, bioassays are in progress involving the actual challenge experiment with WSSV.

Conclusions: Our data presents the successful production, oral delivery and expression of an RNA molecule packaged in a shrimp viral vector. With this oral delivery viral vector platform, antiviral therapeutics by RNAi shall finally reach farm-level application.

Financial Support: The research was funded by a grant from the USDA NIFA to AKD and RRR Alenton.

Notes:

82 - Peptidoglycan hydrolases as alternatives to antibiotics to treat *Streptococcosis* in fish

Annik T. Segree[1], David M. Donovan[2], Jason Abernathy[3], Craig A. Shoemaker[3], Grace T. Ramena[1]

[1]Department of Aquaculture & Fisheries, University of Arkansas at Pine Bluff, [2]Department of Biology, Morgan State University, [3]United States Department of Agriculture. segreea1968@uapb.edu

Session: Aquaculture, 2024-01-22, 9:45 - 10:00

Objective: Infectious diseases are the chief cause of production loss in aquaculture, severely limiting growth and sustainability. *Streptococcus iniae* is a gram-positive fish pathogen that infects ~30 fish species. The economic impact of Streptococcosis on the global aquaculture industry amounts to hundreds of millions of dollars annually. While antibiotics are effective against many bacterial infections in fish, their increased resistance and use in aquaculture may cause severe environmental and human health problems. Additionally, multi-drug resistant strains may lead to a potential transfer of antibiotic-resistance farms to clinics. These potential complications have led to an intensive effort to develop safer alternatives to traditional antibiotics. These novel (non-antibiotic) antimicrobials should be refractory to resistance development. Phage endolysins are cell wall degrading peptidoglycan hydrolases (PGHs), enzyme antimicrobials that digest peptidoglycan, the major structural component of the bacterial cell wall.

Methods: Bioinformatic tools were used to identify streptococcal PGHs. Each PHG was expressed in competent *Escherichia coli* using a pET expression vector pET21a (+) containing an ampicillin resistance gene and a c-terminal 6xHis tag was used for protein purification via a Ni-NTA column. Purified PGHs were tested against multiple *S. iniae* strains in standard endolysin assays to determine their activity.

Results: We identified ten streptococcal PGHs enzymes that can potentially prevent and/or eradicate systemic and topical *S. iniae* infections in fish. Our results indicate that purified PGHs have the ability to kill different *S. iniae* strains in plate lysis assay and zymography.

Conclusion: These results indicate PGH has the potential to be an alternative to antibiotics for treating *S. iniae infections,* and future in vitro and in vivo studies are underway to determine if the identified PGHs can indeed serve as alternatives to antibiotics for treating Streptococcosis in fish.

Financial Support: USDA-NIFA-AFRI.

Notes:

83 - Optimize the PEDV recombination-resistant platform for safe, efficacious live attenuated vaccine development

Mingde Liu[1], Xiaoyu Niu[1], Qiuhong Wang[1]

[1]Ohio State Univerisity. liu.6202@buckeyemail.osu.edu

Session: Vaccinology 2, 2024-01-22, 8:30 - 8:45

Objective: Porcine epidemic diarrhea virus (PEDV) is a coronavirus deadly for neonatal piglets and for which no effective vaccines are available. Immunization of pregnant sows with feedback materials, which are autogenous virus preparations, are still used to control PEDV outbreaks at farms. The drawback of the feedback method is that other pathogens can be transmitted on farms. Therefore, live attenuated vaccines (LAVs) are urgently needed to replace feedback materials to prime pregnant sows and protect suckling piglets against virulent PEDV through lactogenic immunity. However, high rates of coronaviruses recombination complicate the use of PEDV LAVs, which conceivably recombine with wildtype PEDV to result in new variant strains, vaccination failure, and confounded PEDV surveillance. Transcriptional regulatory sequences (TRSs) are critical in regulating coronavirus discontinuous transcription. Previously, our lab generated a recombination-resistant PEDV mutant remodeled-TRS (RMT). RMT carries recoded leader and body TRS core sequences (TRS-CSs) that are incompatible with the wildtype TRS-CSs. So, a recombinant virus between the RMT and field PEDV strains cannot transcribe sub-genomic RNAs, leading to no production of infectious viruses. However, in this RMT mutant, an unexpected 189-nt-insertion containing highly repetitive sequences in front of E gene and one “G”-deletion within the N gene TRS-CS were detected and need correction. Also, the RMT contains an exogenous enhanced green fluorescent protein (EGFP) gene in replace with PEDV’s only accessory gene - ORF3, which should be absent in LAVs. In this study, we aim to optimize the RMT mutant to engineer a ready-to-use platform for LAV development.

Methods: A reverse genetics system composed of five plasmids encoding the full-length genomic cDNA of PEDV RMT mutant was reported and used as the backbone. We stepwise deleted the 189-nt-insertion, inserted the “G”, and removed the EGFP gene to rescue three viruses: 1) RMT-ΔG with the correction for the 189-nt-insertion, 2) RMT-v0 with both corrections for the 189-nt-insertion and the "G"-Del, and 3) RMT-v1 with the removal of the EGFP gene from RMT-v0. The recombinant viruses were plaque-purified, and their genome sequences were confirmed. We investigated their viral growth kinetics, the capability for inducing IFNs, and sensitivity to IFN treatment. Viral RNA profiles were analyzed by RNA-seq.

Results: We rescued RMT-ΔG, RMT-v0, and RMT-v1. In vitro assessments of viral growth kinetics indicated that RMT-ΔG, RMT-v0, and RMT-v1 exhibited lower replication efficiency than RMT. Other data for the three viruses is under analyzing.

Conclusions: The 189-nt-insertion in front of the E gene is the only difference between viruses RMT and RMT-ΔG, suggesting its potential enhancement function for viral replication. These findings highlight the importance of fine-tuning genetic alterations in vaccine development to strike a balance between attenuation and vaccine efficacy. Further research and optimization are still needed to harness the potential of the RMT-v1 as a safer and more effective PEDV LAV development platform.

Financial Support: This project was supported by Agriculture and Food Research Initiative Competitive Grant no. 2019-67015-29843 from the USDA National Institute of Food and Agriculture.

Notes:

84 - Encapsulation of oil-based adjuvants into nanoparticles to make semen friendly vaccine formulations.

Pooja Choudhary[1], Ramin Mohammadi[2], Siew Ng[1], Kezia Fourie[1, 3], Dylan Chand[1, 4], Donaldson Magloire[1, 3], Zahed Khatooni[1], Haoming Liu[1, 3], Azita Haddadi[2], Heather Wilson[1, 3, 4]

[1]Vaccine and Infectious Disease Organization, [2]College of Pharmacy and Nutrition, University of Saskatchewan, [3]Department of Veterinary Microbiology, University of Saskatchewan, [4]School of Public Health, University of Saskatchewan. pooja.choudhary@usask.ca

Session: Vaccinology 2, 2024-01-22, 8:45 - 9:00

Objective: Our overall objective is to optimize vaccine formulations for an intrauterine vaccine (i.u.) that could induce a robust immune response when administered during artificial insemination. To help ensure the vaccine was non-spermicidal we encapsulated antigens and adjuvants with Poly (lactic-co-glycolic acid) (PLGA nanoparticles) that will not negatively affect sperm and will, upon breakdown in the uterus, trigger a strong immune response. To reduce cost and improve flow through, we tested the several nanoparticle formulations consisting of Porcine Epidemic Diarrhea Virus Spike (PEDV S)-antigen and adjuvants first in weaner piglets for immunogenicity.

Methods: Weaner piglets (n=4, each group) were mock immunized (PBS) or immunized with PLGA nanoparticles encapsulating antigen alone (PLGA-PEDVS) or co-formulated with oil-based adjuvants, Adjuvant A (Adj A+PEDVS), Adjuvant B (Adj B+PEDVS), Adjuvant C, (Adj C+PEDVS), Adjuvant D (Adj D+PEDVS) or Adjuvant E (Adj E+ PEDVS) on day 0 and day 21. Blood samples were collected at 0, 21 and 42 to quantify PEDVS-specific antibodies, neutralizing antibody titers and IFN γ, IL-13 and IL-10 secretion from T-cells. Intestinal tissues were harvested at the end of the trial day 42 post vaccination, and intestinal PEDVS1-specific IgG (C) and IgA (D) were quantified.

Results: Adj A+PEDVS showed the highest antigen-specific IgG antibodies in serum, followed by Adj D+PEDVS, Adj C+PEDVS and Adj B+PEDVS formulation. Adjuvant A formulations also had increased antigen-specific IgG antibodies in both jejunum and Ileum Intestinal samples, followed by Adjuvant D, Adjuvant B, and Adjuvant C formulations. While antigen-specific IgA titers were higher in only the Adjuvant A group measured in intestinal ileum samples. Cell-mediated immune responses were quantified in PBMCs isolated on day 42 after 2 doses of vaccine, and IFN γ production was increased in Adjuvant A group restimulated with PEDVS antigen. Virus neutralizing antibody titers were higher in Adjuvant A followed by Adjuvant C and B formulations.

Conclusions: Among several tested adjuvants, Adj A+PEDVS formulations trigger a robust immune response in weaner piglets. Using this optimal nanoparticle formulation, we will administer i.u. vaccine in gilts during breeding and immune response in gilts (serum, colostral/milk neutralizing antibodies) and piglet birth rate will be measured. Piglets born from vaccinated animals will be assessed for average daily and growth kinetics. Piglets will be challenged with infectious PEDV at weaning to establish whether vaccinated gilts passively protect weaners.

Financial Support: Funding was provided by Saskatchewan Agriculture Development Fund. VIDO also receives operational funding from the Government of Saskatchewan through Innovation Saskatchewan and the Ministry of Agriculture and from the Canada Foundation for Innovation through the Major Science Initiatives fund.

Notes:

85 - A triple gene-deleted PRV-vectored subunit PCV2b and CSFV vaccine protects pigs against virulent CSFV challenge

Ediane Silva[1], Elizabeth Medina-Ramirez[1], Selvaraj Pavulraj[2], Douglas P Gladue[1], Manuel Borca[1], Shafiqul I Chowdhury[2]

[1]U.S. Department of Agriculture, ARS, Plum Island Animal Disease Center, [2]Department of Pathobiological Sciences, School of Veterinary Medicine, Louisiana State University. pselvaraj1@lsu.edu

Session: Vaccinology 2, 2024-01-22, 9:00 - 9:15

Objective: Pseudorabies viruses (PRV), classical swine fever virus (CSFV), and porcine circovirus type 2b (PCV2b) are devastating viral diseases of pigs causing huge economic losses to the swine industry. The goal of this study was to develop a PRV-vectored subunit vaccine against PCV2b and CSFV (PRVtmv+) and determine its protective efficacy against PCV2b and CSFV in pigs. Previously we have shown that the vaccinated pigs are protected fully against PCV2b. Further, we showed that the vaccine virus upon reactivation from latency did not replicate in the trigeminal ganglia (TG) and shed in the nasal secretions. In this study, our objective was to determine the protective efficacy of the vaccine in pigs against CSFV challenge.

Methods: Two groups of pigs (n=5) were vaccinated with our vaccine both intranasally and subcutaneously or mock vaccinated. At 28-day post-vaccination, all pigs were challenged with virulent CSFV. To determine the protective efficacy of the vaccine after challenge, we performed a serum neutralization assay to determine the CSFV-specific humoral immune response, real-time PCR to detect challenge virus genome copies in the blood, clinical pathology and gross lesions at necropsy.

Results: A single dose of the PRVtmv+ vaccine stimulated an optimal virus-neutralizing antibody response against CSFV at day 28 post-vaccination (dpv; the day of challenge). The sham-vaccinated control pigs developed severe CSFV-specific clinical signs characterized by pyrexia, and diarrhea and became moribund on or before the seven-day-post challenge (dpc). However, the PRVtmv+ -vaccinated pigs survived until the day of euthanasia at 21 dpc. A few vaccinated pigs showed transient diarrhea but recovered within a day or two. One pig had a low-grade fever for a day but recovered. The control pigs had a high level of viremia, severe lymphocytopenia, and thrombocytopenia. In contrast, the vaccinated pigs had a low-moderate degree of lymphocytopenia and thrombocytopenia on four dpc but recovered by seven dpc. Based on the gross pathology, none of the vaccinated pigs had any CSFV-specific lesions. Therefore, our results demonstrated that the PRVtmv+ -vaccinated pigs were protected against virulent CSFV challenge.

Conclusions: A single dose intranasal/subcutaneous trivalent PRVtmv subunit vaccine against PCV2b and CSFV protects the pigs completely against fatal CSFV disease. Moreover, the vaccinated-challenged pigs did not have any noticeable lesions in the spleen, kidney and tonsils. Therefore PRVtmv+ can be used in the event of a CSFV outbreak in CSFV-free countries.

Notes:

86 - Heterologous immunization with rVSV and rORFV virus vectors provide partial protection against virulent African Swine Fever Virus challenge

Jessica C. Noll[1], Natasha Gaudreault[2], Diego Diel[1], Juergen Richt[2]

[1]Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University, [2]2Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, Kansas State University. nng5757@vet.k-state.edu

Session: Vaccinology 2, 2024-01-22, 9:15 - 9:30

Objective: This study aimed to evaluate the safety and efficacy of a heterologous vaccination protocol against African Swine Fever Virus (ASFV), utilizing recombinant virus vectors, ORF virus (rORFV) and Vesicular Stomatitis virus (rVSV), expressing ten different ASFV antigensselected based on the protein functions and the immune responses they elicit.

Methods: Individual constructs were generated for the development of virus-vectored ASFV subunit vaccine candidates using rORVFs and rVSVs expressing the following tenASFV genes: EP402R, E153R, CP530R, EP364R, B602L, I73R, B646L, E199L and E183L. Expression of each of the ten ASFV proteins was confirmed by western blot analysis and/or immunofluorescence assays, and the recombinant constructs purified and characterized *in vitro.* Next, six piglets were immunized with a cocktail of the recombinant vaccine candidates at day 0 (rORFV-ASFV), day 21 (rVSV-ASFV) and day 35 (rORFV-ASFV); an additional six piglets were included as unvaccinated controls. At day 42, the animals were challenged with a virulent genotype II ASFV strain. The animals were monitored daily for clinical signs, and the study was terminated at day 15 post challenge.

Results: The rORFV and rVSV constructs replicated to similar titers as the WT strain in ovine and swine cells; confocal imaging, flow cytometry, IFA and/or western blots showed that all ASFV proteins were expressed by the recombinant viruses. During thevaccination phase, none of the animals presented any reaction to the immunization, and the body temperature of the vaccinated group remained normal. After ASFV challenge, the vaccinated group presented lower body temperatures compared to the unvaccinated ASFV-infected control animals. Likewise, the average clinical scores of the vaccinated group remained lower than the unvaccinated group. At the end of the experiment, all unvaccinated, ASFV-infectedanimals died by day 14 post challenge, while 50% of the vaccinated animals survived.

Conclusions: Heterologous prime-boost-boost immunization with rVSV-ASFV and rORFV-ASFV vectors are safe, as the vaccinated animals showed no side effects after immunization. Importantly, 50% of the immunized animals survived challenge with a virulent genotype II ASFV. These results indicate that the ASFV proteins encoded by genes EP402R, E153R, CP530R, EP364R, B602L, I73R and E183L may play a role in protection against ASFV infections and are good candidates for subunit vaccine development against ASFV.

Financial Support: This work was supported by USDA NIFA grant nos. NI20AHDRG047 and 2023-67015-39653 and the NBAF Transition Funds.

Notes:

87 - T cell epitope content comparison of a Porcine Reproductive and Respiratory Syndrome vaccine against heterologous type 2 strains

Andres H. Gutierrez[1], Lucas Huntimer[2], William D. Martin[1], Anne S. De Groot[1], J. Mark Hammer[2]

[1]EpiVax, Inc, [2]Elanco Animal Health. annied@epivax.com

Session: Vaccinology 2, 2024-01-22, 9:30 - 9:45

Objective: As reproductive and respiratory syndrome virus (PRRSV) continues to evolve, vaccinedevelopers face a significant challenge; their vaccines must provide cross-protection against highlydiverse strains. Vaccines that contain T cell epitopes well-matched to circulating strains are more likely toinduce strong cell-mediated memory responses upon challenge or field exposure. To quantify therelatedness between a modified live virus (MLV) vaccine and four heterologous type 2 (PRRSV-2) strains, we predicted and compared the T cell epitope content contained in seven proteins and evaluated therelationship between T cell Epitope Content Comparison (EpiCC) scores and vaccine efficacy data from aprevious study.

Methods: Seven structural proteins from the Prevacent® vaccine strain and from four wild-typePRRSV-2 strains (VR2332, NADC20, NADC30, and NC174) were analyzed. Putative class I and II SLA-restricted T cell epitope content in the input sequences were identified using PigMatrix. For eachsequence, putative epitopes of the vaccine were compared to each PRRSV-2 strain to calculate arelatedness score based on T cell epitopes shared between pairs of sequences (EpiCC score). HigherEpiCC scores represent greater relatedness and higher T cell epitope coverage. EpiCC scores and T cellepitope coverage were compared to efficacy data.

Results: Considering the combined T cell epitope content of seven proteins, putative MLV epitopesshared with the PRRSV-2 strains covered on average, 58.09% of their total epitope content, ranging from52.76% (NC174) to 62.72% (NADC20). Although T cell epitope coverage was not significantly differentbetween strains, based on the combined T cell epitope content of seven proteins, protective efficacy wasanticipated to be highest for NADC20 (62.72%), VR2332 (61.34%), and lower for NADC30 (55.55%), andNC174 (52.76%). In the efficacy study, MLV vaccine induced partial protection against VR2332, NADC20, and NADC30 but not against NC174, which suggests that higher EpiCC scores (reflecting broader T cellepitope coverage) were associated with partial protection.

Conclusions: More effective protection following MLV vaccination was associated with higher EpiCCscores, reflecting broader cross-conserved T cell epitope coverage. A similar relationship has beenobserved for PCV2 vaccines and strains. EpiCC was developed to help veterinarians, practitioners, andfarmers selecting the best-matched commercial vaccine for immunization against circulating isolates andsurveillance to identify variants that may represent a threat. EpiCC may complement current methodsfor selecting the best-matched vaccine or for selection of vaccine candidates. EpiCC can be appliedto swine and human viruses beyond PRRSV.

Notes:

88 - Assessing the effects of maternal antibodies on a piglet post-weaning diarrhea vaccine candidate

Kathryn Lauder[1], Chongyang Zhang[1], Siqi Li[1], Ipshita Upadhyay[1], Kristen Massey[1], Ryan Pinto[1], Yiyang Shen[1], Christine Stroink-Pickering[1], Sara Pearson[1], Courtney Hayes[1], Weiping Zhang[1]

[1]University of Illinois at Urbana-Champaign. klauder2@illinois.edu

Session: Vaccinology 2, 2024-01-22, 9:45 - 10:00

Objective: Pre-existing antibodies, obtained through environmental exposure or maternal transmission, are a concern in developing pharmaceutical therapeutics. Endogenous antibodies may react with novel therapeutic antibodies through cross-reactions that adversely or innocuously affect the therapeutics. Influence of passive maternal antibodies in the simulation of pig active immunity from post-weaning diarrhea vaccine candidates have not been characterized. Assessment of the effects of maternal antibodies are essential for the successful development of a vaccine against post-weaning diarrhea for piglets.

Methods: In this study, two gilts were immunized with ProSystem RCE (Merck) and designated "pre-existing maternal antibodies" (Group 1); two gilts were immunized with PBS and designated "no pre-existing maternal antibodies" (Group 2); another gilt without immunization served as a control of opportunistic enterotoxigenic *E. coli* (ETEC) infection. Piglets born from Group 1 and Group 2 gilts were immunized with an experimental post-weaning diarrhea (PWD) vaccine whereas piglets from Group 3 were not immunized and served as the environmental control. Serum samples were collected from each piglet from day 5 to 11 weeks of age, measured for antibody responses derived from the vaccine fimbrial and toxin antigens (K88, F18, LT, Stx2e, STb, and STa) with ELISAs, and analyzed the effect of maternal antibodies at the stimulation of host immunity from the vaccine candidate.

Results: Gilts included in the study had little or low antibodies to ETEC (LT, K88, F18), but the Group 1 gilts developed robust antibody responses after immunization with ProSystem RCE. Piglets born to the immunized Group 1 mothers acquired passive antibodies, whereas piglets born to Groups 2 and 3 had no passive antibodies to ETEC antigens. After active immunization with the PWD vaccine candidate, piglets with maternal antibodies developed vaccine-specific antibodies at levels slightly higher or the same compared to the piglets without maternal antibodies.

Conclusions: Gilts and piglets immunized with a vaccine developed specific antibodies and passive maternal antibodies that did not play a significant role in affecting a post-weaning diarrhea vaccine in stimulating active immunity in young pigs.

Financial Support: USDA University of Illinois at Urbana-Champaign Agriculture Experimental Station.

Notes:

89 - Unraveling the origin of mucosal T cells in Equine herpesvirus type 1 infection

Camille M. Holmes[1], Heather Freer[1], Bettina Wagner[1]

[1]Cornell University. cmh335@cornell.edu

Session: Immunology 1, 2024-01-22, 8:30 - 8:45

Objective: Equine herpes virus type 1 is a highly prevalent respiratory pathogen of the horse. It infects via the upper respiratory tract (URT) causing respiratory disease in susceptible animals, and the subsequent establishment of a cell-associated viremia can cause equine herpesvirus myeloencephalopathy or abortion in pregnant mares. The initial immune response at the site of viral infection likely plays a crucial role in preventing severe disease manifestations. The objective of this work is to characterize the T cell response at the mucosal surfaces during infection and determine the origin of those cells homing to the URT.

Methods: To characterize the T cell response, nasal and peripheral cells were collected from healthy and EHV-1 infected horses. In healthy horses, the immune cells present in the equine URT were quantified and *in vitro* stimulations were conducted to determine specificity. Nasal cells were collected by three different approaches, targeting different regions of the URT. First nasal washes collected lumenal cells, nasal swabs collected cells within the mucus, and nasal brushes collected cells within the mucosa and submucosa. Cells were characterized by flow cytometry for surface expression of LFA-1, CD4, and CD8. Following stimulation with EHV-1, cytokine quantification and intracellular staining for IFN-γ, were used to determine activation. Similarly, nasal cells collected during an experimental EHV-1 infection were characterized for surface expression of LFA-1, CD4, and CD8, and by intracellular staining for granulysin (GNLY), TNF-α, and IFN-γ.

Results: In healthy horses, the distribution of LFA-1$^+$ immune cells within the mucosa significantly differs from the periphery. Mucosal samples collected by nasal brush contain significantly more CD8$^+$ T cells ($p=0.0002$) and CD4$^+$CD8$^+$ T cells ($p=0.0016$), but fewer CD4$^-$CD8$^-$ immune cells ($p=0.0044$), than peripheral samples. Peripheral cells collected from horses protected from EHV-1 produced IFN-γ during viral stimulation, but nasal T cells did not. Interestingly, with a general T cell stimulant, both peripheral and nasal T cells produced IFN-γ, suggesting this is a mature population of cells. Using newly developed equine-specific reagents, we characterized a population of activated CD4$^+$ and CD8$^+$ T cells in the URT during infection, through expression of GNLY, TNF-α, or IFN-γ. This population of activated cells appeared one-week post-infection. While GNLY$^+$ cells could be found in the nasal lumen, they could not simultaneously be detected in the periphery of the same horses.

Conclusions: T cells are a crucial part of the adaptive immune system. While nasal T cells in healthy horses are mature and can become activated, they do not respond to the virus under the same conditions as peripheral cells. However, during active infection, cells within the nasal lumen express markers of activation, establishing their functional relevance in the mucosal immune response. This suggests that EHV-1 specific T cells home to the URT, rather than maintain residency. Work is ongoing to determine the origin of the T cell population that is present in the URT during infection, and further phenotype the resident nasal T cell population.

Financial Support: Harry M. Zweig Memorial Fund for Equine Research, The American Quarter Horse Foundation.

Notes:

90 - Increased secretion of antileukoproteinase during the innate immune response to Equine herpesvirus type 1 infection

Camille M. Holmes[1], Susanna Babasyan[1], Bettina Wagner[1]

[1]Cornell University. cmh335@cornell.edu

Session: Immunology 1, 2024-01-22, 8:45 - 9:00

Objective: The mucosal immune response is an important factor in respiratory infections, where adequate activation is required to clear the pathogen, but overzealous inflammation could interfere with normal respiratory function. Antileukoproteinase (SLPI) wears many hats as an intracellular and secreted protein constitutively expressed at mucosal surfaces, with an overall function as a homeostatic regulator. Previous transcriptomic screening described an early upregulation of nasal SLPI gene expression during equine herpesvirus type 1 (EHV-1) infection, a respiratory virus of the horse. Here, we aimed to define the cell types producing SLPI during EHV-1 infection, compare secretion in EHV-1 protected versus susceptible horses, and understand its position in the inflammatory cascade.

Methods: Monoclonal antibodies (mAbs) against equine SLPI were generated using murine hybridoma technology and validated for flow cytometry, immunofluorescence, and bead-based assay. Horses were intranasally infected with the Ab4 strain of EHV-1, and peripheral and mucosal samples were collected throughout the infection. Serum and nasal secretion samples were used for quantification of SLPI, and cells were isolated from peripheral blood and nasal washes for intracellular staining and characterization of the $SLPI^+$ population. Additionally, nasal explants collected from euthanized horses were infected *ex vivo*, and localization of SLPI production within the mucosa was defined.

Results: During experimental EHV-1 infection, SLPI secretion significantly differed in susceptible compared to immune horses, in both the serum ($p=0.01$) and nasal secretions ($p=0.0004$). Nasal SLPI secretion peaked on day 4 post-infection (pi) and serum SLPI secretion peaked on day 3pi, in susceptible horses. Characterization of the cells producing SLPI in the periphery during EHV-1 infection defined a population of $CD14^+$ $SLPI^+$ monocytes within peripheral blood mononuclear cells (PBMC). The fraction of $SLPI^+$ PBMC increased from day 3pi to day 6pi, returning to normal by day 10pi. A greater percentage of $SLPI^+$ PBMC was present in horses that developed severe clinical disease, compared to those who did not, which was significant on days 5pi ($p=0.02$) and 7pi ($p=0.01$). A population of $CD14^+$ $SLPI^+$ monocytes could also be detected in the nose on d7pi. Finally, immunofluorescence imaging of nasal explants supported both epithelial cell and localized immune cell production of SLPI during EHV-1 infection.

Conclusions: The broad roles of SLPI have been implicated in various diseases in humans and other species, where it can act as an anti-inflammatory, anti-protease, and/or anti-microbial. Here we defined the cellular contributors for SLPI secretion at the mucosal surfaces and systemically during EHV-1 infection. We also demonstrated a conditional upregulation of SLPI in horses that are susceptible and develop disease. Work is ongoing to explore direct interactions of SLPI with EHV-1, and to determine if there is co-expression of anti-inflammatory SLPI with inflammatory cytokines, such as IL-1β and TNF-α. Together this will better characterize the role of this protein in EHV-1 and establish its use as a marker for development of clinical disease.

Financial Support: Harry M. Zweig Memorial Fund for Equine Research.

Notes:

91 - Chitosan-nanoparticle-based oral *Salmonella* vaccine elicits cross-protective immune response in broilers

Sara Dolatyabi[1], Sankar Renu[1], Jennifer Schrock[1], Renukaradhya J Gourapura[1]

[1]The Ohio State University. dolatyabi.1@buckeyemail.osu.edu

Session: Immunology 1, 2024-01-22, 9:00 - 9:15

Objective: Non-typhoidal *Salmonella* infections are a significant public health concern worldwide. *Salmonella* encompasses an extensive range of pathogens that are important sources of foodborne diarrheal illness on a global scale. Developing an oral vaccine to induce cross protective mucosal immunity in the intestines against *Salmonella* in poultry is challenging. Our objective is to develop and evaluate an oral vaccine that can mitigate the load of multiple serotypes of *Salmonella* in broilers.

Methods: We developed *Salmonella Enteritidis* (SE) immunogenic outer membrane proteins (OMPs) and flagellin (FLA) containing mannose chitosan nanoparticle (OMPs-FLA-mCS-NP) vaccine, and evaluated its efficiency to induce cross-protection against *Salmonella Typhimurium* (ST) serotype infection. Broilers were vaccinated with two doses of OMPs-FLA-mCS-NP vaccine orally at age day 3 and booster after three-weeks. As a control, a commercial live vaccine was inoculated to a group of birds at age 3 days by spray method and boosted through drinking water at 3 weeks of age as per the manufacturer's instructions. At 5-weeks of age birds were challenged with ST and after 10-day post-challenge, samples were collected and examined.

Results: Previously, in oral SE subunit OMPs-FLA-mCS-NP vaccinated broilers observed robust mucosal immunity and protection against SE infection. In this study, the orally delivered OMPs-FLA-mCS-NP SE vaccine induced a higher cross-protective immune response against ST compared to the commercial Poulvac® ST vaccine composed of a modified-live ST. The OMPs-FLA-mCS-NP vaccinated birds had increased production of IgA and IgY antibodies specific to OMPs and FLA antigens in samples collected at both post-vaccination and post-challenge compared to mock and commercial vaccine groups. Notably, detected cross protective immunity induced by OMPs-FLA-mCS-NP vaccine associated with reduced ST bacterial load by around 1 $\log_{10}$ CFU in the cecal content which was comparable to the commercial vaccine group.

Conclusions: The findings of our study suggest that the orally administered OMPs-FLA-mCS-NP SE vaccine elicited cross reactive mucosal immune responses against ST infection in broiler chickens. This, OMPs-FLA-mCS-NP vaccine could be a viable option to commercial live vaccine.

Financial Support: Supported by USDA-AFRI 2022-67017-36559.

Notes:

92 - Polyanhydride nanoparticles trigger antiviral responses against bovine RSV in vitro and in a murine RSV model

Fabian E Diaz[1], Elizabeth A Grego[1], Ali Uslu[1, 2], Balaji Narasimhan[1], Jodi L McGill[1]

[1]Iowa State University, [2]Selcuk University. fediaz1@iastate.edu

Session: Immunology 1, 2024-01-22, 9:15 - 9:30

Objective: Despite use of antimicrobials and vaccines, bovine respiratory disease (BRD) remains a highly prevalent disease in the cattle industry, with bovine RSV being a major etiological agent. Efforts to develop immunomodulatory strategies to improve disease resistance in food animals are required as an alternative to antimicrobial use. We have previously shown that polyanhydride nanoparticles (PANPs) loaded with pattern recognition receptor (PRR) agonists have intrinsic immunostimulant properties on bovine epithelial and myeloid cells from the respiratory tract *in vitro*. Here, we evaluated whether PANPs can trigger antiviral responses in epithelial cells *in vitro*, and evaluated their efficacy in preventing RSV disease in a murine model.

Methods: Bovine turbinate cells (BTs) and human A549 cells in 12 well plates were stimulated with one of 15 different PRR-loaded PANPs (at 40 or 200 ug/mL) for 24 h, then infected with Bovine RSV (bRSV) strain 375 or human RSV A/1997 (hRSV) at MOI 0.1. After 48h, the $TCID_{50}$/mL was calculated to quantify infectious titers. Untreated cells (UT) and empty PANPs (200 ug/mL) were used as controls. Selected particles were also tested for antiviral activity in vitro against human RSV (hRSV) and immunogenicity in rodents. Female 6-8-wk old BALB/c mice received PANPs (40 or 100ug/mice) intranasally. Saline (UT) and empty PANPs (100 ug/mice) were used as controls After 72h, animals were either euthanized to collect lung samples for lung cytokine quantification, or infected i.n. with hRSV st. 1997. Infected animals were monitored daily, then euthanized 2 dpi to collect whole lung. Viral copies (N-hRSV/β-actin copies) were quantified by RT-PCR in whole lung tissue.

Results: Several PANPs were able to reduce infectious titers in bRSV-infected BT cells when compared to UT cells, including PRR-loaded and empty NPs. Nanoparticles that contained CL413 (TL2/7 agonist), Pam3CSK4 (TLR2/1 agonist) and MPLA (TLR4 agonist) were able to reduce infectious titers of both hRSV and bRSV *in vitro*. Noteworthy, intranasal administration of a NP made of 1,8-bis-(p-carboxyphenoxy)- 3,6-dioxaoctane and 1,6-bis-(p-carboxyphenoxy)-hexane in a 20:80 ratio loaded with the CL413 agonist (CPTEG:CPH 20:80 CL413) was able to prevent weight loss ($p=0.008$) and reduce lung viral loads ($p = 0.0022$) in RSV infected mice, which was associated to increased IL-1β ($p=0.001$), IL-6 ($p=0.002$), and KC ($p=0.0009$) levels in lungs at the time of infection when compared to the UT group.

Conclusions: Our results suggest that the administration of NPs efficiently prime human and bovine respiratory tract epithelial cells and trigger antiviral defenses. Moreover, selected PANPs trigger local immune responses that are associated to reduced viral replication in murine lungs and clinical benefit during RSV infection. Our results warrant future evaluation in ruminant models of RSV disease and BRD to evaluate their potential application in BRD.

Financial Support: This work was funded by USDA-NIFA grant 2021-07002 and by a Postdoctoral Seed Award from Iowa State University.

Notes:

93 - Bovine epithelial and immune cells express IL-36 in response to toll-like receptor agonists and viral infection

T. Philip Babatunde[1], Jodi L. McGill[1]

[1]Department of Veterinary Microbiology and Preventive Medicine, Iowa State University. btphilip@iastate.edu

Session: Immunology 1, 2024-01-22, 9:30 - 9:45

Objective: Despite the widespread availability of vaccines and antimicrobials, bovine respiratory disease (BRD) continues to cause morbidity, mortality, and economic loss in the cattle industry. Interleukin-36 (IL-36) is a recently described group of cytokines that plays a role in promoting proinflammatory immune responses in the lungs in response to both viral and bacterial infections. IL-36 is comprised of three agonists, IL-36 α, β, and γ, and one antagonist, IL-36 receptor antagonist (IL-36RA). IL-36 signals through a heterodimeric receptor comprised of IL-36R and the IL-1 receptor accessory protein (IL-1RAP). While the importance of IL-36 has been explored in humans and mouse models, its significance in the bovine immune system is not known. The objective of this study was to determine the expression of IL-36 and its receptors in bovine nasal turbinate cells (BTCs) and immune cells in response to stimulation with toll like receptor (TLR) agonists or bovine respiratory syncytial virus (BRSV). The secondary objective was to determine the effect of IL36RA on TLR-induced proinflammatory cytokine production by bovine immune cells.

Methods: Bovine turbinate cells (BTCs) were treated with LPS (1mg/ml), Poly (I:C) (10mg/ml) or Mixed (combination of LPS (1mg/ml), Poly (I:C) (10mg/ml)). In separate cultures, BTCs were infected with a 0.1 MOI of BRSV strain 375. After 6, and 72 h, IL-36 expression was analyzed by qRT-PCR. In Objective 2, peripheral blood mononuclear cells (PBMCs) were isolated from n = 7 4-5-month-old Holstein steers housed at the Iowa State University. PBMCs were treated with IL-36RA (1mg/ml) for 4 h, followed by stimulation with LPS or Poly (I:C) for 48 h. Secretion of IL-1β and IL-6 was measured in supernatants by commercial ELISA.

Results: Six hours after TLR stimulation, BTCs upregulated IL-36β ($p = 0.0022$) in response to Mixed; and IL-36RA in response to both Poly (I:C) ($p = 0.0023$), and Mixed ($p = 0.0005$). The expression of IL-36β ($p = 0.0355$), IL-36γ ($p = 0.0125$), IL-36RA ($p = 0.0188$), and IL-1RAP ($p = 0.0083$) was higher in BRSV-stimulated BTCs compared to unstimulated cells after 72 h. Pretreatment of PBMCs with IL-36RA blocked both IL-1β ($p < 0.001$) and IL-6 ($p < 0.01$) production in response to LPS ($p = 0.0001$) and Mixed ($p = 0.0002$).

Conclusions: We observed that BTCs and PBMCs upregulated expression of IL-36 in response to stimulation with TLR agonists and BRSV. Pretreatment with IL-36RA reduced production of IL-1β and IL-6 by PBMCs stimulated with LPS or Poly(I:C). Future work will focus on elucidating the relevance of IL-36 in vivo in the context of BRD.

Financial Support: Sponsored by John G. Salsbury Endowed Chair in Veterinary Medicine.

Notes:

94 - Characterization of bovine intraepithelial T lymphocytes in the gut

Akanksha Hada[1], Anmol Kandel[1], Lei Li[1], Zhengguo Xiao[1]

[1]Department of Animal and Avian Sciences, University of Maryland. hada@umd.edu

Session: Immunology 1, 2024-01-22, 9:45 - 10:00

Objective: Gastrointestinal (GI) diseases in cattle cause serious health issues and substantial economic losses. Intraepithelial T lymphocytes (T-IELs) are crucial in defending against enteric pathogens and maintaining gut homeostasis. With a composition of one T-IEL for every ten epithelial cells and accounting for 50-60% of all T lymphocytes, the gut becomes the largest immune organ. This makes T-IELs critical targets for developing preventive strategies against GI disorders. Nevertheless, despite their extensive characterization of T-IELs in humans and mice, research on their bovine counterparts is scarce, which is the issue we are addressing in this project.

Methods: T-IELs isolated from various segments of the bovine small intestine were characterized by surface markers such as T-cell receptors (TCR), CD4, and CD8, and intracellular proteins IFNγ, IL17A, and TGFβ1 using flow cytometry, with circulatory and lymphoid lymphocytes used as controls. The cytokine-secreting capability of the freshly isolated T-IELs was assessed after stimulation with either Brefeldin A (BFA) alone or an activation cocktail consisting of phorbol 12-myristate 13-acetate, ionomycin, and BFA for four hours.

Results: Bovine T-IELs displayed several similarities to those in humans and mice, but the composition of their subtypes was distinct. These T-IELs were abundant in the small intestine, especially the jejunum (70%), and they expressed high levels of the activation and tissue retention marker CD69, along with a low level of the peripheral homing marker CD62L. Furthermore, similar to humans and mice, bovine T-IELs produced inflammatory cytokines such as IFNγ and IL17A, and a low level of the immune regulatory cytokine TGFβ1. Importantly, in cattle, the proportions of TCRγδ+ and TCRαβ+ T-IEL subsets were almost equal, and the majority of the TCRγδ+ T-IELs did not express CD8. Additionally, approximately 20% of TCRαβ+ T-IELs were CD4+CD8αβ+. Uniquely, TCRαβ+CD8αα+ cells were not observed in cattle.

Conclusions: Our research provides insights into the unique composition and potential functions of T-IELs in cattle, underscoring both functional conservation across species and specialized adaptations. These findings open avenues for further investigations into bovine T-IELs and their role in developing strategies against gastrointestinal disorders including acute and chronic infections in cattle.

Notes:

95 - Pathogen delivery route impacts disease severity in *Mycoplasma ovipneumoniae* and *Mannheimia haemolytica* coinfection

Bryan Tegner Jacobson[1], Thomas Andrew Sebrell[1], LaShae Zanca[1], Jessica DeWit[1], Sobha Sonar[1], Carol Hardy [2], Michael Throolin[2], Patricia Brewster[1], Katilyn Andujo[1], Kerri Jones[1], Steve Jones[1], Lizabeth Bowen[3], Jonathon Sago[4], Stephen Smith[4], Diane Bimczok[1]

[1]Department of Microbiology and Cell Biology, Montana State University, [2]Department of Mathematical Sciences, Montana State University, [3]United States Geologial Survey, Davis Field Station, University of California, Davis, [4]Anatomic and Clinical Pathology, Histology, and Milk Laboratory, Montana Veterinary Diagnostic Laboratory. diane.bimczok@montana.edu

Session: Host-Pathogen Interaction, 2024-01-22, 8:30 - 8:45

Objective: *M. ovipneumoniae* is a respiratory pathogen associated with mild to moderate pneumonia in domestic sheep, particularly in lambs. Studies on experimental *M. ovipneumoniae* infection have reported highly variable outcomes with regards to clinical signs and pathology. However, previous experimental infection studies also have differed in terms of pathogen source, dose and delivery. Here, we assessed the impact of *M. ovipneumoniae* delivered either to the upper respiratory tract (URT) or to the lower respiratory tract (LRT) on pathogen load and distribution, lung pathology and clinical disease.

Methods: Groups of five two- to three-months-old specific pathogen-free lambs were inoculated with ceftiofur-treated nasal wash fluid obtained from sheep with natural *M. ovipneumoniae* infection. One group received the inoculum to the URT mucosae, a second group to the LRT via tracheal endoscopy, and a control group received PBS alone. Lambs were monitored over a period of eight weeks, with nasal swabs and body weights collected weekly, and blood samples collected biweekly. Eight weeks after inoculation, lambs were euthanized for pathological analysis and sample collection. Bacteria were quantified using qPCR and culture-based approaches, and antibody responses to *M. ovipneumoniae* were measured by ELISA.

Results: All lambs in both infection groups, but not the control group developed a stable infection with *M. ovipneumoniae* and robust systemic antibody responses to *M. ovipneumoniae* after 2 weeks. Interestingly, all inoculated but none of the controls lambs also tested positive for *Mannheimia haemolytica*, a common secondary pathogen in ovine respiratory infections, although *M. haemolytica* was undetectable in the ceftiofur-treated inoculum. LRT inoculation led to lower weight gains and increased clinical signs including fevers and coughing compared to URT inoculation, with control animals showing the lowest clinical scores and the highest weight gains. Notably, monoinfection of lambs with *M. ovipneumoniae* via the URT in a previous study performed using the same protocol did not cause clinical disease or changes in body weight. After eight weeks of infection, lambs inoculated via the LRT had visible consolidation of the cranial lung lobes that was not observed in the other groups. Pathogen loads in the trachea and bronchi, but not the nasopharynx, were significantly higher after LRT than URT inoculation. Histopathological analysis confirmed that pathological alterations were confined to the cranial lungs and consisted of alveolar, bronchiolar, and interstitial inflammation that was significantly more severe for the LRT compared to the URT and uninfected control groups.

Conclusions: Our study demonstrated that bypassing protective mechanisms of the URT by delivering respiratory pathogens to the LRT leads to more severe respiratory disease and lung damage than delivery to the URT. Our findings explain the divergent results on the impact of *M. ovipneumoniae* infection from previous studies. We also confirmed that *M. ovipneumoniae* significantly enhances the susceptibility of lambs to secondary respiratory pathogens such as *M. haemolytica,* and that co-infections are associated with significant clinical disease and weight loss, which in turn decreases productivity in domestic sheep flocks.

Financial Support: This study was supported by USDA NIFA award 2022-67016-36503 and Hatch award #MONB00450 from the Montana Agricultural Experiment Station to D.B., and an NIH TL1 fellowship (#UL1 TR002319) to B.T.J.

Notes:

96 - The effect of bile acids cultured with specific bacteria on *Clostridium perfringens* growth

Tahrir Alenezi[1], Bilal Alrubaye[1], Janashrit Shrestha[1], Samar Algehani[1], Hong Wang[1], Xiaolun Sun[1]

[1]University of Arkansas. alrubaye@uark.edu

Session: Host-Pathogen Interaction, 2024-01-22, 8:45 - 9:00

Objective: *Clostridium perfringens*-induced necrotic enteritis (NE) causes significant economic losses in the poultry industry. The primary bile acid chenodeoxycholic acid (CDCA) is the predominant bile in the chicken small intestine. We aimed to investigate the efficacy of bile acids metabolized by specific bacteria on reducing the growth of *C. perfringens*.

Methods: Bile acids of 0.5, 1.0, and 1.5 mM CDCA, 1.0 mM cholic acid (CA), and 1.0 mM lithocholic acid (LCA) were cultured and metabolized at 37 ºC under anaerobic condition, in the presence of bile-metabolizing bacteria *Eggerthella lenta* or *Parabacteroides merdae*. The pre-cultured media with metabolized bile acids were autoclaved and then used to culture *C. perfringens* for additional 24 h. *C. perfringens* was enumerated by serial dilution and plating on selective *C. perfringens* agar plates. Differences between treatments were analyzed using One-way ANOVA followed by Fisher's LSD test using Prism 7.0 software. Experiments were considered statistically significant if p-values were < 0.05.

Results: *C. perfringens* growth reached 7.4 log10 CFU/ml after 24 h culture, while 0.5, 1.0, and 1.5 mM CDCA reduced the pathogen growth to 7.2, 7.0 (significant), and 6.2 (significant) log10 CFU/ml, respectively. *C. perfringens* cultured in the *E. lenta* pre-cultured media grew to 7.9 log10 CFU/ml after 24 h incubation, whereas its growth was significantly reduced to 4.6, 0, and 0 log10 CFU/ml in the pre-cultured media of *E. lenta* plus 0.5, 1.0, and 1.5 mM CDCA. *C. perfringens* cultured in the *P. merdae* pre-cultured media grew to 5.6 log10 CFU/ml after 24 h incubation, whereas its growth was significantly reduced to 0 log10 CFU/ml in the pre-cultured media of *P. merdae* plus 1.0 mM CDCA. Interestingly, *C. perfringens* grew to 6.2 and 6.1 log10 CFU/ml in 1.0 mM CA and LCA, respectively, while its growth was significantly increased to 7.1 and 7.2 log10 CFU/ml in the pre-cultured media of *E. lenta* plus 1.0 mM CA or LCA, respectively. In addition, *C. perfringens* grew to 7.1 and 5.3 (significant reduction) log10 CFU/ml in the pre-cultured media of *P. merdae* plus 1.0 mM CA or LCA, respectively.

Conclusion: Collectively, these findings indicate that bile acids metabolized by *E. lenta* and *P. merdae* differentially influence *C. perfringens* growth. The findings could be used to intervene *C. perfringens* induced chicken necrotic enteritis.

Financial Support: This research was supported by grants from Arkansas Biosciences Institute, USDA National Institute of Food and Agriculture (NIFA) Hatch project 1012366, NIFA Hatch/Multi State project 1018699, NIFA SAS 2019-69012-29905, and NIFA project 2020-67016-31346 to X.S and B. H.

Notes:

97 - Transcriptomic insights into liver abscesses in beef cattle: A gastrointestinal tissue perspective

J. Okon[1], H. Ford[1], N. Beneduzi[1], T.F. Scull[1], D. Woerner[2], C. Strieder-Barboza[1], O. Benitez[1]

[1]Department of Veterinary Sciences, Texas Tech University, [2]Department of Animal Sciences, Texas Tech University. jokon@ttu.edu

Session: Host-Pathogen Interaction, 2024-01-22, 9:00 - 9:15

Objective: Gastrointestinal (GI) dysfunction is implicated in the pathogenesis of liver abscesses (LA), which remain an issue in the beef cattle industry. However, there are limited studies elucidating the relationship between GI dysfunction and liver abscesses in bovine. The aim of this study was to evaluate the transcriptomic profiles of GI tissues from healthy steers and steers affected with liver abscesses.

Methods: GI tissues from jejunum were collected from 6 beef steers, including animals with (LA; n=3) and without liver abscess (control; n=3). Following sample collection, the tissue was flash frozen and stored at -80C for further isolation of total RNA via RNeasy Fibrous Tissue Mini Kit. Isolated RNA was assessed for quality, and RNA fragmentation, double-stranded cDNA, and adaptor ligation were generated using the Illumina Stranded mRNA Prep kit according to the manufacturer's protocol. The transcriptome sequencing was performed on the barcoded stranded RNA-Seq libraries using the Illumina NovaSeq 6000 flow cell, targeting at least 30 million reads per sample. Differential gene expression analysis was performed using DESeq2 with a cutoff of adjusted p-value ≤ 0.05 and functional analysis was performed using KEGG pathways.

Results: There were 281 differentially expressed genes between LA and control animals with 100 genes upregulated including *H3C10, H2AC16, ITGA5, DEFB10, CD180* and *GP2*. Functional enrichment analysis revealed that the aforementioned genes are associated with neutrophil migration, neutrophil extracellular traps (NETs) formation, necroptosis, shigellosis, bacteria invasion, B cell receptor signaling and Toll Like Receptor (TLR 4) signaling pathways. Contrariwise, a total of 181 genes were found to be down-regulated in steers with LA, including *LCT, ENPP3, APOC2, FABP2, ALPI, and SLC28A2*. The identified genes exhibited associations with several metabolic pathways, including amino acids, carbohydrates, lipids, and vitamins. Additionally, the down-regulated genes were also related to brush border membrane proteins.

CONCLUSION: The upregulation of genes and pathways related to bacterial invasion and shigellosis in GI tissues from steers with LA suggests possible disruption of the adherens junctions and tight junctions, resulting to increased intestinal permeability. This may ease the translocation of bacterial pathogens from intestinal lumen into the circulation, and facilitate systemic infections, and potentially, liver abscess pathogenesis. Additionally, the upregulation of neutrophil migration, NETs formation, necroptosis, and B cell receptor signaling and TLR 4 pathways indicates the activation of both innate and adaptive immune responses in animals with liver abscesses**.** The perceived downregulation of brush border membrane, protein, carbohydrate and lipid digestion and absorption indicates that LA steers may have experienced impaired nutrient biosynthesis and absorption secondary to GI malfunction. This could be attributed to potential damage of the villi and microvilli which may be associated with chronic inflammation led by bacteria colonizing the intestinal epithelium. Therefore, this data suggests that transcriptomic changes in the GI could be associated with liver abscess pathogenesis in beef steers. However, further mechanistic studies are needed to substantiate these outcomes.

Notes:

98 - Transcriptomics analysis of the head kidney and gill of lumpfish in response to *Moritella viscosa* infection

Trung Cao[1], Hajarooba Gnanagobal[1], D.O. Ignacio Vasquez[1], Setu Chakraborty[1], Ahmed Hossain[1], Joy Chukwu[1], Danny Boyce[2], Javier Santander[1]

[1]Marine Microbial Pathogenesis and Vaccinology Laboratory, Department of Ocean Sciences, Memorial University of Newfoundland, [2]Ocean Sciences Centre, Memorial University of Newfoundland. ttcao@mun.ca

Session: Host-Pathogen Interaction, 2024-01-22, 9:15 - 9:30

Objective: Lumpfish (*Cyclopterus lumpus*) is a cleaner fish employed to biologically control sea lice infestation in Atlantic salmon. *Moritella viscosa* is the causative agent of winter ulcer disease in cold-water salmonid-farmed fish and other fish species in the North Atlantic region. While there have been no reported outbreaks of winter ulcer disease in lumpfish, little is known about its susceptibility and immune response to *M. viscosa*. The head kidney is a crucial lymphatic organ, while fish gills act as the mucosal barrier. Both play an essential role in the immune response against pathogen infection.

Methods: Here, we investigated the median lethal dose (LD_{50}) and the transcriptome profile of immune response in lumpfish's gill and head kidney to *M. viscosa* infection. Different groups of lumpfish were intraperitoneally injected with varying doses of *M. viscosa* (3.1×10^6, 3.1×10^7, or 3.1×10^8 CFU dose^{-1}), and sterilized seawater was used as a control. The mortality rates and bacterial infection kinetics were monitored for 30 days post-infection (dpi). Head kidney and gill samples were collected from control (n=3) and infected (n=3) lumpfish at 10 dpi for RNA-sequencing (RNA-Seq).

Results: Lumpfish infected with different doses exhibited typical clinical signs of ulcer disease. The mortality rates for lumpfish infected with high, medium, and low doses were 66.67%, 48.39%, and 30.51%, respectively. The lethal dose (LD_{50}) of *M. viscosa* in lumpfish was calculated as 8.1 x 107 CFU dose^{-1}. We performed a transcriptomic analysis in the head kidney and gill tissues of lumpfish infected with *M. viscosa*. Transcriptome profiling identified 763 and 222 differentially expressed genes (DEGs) in the head kidney and gill, respectively, in infected compared with control fish. In gene ontology (GO) analysis, immune response-related biological processes were enriched in lumpfish head kidney and gill exposed to *M. viscosa*. Gill immune response was mainly associated with cellular extravasation, a complex of collagen trimers, immunoglobulin binding, monosaccharide binding, high-density lipoprotein particles, carnitine metabolic process, and ectodermal placode morphogenesis. The head kidney immune response mostly involved acute inflammatory response, acute-phase response, response to cytokine, cellular modified amino acid metabolic process, receptor-ligand activity, and organic anion transport.

Conclusions: In summary, lumpfish infected with *M. viscosa* showed a typical winter ulcer disease. This study deeply expanded our knowledge about the molecular pathways activated in lumpfish gill and head kidney in response to *M. viscosa* infection.

Notes:

99 - Molecular mechanisms of enhanced resistance to avian influenza in two genetically distinct, highly inbred chicken lines

Liqi An[1], Shenwen Gu[1], Dailu Guan[1], Susan J. Lamont[2], Rodrigo A. Gallardo[1], Hans H. Cheng[3], Huaijun Zhou[1], Ying Wang[1]

[1]University of California, Davis, [2]Iowa State University, [3]USDA-ARS. ucywang@ucdavis.edu

Session: Host-Pathogen Interaction, 2024-01-22, 9:30 - 9:45

Objective: Avian influenza (AI) is one of the most important viral infectious diseases that cause significant economic losses. With the potential of low pathogenic (LP) AI evolving into highly pathogenic (HP) AI, innovative approaches to improve genetic resistance to LPAI are critical to minimize economic losses and promote sustainability. The objective of the project is to discover key genetic components contributing to genetic resistance to LPAI by using the two genetically distinct, highly inbred chicken lines that differ in avian influenza virus (AIV) resistance. The rationale is that understanding key genetic determinants of chicken LPAI resistance will improve host genetic resistance and enhance protection from AIV infection. We hypothesize that immune cell frequencies and MHC molecule expression during antigen presentation in the Harderian gland (HG) are associated with AIV infection. The greater AIV resistance among Fayoumi birds results from gene expression regulation through regulatory elements on the genetic determinants.

Methods: At 3 weeks of age, half of the birds of each line will be inoculated with LPAI. Then genomic assays will be performed on HGs collected at 1- and 4-days post-infection (DPI) from each treatment-line group. In Aim 1, we will investigate the immune response of the HG with LPAI infection by evaluating specific immune cell types that respond to AIV infection by evaluating specific immune cell frequencies and MHC molecule expression involved in antigen presentation through flow cytometry. In Aim 2, the most altered cell population which is responsible for AIV resistance in HGs will be sorted and purified by the FACS. Enhancers and promoters of AIV-associated immune genes in the sorted cells will be annotated by ChIP-seq and ATAC-seq assays. Cell type-specific gene expression and candidate genes through RNAscope will be identified for elucidating the molecular mechanism of host LPAI resistance by the integration analysis.

Results: Through Aim1, the specific cell population in the HG which altered the most with LPAI infection will be identified and purified. With the accomplishment of Aim2, cell type-specific gene regulatory elements and candidate gene expression would provide the molecular mechanisms of chicken LPAI resistance.

Conclusions: The outcome will help to understand the genetic basis of AIV resistance which will bridge the knowledge gap in AIV prevention and protection strategy, reduce the need for

Financial Support: This project was supported by Agriculture and Food Research Initiative Competitive Grant no. 2023-67015-39650 from the USDA National Institute of Food and Agriculture.

Notes:

100 - Colonization kinetics and mechanisms of Listeria monocytogenes in the bovine gastrointestinal tract

Yuxing Chen[1], Mariana Paiva Rodrigues[1], Zepeng Tu[1], Cheta Siletti[1], Tu-Anh Huynh[1]

[1]University of Wisconsin – Madison. thuynh6@wisc.edu

Session: Host-Pathogen Interaction, 2024-01-22, 9:45 - 10:00

Objective: *Listeria monocytogenes* (*Lm*) is an invasive pathogen in both humans and dairy ruminants. Although acquired orally, *Listeria* can breach the intestinal barrier to infect multiple organs. Bovine clinical listeriosis can cause abortion and has high mortality rates. The gastrointestinal (GI) phase of *Listeria* infection precedes systemic spread, and is a determinant of listeriosis outcomes. Thus, it is critical to understand *Listeria* pathogenesis in the bovine GI tract to prevent the progression to clinical infection, and to reduce shedding by subclinical animals. In this project, we develop a calf ligated ileal loop infection model to investigate *Lm* replication and dissemination in the bovine ileum, and identify mucosal immune responses to infection.

Methods: Neonatal calves will be anesthetized, and the ileum from each animal will be tied into ~35 loops of 5 cm long. Each loop will be infected with a phosphate-buffered saline suspension of *Lm* for a total of 10^7 cfu per loop. Mock-infected loops, injected with PBS, will be placed between infected loops. The ileum will then be returned to the abdomen and the calf maintained under general anesthesia until loop harvest and euthanasia. *Lm* burdens will be assessed at 5 and 8 hours post infection to evaluate luminal and tissue-invaded *Lm*. Infected tissue will be examined by histopathology. Mucosal immune responses will be analyzed by RNA sequencing.

Results: Our first infection experiments will be performed in August 2023 and data will be presented in the poster. Based on oral mouse infection studies, we anticipate *Lm* to replicate both in the intestinal lumen and tissue, with the vast majority in the lumen. We also anticipate an inflammatory response with the upregulation of TLR2, TLR5, inflammatory cytokines, and chemokines.

Conclusions: We will analyze data of the August infections.

Notes:

101 - Do our exposure variables tell us what we think they are telling us?

Audrey Ruple[1]

[1]Virginia-Maryland College of Veterinary Medicine, Virginia Tech. aruple@vt.edu

Session: AVEPM - Schwabe Symposium, 2024-01-22, 10:30 - 11:15

With the theme for the 2024 Schwabe Symposium, honoring the lifetime achievements of Dr. Jan Sargeant, being "*Remaining relevant: Conceptual advances in research in veterinary populations*" this talk will explore the critical issue of exposure variable selection and the inherent challenges associated with measurement errors. Epidemiologic research plays a crucial role in understanding the complex relationships between exposures and health outcomes. However, the accuracy of the conclusions drawn from these investigations relies upon the meticulous selection and measurement of exposure variables. Appropriate exposure variable selection is crucial for understanding disease etiologies, but it is often the case that we are not able to directly measure the exposure variable of interest and use proxy measures to assess exposures instead. Inappropriate use of proxy measures can lead to erroneous conclusions being made about the true exposure of interest. These errors may lead to biased estimates of associations between exposures and outcomes. The consequences of such biases extend beyond research concerns as public health decisions can be made based on flawed evidence. Recognizing and mitigating these biases are essential for producing reliable evidence that informs public health policies and interventions, ultimately contributing to improved population health outcomes. To address these challenges, researchers must adopt rigorous methodologies for exposure variable selection and validation studies to minimize measurement errors. Additionally, advancements in technology and data collection tools offer opportunities for improving exposure assessment accuracy. Collaborations between epidemiologists, statisticians, and experts in measurement science are essential for developing robust study designs that enhance the reliability of exposure variable measurement.

Notes:

102 - Aligning valid research outcomes with stakeholder values

David G. Renter[1]

[1]Center for Outcomes Research and Epidemiology, College of Veterinary Medicine, Kansas State University. drenter@vet.k-state.edu

Session: AVEPM - Schwabe Symposium, 2024-01-22, 11:15 - 12:00

With the theme for the 2024 Schwabe Symposium, honoring the lifetime achievements of Dr. Jan Sargeant, being *"Remaining relevant: Conceptual advances in research in veterinary populations"*, the focus of my talk is on how and why to produce outcomes that are valid and have value to the end-users of our research. Given the scope and diversity of topics addressed by animal health researchers, the potential beneficiaries or stakeholders of our research products also varies. These stakeholders or end-users may include veterinary practitioners, other researchers, livestock owners, "pet parents", government officials, corporate entities, or the general public in the case of public health or food security and safety issues. Regardless, our research outcomes must be both valid and relevant - defined such that the outcomes have value for the end-users. While we work toward ensuring validity throughout our research processes, we also should ensure that our resulting outcomes are specified to appropriately inform and enable decision-making by the end-users. Our current research in animal agriculture sustainability provides an interesting opportunity to consider research outcomes in a sustainability framework which concurrently values social, economic, and environment impacts of animal health and management decisions. In companion animals, contemporary issues of affordability and access to care, quality of life metrics, or compliance effects on efficacy, also potentially extend the spectrum of relevant research outcomes. In these cases, the "typical" measures of animal health, such as morbidity, mortality, or growth, may not be the most relevant research outcomes for the end-users. Furthermore, if research studies are not designed and analyzed with primary outcomes that are informed by stakeholders' values, but rather post-hoc considerations of these values are made based on indirect or surrogate measures, there is the potential to incorporate error and bias into the end-users' decision-making processes. My talk will address these topics, providing both examples and challenges for advancing the relevance of research in veterinary populations.

Notes:

103 - Efficacy of Lacticaseibacillus rhamnosus GG in mitigating effects of *Salmonella* infection in chickens

Menuka Bhandari[1], Dhanashree Lokesh[1], Kwonil Jung[1], Gireesh Rajashekara[1]

[1]The Ohio State University. bhandari.72@osu.edu

Session: Antimicrobial Alternatives, 2024-01-22, 10:30 - 10:45

Objective: *Salmonella*, a foodborne zoonotic pathogen, is the leading cause of death associated with foodborne illness in the USA. Salmonellosis is primarily transmitted through the consumption of contaminated poultry meat and eggs. The concern over the evolution of antimicrobial-resistant genes associated with antibiotic use and the lack of cross-protection by the vaccine has limited salmonellosis treatment options. Probiotics, are live beneficial microorganisms when administered in an optimum amount, could be an alternative treatment for salmonella infection in poultry to reduce transmission to humans. The objective of this study is to investigate the effects of *Lacticaseibacillus rhamnosus* GG (LGG) on growth performance, gut microbiota, intestinal integrity, load of salmonella, and immune response of chickens challenged with *Salmonella* Typhimurium (ST).

Methods: A total of 400 specific pathogen-free leghorn chickens were randomly allocated into five groups (n=80 birds/group): negative control (NC-neither infected nor treated with LGG), LGG control (LGG-not infected but treated with LGG), LGG + Salmonella (LT-infected with Salmonella and treated with LGG), and commercial probiotics + Salmonella (CT-infected with Salmonella and treated with commercial probiotic), and positive control (PC-infected with Salmonella not treated). Gut pro was used as a commercial probiotic. The birds were administered 10^8 colony-forming units (CFU/ml) of LGG and commercial probiotics in drinking water from day 1 to day 13. On day 7, birds were challenged with 10^4 CFU of nalidixic acid-resistant ST and necropsied on days 7, 14, and 22, days post infection(dpi). The load of salmonella in the cecum, liver, and spleen was determined by plating on XLT4 plate before and after enrichment in tetrathionate broth (TTB). The microbiota was analyzed by sequencing the V4-V5 variable region of 16S rRNA. The intestinal integrity was measured by morphometry analysis of the Ileum and jejunum. The cecal tonsils were used for the measurement of immune gene expression by RT-qPCR. The microbiota, integrity, and gene expression reflect the effect of LGG on salmonella-infected birds.

Results: At 7 dpi, Salmonella load in the cecum was significantly reduced by 5.95 and 6.11 log CFU in the LT and CT groups, respectively, compared to the PC ($p < 0.05$). Similarly, at 14 dpi, the Salmonella load in the cecum was reduced by 3.74 and 3.49 log CFU in the LT and CT groups compared to the PC ($p < 0.05$), respectively. At 7 dpi, fewer spleen samples were positive for Salmonella in LT and CT compared to the PC. Although similar results were observed for the liver on 14 dpi for LT, there was no difference between CT and PC. On 22 dpi, no Salmonella were recovered in any groups, including the untreated ones. A significant increase in the chicken's body weight was observed in LT group compared to the PC on day 14. In contrast, no significant difference in body weight was observed after 15 days ($p < 0.05$).

Conclusions: These results suggest that administering LGG in drinking water reduces the Salmonella load in the chicken's cecum, liver, and spleen but will significantly affect the body weight gain.

Notes:

104 - Evaluate the efficacy of novel probiotic strains on *Campylobacter* infection in vitro

B. Lamichhane[1], H. True[2], B. Doratt[2], I. Messaoudi[2], Y. A. Helmy[1]

[1]Department of Veterinary Science, University of Kentucky, [2]Department of Microbiology, Immunology and Molecular Genetics, University of Kentucky. bla260@uky.edu

Session: Antimicrobial Alternatives, 2024-01-22, 10:45 - 11:00

Objective: *Campylobacter jejuni* is a common cause of gastroenteritis in animals and humans. Food animals, particularly poultry, serve as a primary source and reservoir for *Campylobacter*. The bacterium is typically transmitted to humans and animals by the consumption of contaminated food and water. Currently, antibiotics are used to treat *Campylobacter* infections in animals and humans. However, the growth of antibiotic resistance to routinely used antibiotics has highlighted the critical need to develop alternative treatment strategies. The objective of our study is to evaluate the effect of selected probiotics on the growth and survival of *C. jejuni in vitro.* We aim to develop probiotics as antibiotic-alternative therapeutics for the control and treatment of *C. jejuni* infections in animals and humans.

Methods: We screened 40 different probiotic strains for their effect on the growth of *C. jejuni* using an agar well diffusion assay. The probiotics with the highest inhibition percentage were then subjected to further development *in vitro*. The probiotics were further evaluated for their ability to inhibit biofilm formation and pre-formed biofilms. They were also tested for their effect on adhesion, invasion, and survival of *C. jejuni* in human intestinal cells. The auto-aggregation and coaggregation properties of the probiotics were also evaluated. Furthermore, the effect of the probiotics on the expression of virulence-associated genes was investigated using RT-PCR. Each of the experiments were repeated at leat two times and the results were analyzed using Two-way ANOVA (or mixed model) followed by Tukey test with $p < 0.05$ to determine the statistical significance.

Results: All probiotics showed inhibition for *C. jejuni* growth using agar well diffusion assay with different levels, however, we selected the eight best probiotics that showed the highest efficacy against *C. jejuni*. All the selected candidates significantly inhibited the growth of the bacteria when cocultured in broth media. They also inhibited the growth of other *Campylobacter* strains such as *C. fetus, C. lari, C. hyointestinalis,* and *C. coli*. Four of the eight selected candidates inhibited up to 100% of *C. jejuni's* biofilm formation and pre-formed biofilms. Interestingly, all 8 candidates significantly ($p<0.05$) inhibited adhesion, invasion, and intracellular survivability of *C. jejuni* in human intestinal cell lines. Additionally, all 8 candidates downregulated the genes related to the expression of virulence factors such as biofilm formation, quorum sensing, motility, and invasion.

Conclusions: Our future studies will focus on understanding how probiotics modulate their action on intestinal cells using transcriptomics. We will also focus on the evaluation of the efficacy of the probiotic on *Campylobacter*'s colonization *in vivo*. Our result will facilitate the establishment of probiotics as alternatives to antibiotics for controlling *Campylobacter* infections in animals and humans.

Financial Support: This research was supported by Igniting Research Collaborations (IRC), Internal Research Support Programs, University of Kentucky.

Notes:

105 - Deoxycholic acid synergizes with butyrate to alleviate necrotic enteritis in broilers

M.A. Whitmore[1], D.M. Kim[1], J. Liu[1], I. Tobin[1], Z. Zhao[1], J. Guo[1], G. Zhang[1]

[1]Department of Animal and Food Sciences, Oklahoma State University. melanie.whitmore@okstate.edu

Session: Antimicrobial Alternatives, 2024-01-22, 11:00 - 11:15

Objective: Necrotic enteritis (NE) causes an estimated annual loss of $6 billion to the global poultry industry, while also posing a great risk to human health as a zoonotic disease. Due to increasing antimicrobial resistance, the FDA banned in-feed antibiotics from livestock production in 2017, highlighting the need for effective antibiotic alternatives to combat diseases like NE. One promising approach involves the modulation of host defense peptides (HDPs), critical components of the innate immune system with antimicrobial and immunomodulatory properties. Butyrate and deoxycholic acid (DCA) have been individually shown to enhance HDP synthesis and alleviate NE; however, their potential synergistic effects have not been investigated. We hypothesized that the combination of butyrate and DCA could synergize to alleviate NE in broilers by enhancing HDP production.

Methods: To test our hypothesis, we treated chicken HD11 macrophage cells and jejunal explants with butyrate and DCA alone or in combination for 24 h, followed by RNA isolation and RT-qPCR to measure the expression levels of HDP and barrier function genes. Additionally, we employed a chicken model of NE to evaluate the treatment efficacy of butyrate and DCA. Cobb broilers were supplemented with butyrate in the presence or absence of DCA from the day of hatch throughout the entire trial. NE was induced through sequential challenges with *Eimeria maxima* and *Clostridium perfringens* on day 10 and day 14, respectively. We recorded animal survival until day 17 and assessed chicken weight gain, intestinal lesion scores, and the impact on the intestinal microbiome using bacterial 16S rRNA gene sequencing of the ileal digesta.

Results: We revealed that butyrate and DCA synergistically induced two HDP genes, avian β-defensin 3 and avian β-defensin 9, and the barrier function gene claudin-1 in a dose-dependent manner in HD11 cells ($P < 0.05$). These results were also observed in jejunal explants for all three genes. In two chicken NE trials, supplementation with 1.5 g/kg DCA or 0.75 g/kg DCA in combination with 1 g/kg encapsulated butyrate resulted in the highest survival rates, showing an approximately 20% improvement over non-medicated chicks. Moreover, these two treatments significantly reversed the body weight loss observed in infected chicks on day 17 ($P < 0.05$). The severity of intestinal lesions was reduced compared to non-treated chicks, with the best outcomes achieved by the butyrate and DCA combination. Furthermore, supplementation with butyrate together with DCA helped restore the richness of the ileal microbial community that was lost in response to NE infection.

Conclusions: In summary, the combination of butyrate and DCA demonstrated synergistic effects in enhancing the expression of several HDP and barrier function genes *in vitro* and *ex vivo*. Moreover, in two chicken NE trials, this combination effectively alleviated NE, as evidenced by improved chicken survival, body weight recovery, and mitigation of intestinal lesions. The combination also restored microbial community diversity in the chicken ileum. These findings accentuate the potential of DCA and butyrate as effective antibiotic alternatives to protect broilers from NE.

Financial Support: This research was funded by a USDA-NIFA Predoctoral Fellowship (2021-67034-35184) and USDA-NIFA (2020-67016-31619 and 2023-67015-39095).

Notes:

106 - In vivo effectiveness of *Enterococcus faecalis* 14 treatment of necrotic enteritis in broiler chickens

Bernard Taminiau[1], Georges Daube[1], Anca Danila-Lucau[2], Djamel Drider[2], Rabia Ladjouzi[2]

[1]Liège university, belgium, [2]Lille university, France. djamel.drider@gmail.com

Session: Antimicrobial Alternatives, 2024-01-22, 11:15 - 11:30

Objective: This study aimed at evaluating the prophylactic and therapeutic properties of *Enterococcus faecalis* 14, a strain producing a two-peptide leaderless bacteriocin, named enterocin DD14 (EntDD14) and its Δ*bac* mutant strain deleted in genes coding for EntDD14 against subclinical induced necrotic enteritis (NE) in broiler chickens kept under battery cage conditions.

Methods: Six different groups of birds consisting in (i) an infected and untreated control (IUC) group, (ii) an infected and amoxicillin-treated control group (ITC), and four treated groups receiving prophylactically or therapeutically either *E. faecalis* 14 (WT) or its Δ*bac* isogenic mutant strain. The average daily weight gain (DWG), Feed Conversion Ratio (FCR), and feed intake were calculated for each treatment group over the different study periods. On D26 and D27, 13 birds per pen were euthanized and necropsied for intestinal lesion scoring. The same chickens have also been scored for typical lesions for coccidiosis with a score from 0 (no lesions) to 4 (severe lesions) for the species relevant to broilers described in this scoring system.

Results: To be noted that 16 mortalities, of which 5 were early mortalities occurred within one week of life of the birds. None of the mortality was however related to necrotic enteritis (NE). Although the birds were uniform in all trial groups with no significant differences prior to challenge on D1 and D12, the weights of birds in treatment groups were lower in comparison to control groups ITC and, interestingly, in the IUC group on D21. Towards the end of the study and post-challenge, the treated birds permitted to gain more weight under the challenge conditions especially the group treated prophylactically with the WT. Next, the DWG was second highest after the amoxicillin treatment. DFI was significantly higher in control groups in periods D12-D21 and D21-D27, whereas the difference was seen in treated groups (*Dbac* prophylactically). In the last study period, the FCR was worst in the IUC group reflecting the occurrence of lesions and lower utilization of feed, with most treated groups performing better in this regard except the group (WT therapeutic). On D26 and D27, 13 birds per were euthanized and necropsied for intestinal lesion scoring in different groups. A model was fit with study day, strain (WT and D*bac*) and the interaction between strain and timing (therapeutic vs prophylactic) as fixed effects. NE lesion scores were assessed at D26 and D27 in the different groups. Following, NE-specific lesion scores were recorded in animals of all groups except the control group (amoxicillin-treated) and groups treated prophylactically and therapeutically with the WT strain on D26. The highest number of birds showing the lesions on D26 were recorded in group IUC. On D27, no lesions were found in groups ITC and WT prophylactically treated.

Conclusions: The WT strain administered prophylactically demonstrated better performance in reducing the necrotic enteritis lesions caused by *C. perfringens* and prevented dysbiosis in comparison to Δ*bac* mutant under the current experimental conditions of subclinical NE.

Notes:

107 - Effect of oral supplementation of eugenol in reducing colonization of *Listeria monocytogenes* in guinea pig model

Chetna Shah[1], Trushenkumar Shah[1], Ana Leticia[1], Chen Zhu[1], Sierra Dean[1], Abhinav Upadhyay[1]

[1]Department of Animal Science, University of Connecticut. chetna.shah@uconn.edu

Session: Antimicrobial Alternatives, 2024-01-22, 11:30 - 11:45

Objective: *Listeria monocytogenes* (LM) is a major foodborne pathogen that leads to life-threatening illness in humans. The current listeriosis treatment includes antibiotic therapy. However, there are reports of antibiotic resistance development in LM. This situation warrants the development of novel strategies for controlling LM infection in humans. This study investigated the effect of generally recognized as safe status phytochemical eugenol (EG) in reducing colonization of LM in guinea pig model. In addition, the effect of EG on the proteome profile of LM, especially the expression of proteins critical for infection in humans was investigated.

Methods: Sub-inhibitory concentration (SICs; compound concentrations below the MIC that do not affect bacterial growth) of EG against LM ATCC-19115 was determined by growth curve assay at 37ºC. For proteome profiling, LM ATCC-19115 was cultured either in the presence or absence (control) of SIC of EG for 12 h followed by protein extraction, quantification, and LC-MS/MS analysis. Differentially expressed proteins between control and treatment samples (n=3) were analyzed using Student's t-test on Scaffold-5 at $P<0.05$. For guinea pig studies, 16 female guinea pigs were randomly divided in 4 groups (n=4) namely, negative control, LM control, EG oil treatment dose-1 (32 mg/kg bw) and EG oil treatment dose-2 (64mg/kg bw). All treatments were administered by oral bolus after completion of 7 days acclimatization period. Guinea pigs were challenged on day 14 with 9 log CFU/ml cocktail of LM (except negative group) and necropsy was performed on day 28 followed by collection of small intestinal content for enumeration of LM counts. Data were analyzed using one way ANOVA. Differences between the means were considered significantly different at $P<0.05$.

Results: The SIC of EG was 0.04%. SIC of EG down-regulated critical virulence proteins contributing to host cell invasion (InlA, InlB and InlH), intra-cellular spread (ActA), toxin production (Hly), protein synthesis (InfA), and catalytic activity (PyrC, PbpA, PyrF, NagB and PlcA) when compared to control ($P<0.05$). The expression of few proteins involved in catalytic activity and cellular metabolism (FusA, FabH, PyrG, AlaS, HemB, PycA, UvrA and MurD) were upregulated by SIC of EG ($P<0.05$). In the guinea pig study, 60% and 50% duodenum samples were positive for LM in control and EG oil treatment dose-1, respectively. All animals were negative for LM in duodenum in EG oil treatment dose-2 group. In the jejunum, 80% and 75% samples were positive for LM in control and EG oil treatment dose-1 groups respectively, whereas 25% samples were positive for LM in EG oil treatment dose-2 group. In the ileum, 80% and 75% samples were positive in control and EG oil treatment dose 1 groups respectively, whereas all animals were negative for LM in EG oil treatment dose-2 group.

Conclusions: The results of guinea pig and proteome study suggest that oral supplementation of eugenol could be used as a prophylactic treatment to reduce colonization and pathogenicity of LM in humans.

Financial Support: National Institute of Food and Agriculture A1332 program Seed grant (2022-67018-36557)

Notes:

108 - Bioluminescent tagging of therapeutics in recombinant lactic acid bacteria

I.Y. Choi[1], J. Oh[1], Z. Wang[1], J.P. van Pijkeren[1]

[1]University of Wisconsin-Madison. ichoi37@wisc.edu

Session: Antimicrobial Alternatives, 2024-01-22, 11:45 - 12:00

Objective: *Streptococcus suis* is as an important swine and zoonotic pathogen that causes globally a significant financial strain on the pig industry and human health care. Also, the continuous increase in antibiotic resistance, including in *S. suis*, highlights the urgent need to develop alternative therapeutic strategies. Our long-term goal is to develop lactic acid bacteria (LAB) as microbial therapeutics, including antimicrobials. However, tools are lacking that allow high-throughput monitoring of recombinant protein production, which hampers the development and optimization of recombinant antimicrobial production. Therefore, the objective of this study is to develop a luminescent tagging system for use in LAB.

Methods: We constructed *Limosilactobacillus reuteri* strains that each produce a recombinant therapeutic protein with an eleven amino acid peptide tag referred to HiBiT (Promega), crucial for generating a luminescent signal. The system was compared with commercial ELISA by quantifying three different recombinant proteins derived from recombinant *Lm. reuteri*. To track the protein production in live cells, we expressed HiBiT complementary protein. We used conventional mice to quantify bioluminescent bacteria throughout the gastrointestinal tract. The versatility of this system was assessed across 11 different LAB species and one strain of *B. bifidum*.

Results: The bioluminescent peptide tagging system allows quantification of recombinant proteins with a linear correlation between bacterial cell number and luminescence signal in the dilution range of 10^0 to 10^6. Luminescent-based quantification of recombinant protein was more sensitive than commercially available immunoassays. In addition, we demonstrated that the bioluminescent peptide tagging system allows *in situ* recombinant protein detection in a continuous-culture parallel bioreactor system. This presents an exciting opportunity to determine recombinant protein production dynamics in response to different stimuli. Finally, following oral administration of recombinant microbes, luminescence in intestinal and fecal samples allowed for rapid detection of microbes with equal sensitivity to conventional plate count. Because we demonstrated the functionality of this bioluminescent peptide tagging system in 12 species encompassing 9 genera, our approach will create previously unexplored opportunities to develop, optimize and validate antimicrobial production by LAB.

Conclusions: With our advanced bioluminescent peptide tagging system in place, we're poised to assess and normalize lysin, advancing our quest for potent antimicrobial solutions against *S. suis*.

Financial Support: This work was supported by Animal Health and Production and Animal Products: Animal Health and Disease [2021-67015-34316] from the USDA NIFA, the Ministry of Education [NRF-2021R1A6A3A03045314], the NIGMS [R01GM135483] and the NCCIH [R01AT011202] of the NIH, and the UW-Madison FRI.

Notes:

109 - Ixodid ticks from animals in Asian countries

H.R. Byun[1], M.S. Rieu[1], S.W. Han[1], S.R. Ji[1], C.R. Choi[2], B.K. Linh[3], H. Thanh[4], M. Kaewthamasorn[5], A. Sahara[6], R.L. Galay[7], I. Prado[8], S.L. Alota[9], S. Wang[10], T. Erdeneshimeg[11], N. Batbayar[11], M. Avais[12], J.S. Chae[1]

[1]Research Institute for Veterinary Science and College of Veterinary Medicine, Seoul National University, [2]Department of Agriculture, Forestry, and Bioresources, College of Agriculture and Life Sciences, Seoul National University, [3]Vietnam National University of Agriculture, [4]Department of Infectious Diseases and Veterinary Public Health, Faculty of Animal Science and Veterinary Medicine, [5]Veterinary Parasitology Research Unit, Faculty of Veterinary Science, Chulalongkorn University, [6]Department of Parasitology, Faculty of Veterinary Medicine, Universitas Gadjah Mada, [7]Department of Veterinary Paraclinical Sciences, College of Veterinary Medicine, University of the Philippines Los Baños, [8]Department of Veterinary Clinical Sciences, College of Veterinary Medicine, University of the Philippines Los Baños, [9]School of Veterinary Medicine, Isabela State University, [10]Institute of Veterinary Clinical Sciences, Veterinary School, National Taiwan University, [10]Institute of Veterinary Clinical Sciences, Veterinary School, National Taiwan University, [11]Wildlife Science and Conservation Center of Mongolia, [12]Department of Veterinary Medicine, Faculty of Veterinary Science, University of Veterinary and Animal Sciences. hyeryung.byun@snu.ac.kr

Session: Parasitology 1, 2024-01-22, 10:30 - 10:45

Objective: Tick-borne disease has an influential impact on the human and animal populations, posing an increasing prevalence rate every year. The risk of transmitting pathogens and infestation is related to the close connection with humans and animals. Herein, the objective study is collecting Ixodid ticks in Asian countries from wild and domestic animals and detecting pathogens from collected ticks.

Methods: Within the Asian regions, fed ticks were collected from companion animals, livestock and wild animals including migratory birds. Unfed ticks were collected from pastures, bushes and throughout livestock barns. All collected ticks have been stored at -80℃ deep freezer. Ticks were identified under the stereo microscopes and detected targets of both RNA and DNA pathogens by nested PCR.

Results: A total 6,727 of ticks were collected from 12 Asian countries, including the Republic of Korea, Japan, Thailand, Philippines, Indonesia, Cambodia, Vietnam, Taiwan, Hong Kong, Mongolia, Pakistan and Sri Lanka. A total of 18 tick species were collected including *Haemaphysalis longicornis, H. flava, H. concinna, H. bispinosa, H. hystricis, H. formosensis, Ixodes persulcatus, I. scapularis, I. nipponensis, I. granulatus, I. turdus, Amblyomma testudinarium, A. varanense, A. gervaisi*, *A. javanense, Rhipicephalus microplus, R. sanguineus, Hyalomma anatolicum*. Host animals were dogs, cattle, birds, Korean water deer, snakes, etc. Also, we detected target pathogens including SFTSV (Severe Fever with Thrombocytopenia Virus), CCHFV (Crimean-Congo haemorrhagic fever Virus), Langya virus, *Anaplasma phagocytophilum, A. bovis, Ehrlichia chaffeensis, E. canis, Borrelia* spp. *Rickettsia* spp. and *Bartonella* spp. The most detected pathogen was *Anaplasma phaogocytophilum* in Korean water deer.

Conclusions: The results suggest ticks have a potential risk threat to public health, as 3 RNA viruses and 7 DNA pathogens were detected and a total of 18 tick species within the Asian countries. Further studies are needed on wild range investigation of ticks and tick-borne pathogens to protect human and animal symbiosis.

Financial Support: Korea Institute of Planning and Evaluation for Technology in Food, Agriculture and Forestry (IPET 122062-2).

Notes:

110 - Detection of Dabie bandavirus in ticks collected from migratory birds

S.R. Ji[1], M.S. Rieu[1], H.R. Byun[1], S.W. Han[1], C.Y. Choi[2, 3], H.Y. Nam[4], S.G. Seo[3], J.S. Chae[1]

[1]Research Institute for Veterinary Science and College of Veterinary Medicine, Seoul National University, Seoul, Republic of Korea. , [2]Department of Agriculture, Forestry, and Bioresources, College of Agriculture and Life Sciences, Seoul National University, [3]Research Institute of Agriculture and Life Sciences, College of Agriculture and Life Sciences, Seoul National University, [4]School of Biological Sciences, College of Natural Sciences, Seoul National University. doe05062@snu.ac.kr

Session: Parasitology 1, 2024-01-22, 10:45 - 11:00

Objective: *Dabie bandavirus*, also known as severe fever with thrombocytopenia syndrome virus (SFTSV), is a tick-borne disease caused by the *Bunyaviridae* family and transmitted through hard ticks. Ticks feed on the blood of host animals three times in their lifetime. Recently, migratory birds have been mentioned as potential reservoir hosts for the SFTS virus, although this has not been confirmed. The goal of this study is to investigate the presence of SFTS virus antigens in ticks collected from migratory birds in Asia.

Methods: Hard ticks were collected from migratory birds in various locations, including western islands (Daechengdo and Heuksando), a southern island (Jejudo), and inland areas (Gangwon-do and Ulsan) of the Republic of Korea (ROK), Japan, and Mongolia from April 2022 to July 2023. Viral RNA was extracted from the ticks collected in migratory birds using the viral DNA/RNA extraction kit. One-step RT-nested PCR was performed to confirm the presence of the S segment amplicon of the SFTS virus. The nucleotide sequence data were analyzed using Chromas and were aligned using Clustal W. The phylogenetic tree was constructed using the maximum likelihood tree method in MEGA 7.

Results: A total 180 of ticks were collected from 114 migratory birds. Total 7 species of ticks were collected; *Ixodes persulcatus* (77 ticks), *Haemaphysalis conccina* (31 ticks), *H. flava* (24 ticks), *H. longicornis* (24 ticks), *H. formosensis* (5 ticks), *I. nipponensis* (7 ticks), *I. turdus* (12 ticks). SFTSV were detected from 2 of 180 (1.1%) ticks. Both of SFTSV-positive ticks were collected from captured migratory birds on islands in the ROK. One was a *H. concinna* nymph collected from *Emberiza spodocephala* (Black-faced bunting) in Daechengdo in April 2022, and the other was *I. turdus* nymph collected from *Anthus hodgsoni* (olive-backed pipit) in Heuksando in March 2023. These two migratory bird species are common passage migrants passing through China, Japan and Korea during their northward migration, and Korea is a stop-over site for both birds. All SFTSV S segment sequences (346 bp) belonged to sub-genotype B-2. The nucleotide sequences of the SFTS virus S fragment in Daechengdo and Heuksando cases were more than 99% identical to the dog isolate from the ROK.

Conclusions: This result suggests that migratory birds could serve as reservoir hosts for the transmission of the SFTS virus. In this study, we confirmed the possibility of SFTSV transmission through migratory birds flying from China to the ROK. Due to the geographical proximity between China and Korea, it is expected that sub-genotype B-2 also can be spread from China to Korea through migratory birds. Therefore, it is important to investigate the SFTS virus infection rate in migratory birds and migration pathways.

Financial Support: Korea Institute of Planning and Evaluation for Technology in Food, Agriculture and Forestry (IPET 122062-2).

Notes:

111 - Acaricidal effect of Sulpher nanoparticles on different developmental stages of hard ticks (Acari: Ixodidae)

M.S. Sajid[1], S. Younas[1], A. Naeem[2], H.M. Rizwan[2]

[1]Department of Parasitology, University of Agriculture, Faisalabad, Pakistan, [2]KBCMA College of Veterinary and Animal Sciences, UVAS, Lahore (Narowal Campus), Narowal, Pakistan drsohailuaf@hotmail.com

Session: Parasitology 1, 2024-01-22, 11:00 - 11:15

Objective: Tick (Acari: Ixodidae) control is mainly based on traditional chemical control. Unfortunately, the method is exhibiting several side effects primarily tick resistance against these available acaricides. The ever-growing fret of acaricidal resistance marks the significance of the development of safer and more effective approaches that can aid to control the tick population, promoting animal health, and maximizing their productive and reproductive potential.

Methods: The aim of this study was the synthesis, characterization, determination, and evaluation of the acaricidal activity of sulphur nanoparticles in vitro. Characterization was done using a scanning electron microscope (SEM) for morphological assessment which revealed the amorphous morphology of the sulphur nanoparticles. The formulated nanoparticles were evaluated for acaricidal activity against different life stages i.e., eggs, larvae and adults of the hard ticks (*Hyalomma anatolicum*). This was performed through tick bioassays such as the adult immersion test (AIT), Egg hatchability test (EHT), larval immersion test (LIT), and larval packet test (LPT).

Results: Probit analysis concluded that sulphur nanoparticles have caused adult tick mortality with LC50 36.16 mg/L. The hatchability was observed least at the highest concentrations of Sulphur indicating a concentration-dependent hatchability response in eggs. In comparison, ivermectin showed 85% hatching inhibition with LC50 0.64mg/L, 0.002mg/L, and 0.0012mg/L at 24hrs. In the case of the larval packet test the observed LC50 was 11.47mg/L and 2.21mg/L for sulpher nanoparticles and ivermectin respectively. As per time-bound activities, the ticks were more susceptible to nanoparticles than ivermectin. The study revealed that all the ticks died against sulphur nanoparticles while in the case of ivermectin trials ticks were found resistant.

Conclusions: This study concluded that sulphur nanoparticles are eco-friendly, safer, and effective candidates to control various developmental stages of ticks. *In vivo,* studies are suggested to investigate their efficacy against ticks.

Financial Support: Sponsored by Agricultural Linkages Program at the Pakistan Agricultural Research Council, Islamabad vide Project No. AS-106

Notes:

112 - Towards novel acaricide development against cattle fever tick: GPCR chemical hits and neuropeptide physiology

Patricia Pietrantonio[1], Jonathan Hernandez[1], Waleed Ahmed[1]

[1]Department of Entomology, Texas A&M University. p.pietrantonio@ag.tamu.edu

Session: Parasitology 1, 2024-01-22, 11:15 - 11:30

Objective: This new project advances cattle disease prevention through the discovery of the functions of neuropeptide G protein-coupled receptors in ticks and of candidate small molecules as potential acaricides. Specific aims: 1) Evaluate anti-tick bioactivity of small molecules antagonists and peptidomimetics of tick kinins already validated on the receptor in dose-response assays. 2) Determine the physiological function of tick neuropeptides through tissue localization analyses and by identification of gene networks affected by silencing the kinin receptor or kinin gene using transcriptomics, studies which complement the previously performed silencing of periviscerokinin and pyrokinin receptors.

Methods: Ticks *Rhipicephalus sanguineus* were used for methods validation at Texas A&M University in College Station because *R. microplus* can only be tested at the USDA facility in Edinburg, TX. Solvents were also tested on *R. microplus* larvae at the USDA-ARS facility, Edinburg, TX.

1.a. Tested the toxicity of two solvent combinations: 1) 5% dimethylsulfoxide (DMSO)/ 1% MERO® (Bayer, Germany) and 2) 5% DMSO/ 10% JEFFSOL® AG 1555 (Huntsman Co., TX, USA) on ticks to determine solvent suitability for testing discovered candidate acaricidal molecules. On *R. sanguineus* unfed females solvents were applied topically (2 µl) on the scutum. Positive control for toxicity was permethrin (0.125%). Mortality was assessed after 24h. The same solvent combinations were tested on *R. microplus* larvae using the larval immersion test.

2.a. One of the *R. microplus* kinin peptides, Rhimi-kinin 8, was labeled with tetramethyl rhodamine (TMR-labeled Rhimi-K-8) and applied to midguts of *R. sanguineus* ex-vivo. The sequence of this kinin peptide (GTGEDQAFSPWGa) is identical in *R. sanguineus*. Similarly, a labeled scrambled-sequence of Rhimi-K-8 peptide was applied as negative control. Phalloidin and DAPI stained F actin and nuclei, respectively. Tissues were analyzed by confocal microscopy. The gut myotropic activity of the TMR-labeled Rhimi-K-8 peptide was quantified by video-analyses using EthoVision software (Noldus).

Results: 1.a. The tested solvent combinations were non-toxic for both *R. sanguineus* unfed females and *R. microplus* larvae.

2.a. The labeled kinin 8 peptide (Rhimi-kinin 8) specifically localized the kinin receptor to the midgut circular and longitudinal muscles exhibiting a typical grid-like pattern. The kinin receptor labeling co-localized with the phalloidin staining, supporting receptor expression in muscles. The labeled peptide was myotropic on the ex-vivo midgut, as expected of an active kinin peptide, while the scrambled-sequence peptide showed no significant activity.

Conclusions: 1.a. The tested solvents are suitable to test small molecules in tick bioassays.

2.a. The use of a fluorescently labeled endogenous kinin peptide allowed the localization of the kinin GPCR on the midgut musculature. Further, the labeled peptide exhibited myotropic activity, consistent with the known function of kinins in insects, and therefore validating the observed localization. More broadly, this technique may allow localization of other peptide GPCRs eliminating the need for anti-receptor antibody development and validation.

Financial Support: U.S. Department of Agriculture, National Institute for Food and Agriculture, AFRI; USDA Animal Health and Disease Research Capacity Program; Texas A&M AgriLife Research - Insect Vector Diseases Grant Program.

Notes:

113 - Assessing genetic diversity and coinfection of tickborne pathogens in small and large ruminants of Punjab, Pakistan

Sabir Hussain[1, 2], Abrar Hussain[3], Muhammad U. Aziz[1], Baolin Song[1], Jehan Zeb[1], Sara Moutailler[4], Angélique F. Simonin[4], Rebecca L. Smith[3], Alejandro C. Cruz[4], David George[5], Olivier Sparagano[1, 6]

[1]Department of Infectious Diseases and Public Health, City University of Hong Kong, [2]School of Biological, Environmental, and Earth Sciences, University of Southern Mississippi, [3]Department of Pathobiology, University of Illinois, Urbana-Champaign, [4]Ecole Nationale Vétérinaire d'Alfort, [5]School of Natural and Environmental Sciences, Newcastle University, [6]Agricultural Sciences and Practice, Royal Agricultural University. sahussain8-c@my.cityu.edu.hk

Session: Parasitology 1, 2024-01-22, 11:30 - 11:45

Objective: Tick-borne diseases affecting domestic animals and humans have increased globally in recent years. Pakistan faces a significant economic threat from ticks, where two specific species, *Rhipicephalus microplus,* and *Hyalomma anatolicum*, act as vectors for various pathogens such as piroplasma, *Anaplasma*, *Ehrlichia*, and *Rickettsia* that pose a significant burden on livestock production in the country. These tick-borne pathogens, responsible for endemic diseases in Pakistan, have never been studied in ticks and blood of tick-infested animals simultaneously. This study aims to use molecular techniques to study the impact of tick-borne pathogens on livestock animals in Pakistan, exploring the diversity and co-infection of tick-borne pathogens in both small and large ruminants and analyzing e ticks collected from the animals.

Methods: To better understand the risk that tick-borne pathogens pose to livestock in Pakistan, we conducted a cross-sectional study of the occurrence, diversity, and co-infection of these pathogens in small and large ruminants owned by small farms and in ticks collected from these animals. We collected blood samples from 224 cattle, 224 buffalo, 69 goats, and 56 sheep from 112 farms in the seven Punjab districts, one of Pakistan's largest provinces. Additionally, we collected a total of 476 ticks attached to these animals. Ticks and blood samples were processed for pathogens identification, such as piroplasma, *Anaplasma*, *Ehrlichia*, and *Rickettsia* and their coinfection using conventional and microfluidic PCR.

Results: Based upon the identification of tick species through morphology and sequence analysis of the 16S rRNA and cytochrome c oxidase subunit 1 (cox1) gene, we confirmed that the most commonly collected tick species were *R. microplus* (38.65% of all individuals), *H. anatolicum* (31.93%) and *R. decoloratus* (8.40%). Notable pathogens detected in the collected ticks included *Theileria annulata* (18.4%), *Anaplasma ovis* (15.79%), *A. centrale* (13.16%), and *Rickettsia slovaca* (13.16%). In blood samples, the most frequently detected pathogens were *T. annulata* ($n = 8$), *Babesia bovis* ($n = 7$), *A. centrale* ($n = 6$), and *B. bigemina* ($n = 5$). In some cases, both cattle and buffaloes were found to be co-infected with *B. bovis*, *T. annulata*, and *A. centrale*.

Conclusions: Overall, 13 tick-borne pathogens were identified from ticks and blood samples of animals, encompassing the variability in their distribution among districts of Pakistan. Co-infection of multiple pathogens were observed in both small and large ruminants, highlighting the complexity of pathogen associations. These findings contribute to our understanding of the epidemiology and distribution of tick-borne diseases, which can aid in developing effective control and prevention strategies in veterinary medicine.

Financial Support: This work has been funded by City University of Hong Kong project Number 7005758.

Notes:

114 - Comparative proteomics of salivary gland and midgut extracellular vesicles from different tick species

Sydney Orsborn[1], Emily Bencosme Cuevas[2], Thuy Nguyen[2], Cross Chambers[1], Brenda Galvan-Leal[1], Cristina Harvey[1], Carita de Souza Ribeiro-Silva[3], Branden Pierce[1], Perot Saelao[4], Kim Lohmeyer[4], Susan Noh[5], Tammi Johnson[6], Raul Medina[1], Albert Mulenga[2], Adela Oliva Chavez[1]

[1]Department of Entomology, Texas A&M University,[2]Department of Veterinary Pathobiology, School of Veterinary Medicine and Biomedical Sciences, Texas A&M University, [3]Departamento de Biociencias e Tecnologia, Instituto de Patologia Tropical e Saude Publica, Universidade Federal de Goiasl, [4]USDA-ARS Knipling-Bushland U.S. Livestock Insects Research Laboratory, Veterinary Pest Genetics Research Unit, [5]Program in Vector-borne Disease, Department of Veterinary Microbiology and Pathology, Washington State University, [6]Texas A&M AgriLife Research. aolivachavez@tamu.edu

Session: Parasitology 1, 2024-01-22, 11:45 - 12:00

Objective: Ticks are obligate hematophagous arthropods that can transmit a plethora of pathogens of public health and veterinary importance. In tropical and subtropical countries around the world, the animal industry suffers important economic losses due to tick infestations. Anti-tick vaccines have shown promising results, but antigens that are conserved and protective against different tick species are unknown. We have shown that extracellular vesicles (EVs) are essential for tick feeding and the manipulation of host immune responses, highlighting their physiological importance. For this reason, EVs have been explored recently as vaccine candidates against several parasites. Nevertheless, the level of conservation of the cargo of these vesicles among tick species and its variation during feeding is unknown. The objective of this study was to compare the proteome of EVs from different tick species and define their differences among species, changes during feeding, and between hosts.

Methods: To characterize salivary and midgut EVs in two different tick species (i.e., *Amblyomma americanum* and *Dermacentor andersoni*), ticks were fed for 7 days. *Ex vivo* organ cultures of salivary glands and midguts were used to isolate EVs. A Global Proteomic Analysis was performed to identify core proteins that are shared between salivary and midgut EVs in all two species and to detect differences in protein cargo between tick species. Only proteins with 0.1% peptide FDR, >1 peptide spectral count per protein were reported. To define changes in the proteomic profile throughout tick feeding and in different hosts, we performed label-free quantitative proteomics on EVs isolated from *A. americanum* females fed on rabbits for 3, 5, and 7 days and *A. americanum* fed on cattle or rabbits for 7 days. The following criteria were used for protein identification: FDR <1%; 1 unique peptide per protein; a protein was identified in two samples per group and 2 spectrum counts in one sample. Difference in expression per day was evaluated by One-way ANOVA and p-values were corrected by Benjamini-Hochberg FDR (BH-FDR) method. Host proteins differences were evaluated by t-test followed by adjusted p-value with BH-FDR method. Western blot analysis with serum from experimentally infested animals was performed.

Results: Transmission electron microscopy (TEM) and Nanoparticle tracking analysis (NTA) showed a wide distribution of vesicle sizes. Global proteomics of the core cargo within vesicles from midguts and salivary glands from all two species showed the expression of core proteins between the species. Further, quantitative analysis showed that vesicle cargo changed during feeding within both salivary (65 proteins) and midgut (625 proteins) vesicles. Interestingly, tick salivary vesicle cargo changed significantly when ticks fed on different hosts (160 proteins), but midgut proteins showed little change (2 proteins). Vesicle proteins were recognized by experimentally infested animals.

Conclusions: Our results indicate that tick EVs contain a core set of proteins that are found within midgut and salivary glands. However, organ specific cargo varied among tick species and changes throughout feeding in *A. americanum*. Moreover, *A. americanum* salivary gland cargo varies, depending on the host that they feed on, but midgut vesicles are more conserved.

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Notes:

115 - Rat hepatitis E virus (HEV) cross-species infection and transmission in pigs

Kush Kumar Yadav[1], Patricia Boley[1], Carolyn Lee[1], Saroj Khatiwada[1], Kwonil Jung[1], Thamonpan Laocharoensuk[1], Jake Hofstetter[1], Ronna Wood[1], Juliette Hanson[1], Scott P Kenney[1]

[1]Center for Food Animal Health, The Ohio State University. yadav.94@osu.edu

Session: Virology 2, 2024-01-22, 10:30 - 10:45

Objective: *Rocahepevirus ratti*, an emerging Hepatitis E Virus (HEV), has recently been found to be infectious to humans. Rats are the only known reservoirs of the virus, thus it is referred to as "rat HEV". Both immunocompromised and immunocompetent adults are susceptible to infection by rat HEV. Like swine HEV from pigs, rat HEV is also genetically and antigenically related to human HEV. In response to the increase in human infection by rat HEV, we sought to construct and characterize an infectious cDNA clone of rat HEV to understand whether swine may serve as a transmission host for rat HEV.

Methods: The complete genome of rat HEV strain LCK-3110 was cloned downstream of SP6 promoter. Capped genomic RNA was generated by in vitro transcription. The infectious clone replication was assessed by in vitro transfection of human hepatoma (huh7), mouse subcutaneous tissue (LMTK), human carcinoma lung tissue (A549) and swine testicular (ST) cells. Direct intrahepatic inoculation of gnotobiotic pigs with capped RNA transcripts was performed to study the replication competence of transcripts of rat HEV. Ten percent fecal suspension of intestinal content derived from the HEV positive gnotobiotic pigs was intravenously inoculated via ear vein in conventional pigs. Sentinel pigs were comingled with the rat HEV inoculated pigs after 7 days post inoculation (DPI). Pigs were also inoculated with human HEV (US-2) strain and PBS, as positive and negative control, respectively.

Results: The results demonstrated that capped RNA transcripts from the rat HEV infectious clone were replication competent when transfected into A549, LMTK and ST cells as shown by detection of HEV ORF2 positive cells using immunofluorescence and flow cytometry. Transcripts of rat HEV developed active HEV infection as evidenced by viremia and fecal virus shedding in gnotobiotic pigs. The infectivity was further confirmed by the successful infection of conventional pigs and added sentinel pigs as shown by seroconversion, viremia, fecal virus shedding and immunohistochemistry. Our results indicate that the LCK-3110 strain of rat HEV is capable of cross-species infection in pigs.

Conclusions: Rat HEV has an expanding host range, including pigs, that could be a transmission source to humans.

Notes:

116 - Characterizing the role of cellular protein, ZFP36L1 in suppressing viral gastroenteritis in pigs

Tooba Momin[1], Malabika Bhowmik[1], Mrigendra Rajput[1]

[1]Department of Biology, University of Dayton. momint1@udayton.edu

Session: Virology 2, 2024-01-22, 10:45 - 11:00

Objective: Viral gastroenteritis in pigs cause substantial economic losses by increasing morbidity and mortality in piglets, decreasing productivity, and increasing production costs. Transmissible gastroenteritis virus (TGEV: porcine coronavirus), porcine epidemic diarrhea virus (PEDV: porcine coronavirus), and porcine rotavirus (PRV) are the major etiological agents for viral gastroenteritis in pigs in the United States. These viruses cause direct damage to enterocytes when they replicate in them. These damages impair the structure and function of intestinal villi and thus interferes with nutrient absorption. These viruses belong to RNA virus class, which are known to have a very high mutation rate. A high mutation rate facilitates these viruses to change frequently and create new virus variant as we recently observed for PEDV, TGEV as well as in rotavirus due to genomic reassortment. Additionally, these viruses have great potential for causing zoonotic disease in human. Recent studies showed spillover of porcine coronavirus to other non-porcine host including human beings and caused acute febrile illness in children. Similarly, reassortment of human and porcine rotavirus indicated the emergence of new virus variant which could infect both human and porcine population. Therefore, current study is designed to identify and characterized the cellular proteins which can suppress wide rage of viruses responsible for viral gastroenteritis in pigs such as TGEV, PEDV and PRV. Additionally, to identify biocompatible compound which can modulate that cellular protein to suppress the broad range of virus replication without affecting the normal physiology of the host cell.

Methods: We have screened 68 RNA binding proteins which suppresses RNA replication and moderate virus induced hyperinflammation by targeting the conserved RNA replication cycle using literature search, bioinformatics tools such as RNA-Protein Interaction Prediction (RPISeq) software and lab experiments. Using that approach we selected ZFP36L1 for initial characterization. We used Human Coronavirus OC43 (HCoV-OC43) and Murine Norovirus 1 (MNV1) as representative of viral gastroenteritis agents.

We overexpressed or knockdown ZFP36L1 in HCT-8 cells and RAW264.7 cells. Wild-type, ZFP36L1 overexpressed, and ZFP36L1 knockdown cells were infected with HCoV-OC43 or MNV1. Time course virus titer, virus induced cytokine and cytopathic effect were measured and analyzed for statistical significance.

Results: Our results showed that ZFP36L1 overexpression significantly suppressed HCoV-OC43 and MNV1 titer while knockdown of ZFP36L1 significantly enhanced the titer of these viruses as compared to virus infected wild type cells ($p<0.05$). Similarly, ZFP36L1 overexpression significantly reduced MNV1 titer as compared to control wild type cells at 12-hour, 24 hour and 36 hours post-infection (p.i.) ($p<0.05$). ZFP36L1 also moderated the virus induced TNFα and IL-6 and suppressed virus mediated cytopathic effect for both HCoV-OC43 and MNV1 in cell culture.

Conclusions: These results showed that ZFP36L1 can simultaneously suppress multiple viruses responsible for gastroenteritis. Current results indicate the potential role of ZFP36L1 as a therapeutic target which can be overexpressed/ modulated to control virus replication. Further research is needed to understand the underlying mechanisms through which ZFP36L1 suppresses virus replication along with its efficacy in practical application.

Financial Support: Department of Biology, University of Dayton, Ohio.

Notes:

117 - Investigating cellular dynamics of porcine lung environment in PRRSV infection by single-cell RNA sequencing

HJ Kim[1], SC Kim[1], B. Lim[2], JM Kim[2], WI Kim[1]

[1]College of Veterinary Medicine, Jeonbuk National University, [2]Functional Genomics & Bioinformatics Laboratory, Department of Animal Science and Technology, Chung-Ang University. felix1116@jbnu.ac.kr

Session: Virology 2, 2024-01-22, 11:00 - 11:15

Objective: Porcine Reproductive and Respiratory Syndrome is one of the most important viral diseases in the swine industry. The primary target cell of the virus is pulmonary alveolar macrophages (PAM) in the lung. Upon PRRSV infection in pigs, PAMs produce various cytokines and chemokines, and the destruction of infected PAMs induces the influx of other immune cells into the lungs, altering the cellular composition in the pulmonary environment. After pigs recover from PRRSV infection in approximately 14 days-post-infection, alveolar macrophage populations return to normal levels. In this study, changes in the lung environment according to PRRSV strains with different pathogenicity and each time point of PRRSV infection were investigated through transcriptome analysis by single-cell RNA (scRNA) sequencing.

Methods: 36 4-week-old, three-way crossbred, PRRSV-free piglets were grouped into 4 groups: NC as a negative control group (12 pigs) and NA8 (6 pigs), JA142 (12 pigs), and NA10 (6 pigs) groups challenged with low, medium, and high virulence strains of PRRSV-2, respectively. Each challenge group was inoculated with 2ml of each PRRSV strain at 1 x 10^3 $TCID_{50}$/ml by I.M. At 3, 7 days per challenge (dpc), 3 pigs from each group and at 14, 21 dpc, 3pigs from NC and JA142 groups were euthanized, and bronchoalveolar lavage (BAL) cells from the lungs by PBS were collected. Each BAL cell pellet was aliquoted as 2 x 10^6 cells/ml and frozen. The scRNA sequencing was performed by Chromium Next GEM Single Cell 3' (10x Genomics) and Illumina platform according to the manufacturer's instructions. After pre-processing, a total of 306,502 cell data were obtained, which were subsequently clustered and cell type annotated.

Results: The presence of PRRSV-infected mono/macrophage-type cells varied by strain and time point. In the NA10 group, infected cells were detected at 3 and 7 (1.15% and 2.01%) dpc; in the JA142 group, infected cells were detected at 7, 14, and 21 (5.29%, 1.21%, and 0.06%) dpc; and in the NA8 group, infected cells were detected only at 7 dpc (0.047%). The dpc-dependent changes in monocyte/macrophage subpopulations in the infection groups also varied. C1QB-, PHYH-, and S100A2-high macrophages were further decreased in 7 dpc of the NA10 group and 14 dpc of the JA142 group. However, JA142 group at 21 dpc, the proportion of these cells was recovered. In the JA142 group, SPP1-high macrophages were few in 3 and 7 dpc but increased up to 20% of total cells in 14 dpc. Gene set enrichment analysis (GESA) indicated that infected cells showed lower immune and apoptotic signaling than bystander cells in the NA10 group. On the other hand, bystander cells in infected groups showed higher immunogenic signals than negative group cells.

Conclusions: In the present study, changes in the pulmonary environment following PRRSV infection were observed at the single-cell dimension. As certain types of mono/macrophage cells change in association with PRRSV infection and the recovery process, further studies are needed to investigate the detailed function and properties of these specific cells in the future.

Financial Support: This research was supported by Bio & Medical Technology Development Program (2021M3E5E6019133) of the National Research Foundation of Korea.

Notes:

118 - PRRS virus nonstructural protein 1 restricts TRIM19 expression and promotes viral replication

Dongwan Yoo[1], Chai-Ming Su[1], Junyu Tang[1], Yu Fan Hung[1], Mingyuan Han[1], Roger Everett[2]

[1]University of Illinois at Urbana-Champaign, [2]MRC-University of Glasgow Center for Virus Research. dyoo@illinois.edu

Session: Virology 2, 2024-01-22, 11:15 - 11:30

Objective: Porcine reproductive and respiratory syndrome virus (PRRSV) is an arterivirus inhibiting the IFN production and signaling during infection, and the nsp1 protein is the major viral IFN antagonist. Promyelocytic leukemia (PML) protein, known as TRIM19, is an IFN-stimulated gene product and plays a role in antiviral response. Preliminary data show the reduction of PML nuclear bodies in PRRSV-infected cells. A hypothesis has been established that PRRSV nsp1 protein may downregulate PML expression so that viral replication can be promoted.

Methods: Gene silencing for endogenous PML and overexpression of individual isoforms of PML were employed to determine the role of PML in viral replication. The interaction of PML and PRRSV proteins was determined by co-IP, pull-down, and co-staining assays. SUMO-interacting motifs (SIMs) in nsp1 were mutated by site-directed mutagenesis. SIM mutant PRRS viruses were generated by reverse genetics.

Results: PML nuclear bodies were significantly reduced in PRRSV-infected cells, and PRRSV nsp1b protein was determined to be the negative regulator for PML. Gene silencing confirmed the inhibitory role of PML for PRRSV. In contrast, coexpression of all 6 isoforms of PML restricted PRRSV replication. Among the isoforms, PML-II and PML-IV were the most significant suppressor for viral replication. The reduction of PML was post-translational and mediated via ubiquitination-dependent proteasomal degradation. PRRSV nsp1b was directly bound to PML, and the interaction of nsp1 to PML was common for representative member viruses in the Arteriviridae family. PRRSV nsp1b contained 4 SIMs, and SIM1 and SIM4 were PML binding domains. Double mutation of SIM1 and SIM4 completely abolished PML binding. A series of SIM mutant infectious PRRSV were constructed by reverse genetics, and the role of PML in viral replication was confirmed using SIM mutant viruses.

Conclusions: Our study reveals a novel strategy of arteriviruses for immune evasion to promote viral replication.

Financial Support: This project was supported by Agriculture and Food Research Initiative (AFRI) Competitive Grants nos. 2018-67015-28287 and 2023-67015-39710 from the U.S. Department of Agriculture (USDA) National Institute of Food and Agriculture (NIFA) awarded to DY.

Notes:

119 - Farm infection and pathological characteristics of a NADC34-like PRRSV variant in Korea

Seung-Chai Kim[1], Hwan-Ju Kim[1], Sang Chul Kang[2], Won-Il Kim[1]

[1]College of Veterinary Medicine, Jeonbuk National University, [2]Optipharm Inc., Republic of Korea. leesor2@jbnu.ac.kr

Session: Virology 2, 2024-01-22, 11:30 - 11:45

Objective: Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) is one of the most important viruses in the global swine industry due to enormous economic losses and its high genetic variation. Among its genetic lineages and subtypes, Lineage 1A viruses or NADC34-like PRRSV, which was first identified in the US in 2014 and subsequently spreading in China since 2017, has been recently identified in Korea with reports of an "abortion storm" similar to the cases from abroad. Although the virus is now spreading within the nation, the pathogenesis is largely unknown. The objective of this study is to characterize recently emerging NADC34-like PRRSV in Korea through the analysis of infection patterns within the affected farm as well as a challenge experiment.

Methods: Retrospective 14-week period productive performance data (7 weeks prior to the PRRS outbreak and 7 weeks after the outbreak) of NADC34-like PRRSV affected farm was thankfully shared by the farm and analyzed. The challenge experiment was conducted for 14 days with twenty-four 4-week-old PRRSV-negative piglets assigning six piglets for each group: negative control, reference strain VR2332, NADC30-like strain PJ73, and NADC34-like strain JBNU-22-N01. At 7- and 14-days post-challenge, three piglets from each group were euthanized at each timepoint, and histopathological evaluation as well as quantification of cytokine protein levels from lung tissues were conducted. Bronchoalveolar lavage (BAL) cells were also collected to conduct flow cytometry and to quantify the mRNA expression level of chemokines and immune checkpoint molecules. Lung tissues were used to quantify cytokine levels by Luminex assay. Quantification of serum viremia, nasal virus discharge, and PRRSV-specific antibodies was also conducted throughout the experiment period.

Results: In the affected farm, only the sow population, especially late-term pregnant sows, were significantly affected by NADC34-like PRRSV infection, in which 18.5% (159/861) of pregnant sows died during the post-infection period whereas only 1.7% (14/805) died during the pre-infection period. Abortion rates were increased up to 33.3%. However, weaned piglets were not severely affected by PRRSV infection during the same period, implicating the age-specific pathogenicity of NADC34-like PRRSV. In the challenge experiment with piglets, JBNU-22-N01 strain infected piglets show relatively lower weight gain and higher viremia compared to VR2332- or PJ73-infected piglets, but did not show fatal clinical symptoms. However, JBNU-22-N01 infection induced significant destruction(?) of BAL cell population, and upregulation of cytokines (IFN-α, IFN-γ, IL-1β, IL-10, and IL-12p40), chemokines (CCL2, CCL5, CCL8, and CXCL10) and chemokine receptors (CCR5 and CXCR5) as well as immune checkpoint molecules (PD1, PDL1, CTLA4, LAG3, and IDO1) at the earlier time point (7-day post-challenge).

Conclusions: Retrospective analysis of infection patterns within NADC34-like PRRSV infected farm as well as laboratory challenge experiment with piglets suggest that recently emerging NADC34-like PRRSV in Korea only heavily affect the pregnant sow population. Although the sow challenge experiment has not yet been conducted, the unique immunomodulation properties of NADC34-like PRRSV infection identified in the piglet could provide clues to understanding the pathogenesis of the virus.

Financial Support: This research was supported by Bio & Medical Technology Development Program (2021M3E5E6019133) of the National Research Foundation of Korea.

Notes:

120 - Inhibition of swine viruses by targeting viral proteins and host factors

Yunjeong Kim[1], Luija Wijesekara[1], Kyeong-Ok Chang[1]

[1]Kansas State University. ykim@ksu.edu

Session: Virology 2, 2024-01-22, 11:45 - 12:00

Objective: Viral infections such as porcine reproductive and respiratory syndrome (PRRS), foot-and-mouth disease (FMD) and African swine fever (ASF) are serious threats facing the swine industry worldwide. The current control measures are heavily dependent on implementing strict biosecurity measures and vaccinations, if available. The use of antiviral agents to reduce viral spread would be helpful to enhance biosecurity and to close the gap in vaccine-induced protection in potential outbreaks of FMD and ASF in the US, as well as reduce losses from highly virulent strains of PRRSV. FMDV, ASFV and PRRSV encode proteases that process viral polyproteins generated during virus replication to yield individual, mature virus proteins. Viral proteases are generally conserved well among different virus strains, which make them attractive targets for antiviral discovery. Here we analyzed the sequence homology of virus proteases and established assay platforms for screening compound libraries for FMDV, ASFV and PRRSV to identify inhibitors targeting virus protease or host factors involved in virus replication.

Methods: The amino acid protease sequences of various strains of each virus were analyzed by multiple sequence alignment analysis. To establish the protease inhibition assay, the codon-optimized, full-length genes of FMDV 3C protease and ASFV pR273S were expressed, and the expressed proteases were incubated with serial dilutions of a compound and a substrate containing a donor/quencher pair. Cleavage of substrate by a protease increases fluorescence readings, which is inhibited by the presence of an inhibitor. Following measuring fluorescence readings, the 50% inhibitory concentration (IC_{50}) was calculated for each compound. Cell-based assay was conducted by co-transfecting 293T cells with circular permutated firefly luciferase gene containing protease cleavage site and virus protease gene in a mammalian expression plasmid. Serially diluted compounds were added to the cells, and the luminescence was measured for the determination of the 50% effective concentration (EC_{50}) for each compound. For PRRSV inhibition assay, MARC-145 cells were incubated with serial dilutions of compound that are targeting viral proteins of host factors and infected with GFP-encoding infectious clone of PRRSV to determine the EC_{50} values.

Results: The amino acid homology of virus proteases is highly conserved among various strains with homology of >84% for ASFV pR273S, and >93% for FMDV 3Cpro. The screening efforts have identified compounds that show low micromolar range of inhibitory activities against viral proteases or viral replication, and the results from the cell-based studies correlated well with the protease inhibition assays.

Conclusions: The results confirmed the utility of the assay platforms which can be utilized in BSL-2 facility and the feasibility of identifying potent inhibitors of these important swine viruses.

Financial Support: This work was funded by USDA-NIFA AFRI grant # 2019-67015-29864.

Notes:

121 - Selection and immunogenicity of a VapA mRNA construct for immunizing foals against *Rhodococcus equi*

R.M. Legere[1], C. Poveda[2], J.A. Ott[3], J.M. Bray[1], B.P. da Silveira[1], S.K. Kahn[1], C.L. Martin[4], M.F. Criscitiello[3], L.R. Berghman[3, 4], A.I. Bordin[1], J. Pollet[2, 5], N.D. Cohen[1]

[1]Department of Large Animal Clinical Sciences, Texas A&M University, [2]National School of Tropical Medicine, Baylor College of Medicine, [3]Department of Veterinary Pathobiology, Texas A&M University, [4]Department of Poultry Science, Texas A&M University, [5]Texas Children's Hospital Center for Vaccine Development, Baylor College of Medicine. rlegere@cvm.tamu.edu

Session: Immunology 2, 2024-01-22, 10:30 - 10:45

Objective: To investigate a mRNA vaccine for foals targeting the virulence-associated protein A (VapA) of *R. equi* by designing, selecting, and evaluating immunogenicity of a mRNA vaccine candidate expressing VapA in neonatal foals.

Methods: Four variants of a mRNA vaccine coding for VapA were designed and *in vitro* transcribed: 1) native VapA, including its transmembrane domain (TM); 2) a codon-optimized version of construct 1; 3) VapA mRNA without the TM domain; and 4) a codon-optimized version of construct 3. Equine bronchial fibroblasts (EBFs) and equine bronchial epithelial cells (EBECs) cultured from healthy adult horses were transfected with each of the 4 mRNA constructs formulated in lipid nanoparticles (LNPs). Expression of VapA was evaluated by western immunoblot of cell lysates and supernatants using a commercial anti-VapA monoclonal antibody to identify a vaccine candidate. Immunogenicity of the mRNA formulated in LNPs was evaluated by comparing foals in the following study groups: 1) mRNA delivered by nebulization at doses of either 300 µg (n=6) or 600 µg (n=6); 2) 300 µg mRNA administered intramuscularly (IM) (n=6); 3) negative control foals nebulized with RNase-free water (n=6); and 4) positive controls immunized IM with 300 µg purified recombinant VapA plus adjuvant (n=6). All foals were immunized at ages 2 and 21 days. Serum and bronchoalveolar lavage (BAL) fluid samples were collected from foals at ages 3, 22, and 35 days to test for relative anti-VapA IgG_1 and $IgG_{4/7}$ activities by ELISA (i.e., OD of sample/OD of positive control).

Results: Among the mRNA vaccine variants tested, only constructs 3 and 4 lacking the TM domain were expressed in both EBFs and EBECs. Construct 3 exhibited higher expression than construct 4 in both cells and supernatants. Activities of anti-VapA IgG_1 were highest at 35 days for the foals that received either IM VapA mRNA or IM VapA protein. The proportion of foals with anti-VapA IgG_1 ratio > 30% of the positive control in the 2 IM groups (100%; 11/11) was significantly greater than either all other foals (36%; 5/16; $P < 0.001$) or foals in the negative control group (50%; 3/6; $P = 0.029$). Increased anti-VapA activity in controls was attributed to natural exposure to environmental *R. equi*. The only significant differences for $IgG_{4/7}$ were that foals in the IM VapA mRNA group were increased on day 35 relative to themselves and control foals at ages 3 and 22 days ($P < 0.05$ for all). Anti-VapA IgG_1 and $IgG_{4/7}$ activities in serum were significantly ($P < 0.0001$) correlated with those in BAL fluid.

Conclusions: As formulated, nebulized mRNA resulted in inconsistent antibody responses in serum and BAL fluid; however, IM administration of the mRNA appeared immunogenic. Natural exposure to *R. equi*, which is common in foals, must be considered in designing *R. equi* vaccine studies.

Financial Support: The project was funded by The Foundation for the Horse, the Grayson-Jockey Club Research Foundation, the Link Equine Research Endowment, Texas A&M University, and the Department of Large Animal Clinical Sciences, School of Veterinary Medicine & Biomedical Sciences, Texas A&M University.

Notes:

122 - Induction of innate immunity by enteral virulent and avirulent *R. equi* in foals

Bibiana P. da Silveira[1], Rebecca M. Legere[1], Jocelyne M. Bray[1], S. Garrett Wehmeyer[1], Michael C. Golding[1], Joanne Hardy[1], Noah D. Cohen[1], Angela I. Bordin[1]

[1]School of Veterinary Medicine & Biomedical Sciences. abordin@tamu.edu

Session: Immunology 2, 2024-01-22, 10:45 - 11:00

Objective: *Rhodococcus equi* pneumonia in foals is economically detrimental to the equine industry and no vaccine is licensed. Enteral live, virulent *R. equi* (VRE), but not avirulent *R. equi* (AvRE), has been shown repeatedly to protect foals against infection. These previous studies focused on adaptive immune responses, and overlooked the likely role of innate immunity. We have previously shown that enteral VRE induces epigenetic modifications in circulating monocytes of foals, but whether these changes happen in progenitor cells in the bone marrow remains unknown. Our objectives were: 1) to determine whether enteral AvRE protects foals against intrabronchial challenge; and, 2) to determine whether enteral administration of either VRE or AvRE induces transcriptome changes in myeloid progenitor cells in the bone marrow and circulating monocytes from newborn foals.

Methods: This project will be performed during 2 years, and this abstract reflects the work from Year 1. Foals were gavaged at age 2 days with either virulent *R. equi* (VRE; 10^{10} CFU in 50 ml of saline; n = 4), avirulent *R. equi* (AvRE; 10^{10} CFU in 50 ml of saline; n=4), or saline (control; 50 ml; n=4). Blood was collected at ages 2 and 28 days, and bone marrow at age 12 days. Foals were infected intrabronchially with 2×10^6 CFU of virulent *R. equi* at age 28 days, and clinically monitored until age 12 weeks. Monocytes were isolated from blood and submitted for bulk RNA-sequencing (RNA-Seq). Bone marrow cells were processed and submitted for single-cell RNA sequencing (scRNA-Seq). Data analysis will be performed in R with significance set at $P<0.05$ and included FastQC and Cutadapt (RNA-Seq library quality), HISAT2 (mapping to equine genome), and DESeq2 (DEG RNA-Seq between groups). Data from scRNA-Seq will be analyzed using Seurat package in R.

Results: All foals (4/4) gavaged with VRE remained healthy, while 75% (3/4) of foals gavaged with AvRE and 75% (3/4) of control foals developed pneumonia. Bone marrow scRNA-Seq and monocyte RNA-Seq data are pending but will be presented at the conference.

Conclusions: Enteral VRE, but not AvRE, protected foals against *R. equi* pneumonia. Further studies are needed to elucidate the mechanistic pathways by which VRE induces protection, and to determine whether a different formulation of AvRE (*i.e.*, higher dose, increased frequency) might elicit a protective response similar to VRE.

Financial Support: This project was supported by Agriculture and Food Research Initiative Competitive Grant no. 2023-67015-39097 from the USDA National Institute of Food and Agriculture, Grayson-Jockey Club Research Foundation, and Link Equine Research Endowment - Texas A&M University.

Notes:

123 - Low serum activities of C1q and anti-R. equi IgG1 predict rhodococcal pneumonia

A.B. Bettencourt[1], D. Reiskind[1], P. Flores-Ahlschwede[2], S.K. Kahn[1], J.M. Bray[1], E.G. Villafone[1], S. Ahlschwede[2], A.I. Bordin[1], N.D. Cohen[1]

[1]Department of Large Animal Clinical Sciences, School of Veterinary Medicine & Biomedical Sciences, Texas A&M University, [2]Rood & Riddle Equine Hospital in Saratoga. abordin@tamu.edu

Session: Immunology 2, 2024-01-22, 11:00 - 11:15

Objective: To determine the effects of transfusion of *Rhodococcus equi* hyperimmune plasma (REHIP) on serum concentrations of complement component 1q (C1q), and to examine the association of serum C1q and anti-rhodococcal antibodies of newborn foals with subsequent development of rhodococcal pneumonia.

Methods: Foals (n = 205) from 2 Thoroughbred breeding farms in New York were transfused with REHIP. Blood was collected immediately before transfusion with REHIP, and again from the contralateral vein immediately after transfusion. Foals were followed through weaning for clinical and ultrasonographic evidence of rhodococcal pneumonia. Serum samples were tested by ELISA for concentrations of C1q and for activity of IgG_1 and $IgG_{4/7}$ recognizing the virulence-associated protein A (VapA) of *R. equi*. Logistic regression analysis was used to determine the association between rhodococcal pneumonia and levels of C1q and anti-VapA IgG_1 and $IgG_{4/7}$.

Results: REHIP significantly decreased C1q concentrations after transfusion. Accounting for effects of farm and birth-month, estimated odds of pneumonia were 2.1-fold (P = 0.0330) higher for foals with pretransfusion C1q concentrations less than the median and 3.3-fold (P = 0.0051) higher for foals with posttransfusion IgG_1 activity in the lowest quartile.

Conclusions: Although C1q contributes to protection against *R. equi*, protective effects of REHIP are likely attributable to IgG, and IgG_1 appears to be especially important. Increasing IgG_1 concentrations targeting rhodococcal proteins in REHIP or serum of foals would improve protection against *R. equi* foal pneumonia.

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Notes:

124 - Nebulization of mRNA encoding a monoclonal antibody against Rhodococcus equi in foals

R.M. Legere[1], D.A. Vanover[2], B.P. da Silveira[1], A.R. Woolums[3], M.F. Criscitiello[4], L.R. Berghman[5], A.I. Bordin[1], J. Pollet[6], N.D. Cohen[1]

[1]Department of Large Animal Clinical Sciences, Texas A&M University, [2]Department of Biomedical Engineering, Emory University, [3]Department of Pathobiology and Population Medicine, Mississippi State University, [4]Department of Veterinary Pathobiology, Texas A&M University, [5]Department of Poultry Science, [6]National School of Tropical Medicine, Baylor College of Medicine Texas Children's Hospital Center for Vaccine Development. rlegere@cvm.tamu.edu

Session: Immunology 2, 2024-01-22, 11:15 - 11:30

Objective: Foal pneumonia caused by *Rhodococcus equi* is a major equine health problem worldwide. The only method available for preventing *R. equi* pneumonia in foals is transfusion of hyperimmune plasma, which is expensive, labor- and time-intensive, and carries risks for foals including circulatory volume overload. Our objective was to evaluate whether nebulizing mRNA encoding a monoclonal antibody (mAb) targeting the virulence-associated protein A (VapA) of *R. equi* could generate detectable antibodies in the lungs of foals.

Methods: Foals were nebulized at age 2 days with either mRNA constructs encoding the heavy (H) and light (L) chains of a mAb against VapA at a dose of 10 mg/foal (≈ 0.2 mg/kg; n = 5), or an equal volume of saline (control foals; n=5). The L construct was modified to incorporate the mRNA sequence encoding nanoluciferase (NanoLuc®) at the 3′ end. The mRNA constructs were formulated at a ratio of 3H:1L in a poly-β-amino-thio-ester polymeric nanoparticle carrier agent. Serum and bronchoalveolar lavage (BAL) fluid (BALF) were collected at ages 4, 7, 14, 28, and 56 days. BAL fluid was centrifuged to separate BAL cells. BAL cells and BALF were examined for luminescence activity. Serum and BALF were tested for antibodies recognizing *R. equi* expressing VapA by direct ELISA.

Results: Luminescence indicating mAb translation was detected in BAL cells and BALF of foals nebulized with the mRNA/polymer compound at ages 4 and 7 days but not in controls; mAb translation was not apparent in any samples at age ≥14 days. Luminescence was not detected in the serum of any foals at any age. Significantly ($P < 0.05$) higher *R. equi*-specific antibodies were detected by ELISA in the BALF (but not serum) of mRNA foals than controls at ages 4 and 7 days.

Conclusions: Nebulizing foals with mRNA encoding mAbs in polymeric nanoparticles can generate detectable antibodies in foals for at least 5 days following nebulization. Further studies are warranted to evaluate the efficacy of these antibodies to protect foals against infection with *R. equi*.

Financial Support: This work was supported by a grant from USDA-NIFA (2022-67015-36335). Dr. Rebecca Legere is supported by a fellowship from the Department of Large Animal Clinical Sciences, Texas A&M University. Noah Cohen is supported by the Glenn Blodgett Chair.

Notes:

125 - Administration of attenuated *Salmonella* strains in ovo to induce protective immunity against bacterial pathogens

Vinicius Lima[1], Roy Curtiss III[1], Soo-Young Wanda[1], Douglas Natoce Jr.[1]

[1]University of Florida. v.lima@ufl.edu

Session: Immunology 2, 2024-01-22, 11:30 - 11:45

Objective: Induction of an early and sustained protective immune response is one of the biggest challenges faced by the modern poultry industry. We seek to address this issue using a technology based on self-destructing attenuated adjuvant *Salmonella* (SDAAS) strains. Our SDAAS strains are programed to undergo regulated lysis in various cell compartments within the inoculated embryo to maximize delivery of pathogen and damage associated molecular patterns (PAMPs and DAMPs) to pattern recognition receptors (PRRs) to potentiate induction of immunity. In addition, this feature confers biological containment with no persistence in vivo and no survival if excreted.

Methods: Novogen brown chicken embryos at 18 days of incubation are inoculated in the amniotic fluid with several Family A (regulated lysis phenotype) and Family B (delayed lysis phenotype) SDAAS strains, each possessing unique features to modulate and/or enhance induction of immunity. At day-of-hatch chicks are challenged orally with $1x10^3$ CFU of wild-type *S.* Typhimurium strain χ3761 by oral inoculation. Animals are euthanized at different time points and bacterial titers in different tissues, including caeca content, are measured by plating in *Salmonella-Shigella* agar plates. Animals are also challenged by subcutaneous inoculation with Avian Pathogenic *E. coli* (APEC) strain χ7122 and are observed up to 7 days after challenge. Body weight and mortality is compared with a group of animals derived from non-inoculated eggs. We have also euthanized chicks at day-of-hatch and collected samples of liver, spleen, bursa of *fabricius*, lungs (and others) to evaluate the ability of SDAAS strains to colonize and invade different organs and tissues.

Results: We have demonstrated that administration of SDAAS strains *in ovo* can be safe with no negative impacts in hatchability and chick quality. These strains can also induce a protective immune response against the aforementioned pathogens, leading to lower *Salmonella* titers in caeca content, liver and spleen and reducing mortality in animals challenged with APEC strains. We also demonstrate the ability of SDAAS strains to colonize different mucosal and lymphoid tissues after *in ovo* inoculation.

Conclusions: Our continued work suggests that *in ovo* administration of different SDAAS strains is a safe and effective method to induce a robust immune response against pathogens of animal and public health importance. Our results also suggest that *in ovo* inoculation is a superior means to induce a protective immune response against bacterial pathogens when compared with conventional spray/drinking water vaccination. To our knowledge, this is the first word showing that live attenuated *Salmonella* strains can be safely administered *in ovo.*

Financial Support: This project was sponsored by USDA-NIFA, grant number 2020-67017-33237.

Notes:

126 - MicroRNAs isolated from bovine colostrum modulate the NF-kB pathway activation in vitro

Luciana Kluppel[1], Rafaela Santos[1], Audrey Brown[1], Keerra Holzapfel[1], Sravani Gade[1], Ulrich Bickel[2], Fernanda Rosa[1]

[1]School of Veterinary Medicine, Texas Tech University, [2]Jerry H. Hodge School of Pharmacy, Texas Tech Health Sciences Center. luciana.kluppel@ttu.edu

Session: Immunology 2, 2024-01-22, 11:45 - 12:00

Objective: Bioactive components present in bovine colostrum play an essential role in the regulation of inflammatory responses in early life. Thus, this study aimed to characterize extracellular vesicles (EVs) containing microRNAs (miRNAs) in bovine colostrum and their ability to modulate inflammation through the NF-kB activation pathway.

Methods: Bovine colostrum samples were collected from multiparous Holstein cows in a commercial farm. Colostrum samples were immediately defatted and frozen at -80°C until processing. The EV pellets and the miRNA isolation from the EV pellets were performed using commercial isolation kits. For the characterization of EVs under super resolution microscopy (STED), a subset of EV pellets were labeled with a primary polyclonal antibody (CD63) and with a secondary antibody (Alexa 594). To determine the NF-kB activation by miRNAs, a murine macrophage reporter cell line (Raw-Blue Cells™) was incubated with a pool of miRNAs isolated from bovine colostrum for 24 hours. A colorimetric enzymatic assay (Quanti-Blue™) was used to detect the NF-kB activity over-time using a spectrophotometer (620-655 nm). Additionally, a subset of Raw-Blue cells (control group and treated with miRNAs) were challenged with lipopolysaccharide isolated from *Escherichia coli* (1 µg/well; LPS O111:B4). The miRNA pool used in this *in vitro* assay was subjected to RT-qPCR to determine the expression of key miRNAs. Statistical analyses were performed using PROC MIXED procedure of SAS (v. 9.4) with repeated measurements design. Statistical significance was declared at $P < 0.05$.

Results: The cells treated with bovine-miRNA showed NF-kB activity as early as 6-h post-incubation ($P<0.01$). Similarly, the cells challenged with LPS (including LPS control and LPS+miRNA treated cells) had a significant increase in the NF-kB activity from 6- to 24-h post-challenge ($P<0.01$). Interestingly, the cells treated with miRNAs alone had lower NF-kB activity relative to LPS or LPS+miRNA cells at 24-h ($P<0.02$). Six different miRNAs (miR-29C-3p, miR-101-3p, miR-222-3p, miR-340-3p, let-7a-5p, miR-21-5p) showed a high level of expression in bovine colostrum of lower than 30 cycle threshold (CT) units. Examining the effect of miRNA treated cells, our *in vitro* preliminary findings show that miRNAs can regulate immunological pathways via endosomal toll-like receptor (TLR7 or TLR8) binding independently of inflammation. We also noted that miRNAs involved in cell apoptosis, immune regulation, and oncogenic pathways are found in bovine colostrum.

Conclusions: MicroRNAs present in bovine colostrum can regulate immunological pathways such as the NF-kB activity by binding to endosomal TLRs, regardless of the presence of initial infections. The high expression of target miRNAs in colostrum of multiparous Holstein cows might play a role in the regulation of oncogenic and immune-related pathways in neonatal dairy calves. Studies evaluating bovine colostrum derived extracellular vesicle cargo and its immunomodulatory effects *in vivo* are warranted for future investigation.

Financial Support: This project was supported by Agriculture and Food Research Initiative Competitive Grant no. 2023-67015-39081 from the USDA National Institute of Food and Agriculture.

Notes:

127 - Role of segmented filamentous bacteria in gut immune maturation and resistance to *Enterobacteriaceae* in layer chickens

Melha Mellata[1], Jared Meinen-Jochum[1]

[1]Iowa State University. mmellata@iastate.edu

Session: "Omics" 1, 2024-01-22, 10:30 - 10:45

Objective: The chicken gut is a major site affecting host health and productivity. In commercial farms, newly hatched chicks receive their microbiota from the environment, mainly *Enterobacteriaceae* that leads to altered immune system development, gut inflammation, increased disease susceptibility, and reduced productivity and performance. In the absence of contact with their progenitors, the newly hatched birds will not inherit key gut microbes like segmented filamentous bacteria (SFB) that play a key role in gut maturation during early life. The objectives of this study were to (1) test a treatment for chickens that includes SFB, (2) test ability of the treatment to increase resistance to total *Enterobacteriaceae* and *Salmonella* in layer hens, and (3) elucidate its molecular mechanism.

Methods: SFB-based inoculum was prepared from ilea scrapings. Day-old layers were either non-treated (CON) or SFB treated (SFB) and then challenged with *Salmonella* Typhimurium (ST). Total *Enterobacteriaceae* and *Salmonella* were examined by plating and enumeration in feces at 7-,10- and 14-day-post-inoculation (DPI); and in the ileum, cecum, and spleen at 16 DPI in euthanized birds. The presence and levels of SFB in feces and ilea scrapings were determined *via* both microscopy and RT-qPCR. Relative gene expression of host-derived antimicrobial peptides and cytokines in the distal ileum was determined by RT-qPCR. Data analysis was performed using the GraphPad Prism software.

Results: Treatment with SFB led to 50% increase of the level of SFB in ilea at 6 DPI and significant decrease of both total *Enterobacteriaceae* ($P < 0.001$) and ST ($P < 0.01$) in the feces of layers. RT-qPCR revealed significantly increased expression of β-defensin 14 ($P < 0.01$), and the cytokines IL-10 ($P < 0.0001$) and IFNγ($P < 0.05$).

Conclusions: We have demonstrated the probiotic properties of SFB based inoculum. Overall, the treatment has potential to improve gut homeostasis and diseases resistance, which will increase poultry health and productivity.

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

Notes:

128 - Role of chicken miRNA in the regulation of bacterial plasmid conjugation in vitro

Logan Ott[1, 2], Caleb Skow[1, 2], Melha Mellata[1, 2]

[1]Department of Food Science and Human Nutrition, Iowa State University, [2]Interdepartmental Microbiology Gradaute Program, Iowa State Univsity. ott@iastate.edu

Session: "Omics" 1, 2024-01-22, 10:45 - 11:00

Objective: The gut of animal hosts acts as a potent reservoir for the emergence and spread of antimicrobial resistance (AR) through bacterial plasmid conjugation. Little is understood about the role of the host in regulating this process in the gut. We have shown that many host factors play a role in the incidence of and transfer rate of large AR plasmids, such as genetics, diet, and age. Here we examine the effect of chicken ceca small RNA and specific miRNA mimics effects on bacterial plasmid conjugation *in vitro* explant and broth conjugation models for the chicken gut.

Methods: In silico binding analysis of chicken miRNA to the complete coding sequence record for the large AR plasmid pAPEC-O2-211A-ColV was completed using the miRNA hybridization software RNAHybrid. The top ten hypothetical bindings were identified, and the six unique sequences were obtained as miRVana miRNA mimics. Conjugation reactions were supplemented with miRNA mimics to measure their effect on the transfer of pAPEC-O2-211A-Colv from its natural host, *E. coli* APEC-O2-211, to the plasmid free recipient *E. coli* HS-4. Conjugations were conducted for 1, 2, and 3 hours at 40˚C; and donors, recipients, and transconjugants were enumerated on selective media.

Results: Of the six unique miRNA species screened, all showed a numerical reduction in conjugation frequency after three hours of incubation. The miRNA mimic gga-miR-12279-3P demonstrated a significant ($p < 0.05$) reduction in conjugation frequency with respect to donors from the control, whereas miRNA mimic gga-miR-12235-3P demonstrated a significant ($p < 0.05$) reduction in conjugation frequency with respect to recipients compared to the control.

Conclusions: We identify the potential role of two chicken miRNA species in the regulation of bacterial conjugation *in vitro*. Overall, this study helps elucidate a potential interaction associated with the complex gut environment and the emergence of AR. This information will be invaluable in identifying novel approaches to mitigate the global AR threat.

Financial Support: Funding sources for this study were from the United States Department of Agriculture, National Institute of Food and Agriculture project IOW05679 (LCO) and USDA Hatch project IOW04202 (MM).

Notes:

129 - MAP3773c modulates key metabolic and redox pathways in *Mycobacterium paratuberculosis* under invitro iron starvation

Sajani Thapa[1], Denise K. Zinniel[2], Raul G. Barletta[2], John P. Bannantine[3], Marianne Huebner[4], Srinand Sreevatsan[1]

[1]Pathobiology and Diagnostic Investigation, Michigan State University, [2]School of Veterinary Medicine and Biomedical Sciences, University of Nebraska, [3]National Animal Disease Center, [4]Department of Statistics and Probability, Michigan State University. thapasaj@msu.edu

Session: "Omics" 1, 2024-01-22, 11:00 - 11:15

Objective: *Mycobacterium avium* subsp. *paratuberculosis* (MAP) is a pathogenic mycobacterium with special micronutrient requirements. For optimal growth in laboratory media, MAP requires supplementation of a siderophore (mycobactin), and takes eight to sixteen weeks to produce colonies, a major hurdle in timely diagnosis. MAP carries a *MAP3773c*, a putative metal regulator, on its genome which is absent from other mycobacteria. Homologs of this gene in enterobacteria have roles in global iron regulation and homeostasis. The role of *MAP3773c* in regulating intracellular iron in MAP, is poorly understood. In this study, the transcriptional regulation pathways of *MAP3773c* deployed by MAP to maintain iron homeostasis under iron restriction conditions was investigated.

Methods: A field isolate (K-10) and an in-frame *MAP3773c* deletion mutant (Δ*MAP3773c*) derived from K-10 through homologous recombination were exposed to iron restricted conditions for 5, 30, 60, and 90 minutes with five replicates each. Total RNA was extracted at each time point. Quality and integrity of the RNA was assessed before RNA-Seq was performed. RNA-Seq data analysis was carried out on MSU's High Performance Computing Center (HPCC) clusters and then were processed for differential gene expression analyses using DESeq2. Functional analysis of the differentially expressed genes (DEGs) was performed to identify the set of genes that were involved in several metabolic and cellular processes in both K-10 and *ΔMAP3773c* strain.

Results: A comparison of transcriptional profiles between K-10 and Δ*MAP3773c* showed 425 differentially expressed genes (DEGs) at 30 minutes time post iron restriction. Functional analysis of DEGs in Δ*MAP3773c* revealed that pantothenate biosynthesis, polysaccharide biosynthesis, sugar metabolism and n-acetyltransferase enhanced intracellular survival (eis) genes were downregulated at 30 minutes post iron starvation whereas arginine and proline metabolism, PPE family genes and mammalian cell entry genes were upregulated at 30 minutes post iron starvation in Δ*MAP3773c* strain. Pathway analysis showed that *ΔMAP3773c* strain significantly downregulated *panB, panC, panD and panK* genes suggesting potential impairment of pantothenate (Pan) and CoA biosynthesis pathway due to absence of *MAP3773c* gene at 30 minutes post iron starvation.

Conclusions: Our results suggest that MAP regulates different sets of genes within 30 minutes of encountering iron restriction conditions suggesting high sensitivity of MAP to iron starvation at this specific time point. Furthermore, the absence of *MAP3773c* gene appears to impair MAP's ability to synthesize major cell wall components likely affecting cell wall biosynthesis process. Pathway analysis revealed that *ΔMAP3773c* strain experiences an impairment in pantothenate (Pan) and CoA biosynthesis pathways at 30 minutes post iron starvation suggesting that the absence of those pathways potentially affect overall metabolic process and cellular functions, affecting MAP survival and pathogenesis. Taken together, these findings highlight the critical role of *MAP3773c* in the survival and pathogenicity of MAP.

Notes:

130 - Single-nuclei RNA Sequencing of adipose tissue of dairy cows with subclinical ketosis

Hunter Ford[1], Tainara Michelotti[2], Clarissa Strieder-Barboza[1]

[1]Texas Tech University, [2]INRAE Clermont-Auvergne-Rhône-Alpes. hunter.ford@ttu.edu

Session: "Omics" 1, 2024-01-22, 11:15 - 11:30

Objective: Our objective was to evaluate the depot-specific transcriptome at single-cell resolution in subcutaneous (SAT) and visceral (VAT) adipose tissues in dairy cows with and without subclinical ketosis.

Methods: Ten Holstein dairy cattle (8 ± 2 DIM, parity 3.2 ±1.4, BCS 3.6 ± 0.3) were enrolled in a nonrandomized trial and blocked according to parity, BCS, and blood BHB (Precision Xtra, Abbott). Cows were assigned to two groups: non-ketotic (NK, n=5, BHB ≤ 1.0 mmol/L) and subclinical ketosis (SCK, n=5, BHB > 1.0 mmol/L). Abdominal SAT and retroperitoneal VAT samples were obtained via laparotomy (right paralumbar fossa). Tissue samples were then homogenized and processed to isolate single-nuclei for library construction (10X Genomics), sequencing (Illumina NovaSeq 6000), and alignment using the CellRanger Pipeline (10X Genomics). Single-nuclei transcriptomic data was analyzed using the Seurat package in R (v1.4). Clusters were identified using canonical marker genes for cell types commonly found in adipose tissues and functional analysis was performed using the clusterProfiler package in R for KEGG pathway enrichment.

Results: Analysis identified 11 unique cell clusters in SAT, including two subpopulations of mature adipocytes (AD), two subpopulations of adipocyte progenitor cells (ASPC), three subpopulations of endothelial cells (EC), three subpopulations of immune cells (IMC), and one subpopulation of pericyte/smooth muscle cells (PE/SMC). SAT AD and ASPC made up the majority of cells, composing 35% and 21% of all nuclei respectively. There were no differences in the abundance of cell types in SAT between NK and SCK cows, however, one cluster of EC were primarily found in SCK cows compared to NK cows. Analysis of the VAT nuclei identified 10 distinct cell clusters, including one subpopulation of AD, one subpopulation of ASPC, three subpopulations of EC, one subpopulation of PE/SMC and three subpopulations of IMC. Similar to SAT, the majority of nuclei sequenced in VAT were AD, making up 42% of all cells, while only 10% of cells were identified as ASPCs, suggesting a lower adipogenic capacity of VAT. Interestingly, there was a tendency for a greater abundance of IMC (p=0.09) in SCK compared to NK cows indicating active inflammation. Functional analysis of the ASPCs in VAT revealed activation of inflammation in SCK cows with upregulation of genes associated with chemokine and immune pathways. Furthermore, differentially expressed genes in these cells were also associated with suppressed fatty acid metabolism.

Conclusions: Our findings highlight distinct differences in the abundance of specific adipose tissue cell types between SAT and VAT depots. While there are minimal differences in the cellular composition and functional profile of SAT between NK and SCK cows, VAT cells from SCK contain a more pro-inflammatory profile indicating a potential link between this adipose tissue depot and the pathogenesis of metabolic dysfunction in dairy cattle.

Financial Support: We are appreciative of USDA for funding this investigation.

Notes:

131 - Longitudinal blood RNA-Seq analysis of cattle to determine the impact of vaccination and marketing on clinical BRD

Bradly Ramirez[1, 2, 3], Hudson McAllister[1, 2, 3], Sarah Capik[4], Robert Valeris-Chacin[1, 2, 3], Kelsey Harvey[5, 6], Amelia Woolums[5,7], Brandi Karisch[5, 6], Matthew Scott[1, 2, 3]

[1]Texas A&M University, [2]VERO Program, [3]Large Animal Clinical Sciences, [4]Tumbleweed Veterinary Services, PLLC, [5]Mississippi State University, [6]Department of Animal and Dairy Sciences, [7]Department of Pathobiology and Population Medicine. ramirez_b@tamu.edu

Session: "Omics" 1, 2024-01-22, 11:30 - 11:45

Objective: Bovine respiratory disease (BRD) is a multifaceted syndrome of respiratory distress brought about by the interaction of viral agents, bacteria, and environmental stressors. Mitigation of the viral component of BRD is achieved through broad vaccination strategies. Animal management decisions, specifically in relation to sale strategy, is predicted to influence the way stress contributes to the disease. Current practices variably reduce the impact of BRD on the beef industry. To evaluate and improve the effectiveness of BRD mitigation techniques, we employed a study to assess the interaction between vaccination, marketing strategy, and BRD outcomes on host gene expression to understand the ways in which these techniques stimulate the immune systems of cattle which remain healthy or are afflicted by respiratory disease.

Methods: Jugular whole blood samples (Tempus) were randomly selected from 73 bull calves enrolled in a whole plot, split-plot time course study. Thirty-three cattle received a modified live virus vaccine (Pyramid 5) and booster during the cow-calf phase of production, while 40 cattle did not. Forty animals were then shipped directly from the cow-calf facility to backgrounding, while 33 cattle were placed in a commercial auction market for eight hours, then an order-buyer system for three days prior to shipping to the same backgrounding facility at the same time. Jugular blood was collected at six timepoints: immediately prior to vaccination or not (T1), seven days-post vaccination (T2), and immediately prior to booster or not (T3), at weaning prior to marketing enrollment (T4), backgrounding facility arrival (T5), and end of backgrounding (T6). RNA was extracted and sequenced (150 bp; ~35 million reads/sample), and bioinformatically processed with a HISAT2/StringTie2 pipeline for determining differential gene expression between each treatment group. Differentially expressed genes (DEGs) were determined with edgeR and glmmSeq (FDR<0.05). Functional enrichment terms were identified for DEGs with KOBAS-i (FDR<0.05).

Results: Vaccinated cattle which would later develop BRD demonstrated an increase in gene expression related to airway epithelium differentiation compared to non-vaccinated cattle that later developed BRD. Marketing enrollment strategy was the largest influence of gene expression, with lasting effects at the end of the study period. The animals which were routed through an auction system as opposed to direct transportation showed a decrease in inflammatory mediating molecules and a greater mobilization of toll-like receptor and cytokine activity, type-I interferon signaling, T-helper cell differentiation, and neutrophil degranulation. Cattle directly transported to backgrounding demonstrated an increase in gene expression related to platelet activation, adipose cell metabolism, and striated muscle contraction.

Conclusions: Collective findings from this study indicated that vaccination and direct purchasing of cattle over an auction setting may mitigate the transcription of inflammatory elements. Most critically, identification of key genes expressed by putatively healthy animals highlight potential mechanisms that might be exploited to manage chronic and acute inflammation, in supplement to more established practices of vaccination and purchasing method.

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Notes:

132 - Persistence of prenatal epigenetic alterations into the postnatal period following fetal BVDV infection

J. Kincade[1], H. Van Campen[1], J. Bishop[1], T. Engle[1], H. Georges[1], C. Gonzalez-Berrios[1], T. Hansen[1]

[1]Colorado State University. jkincade@colostate.edu

Session: "Omics" 1, 2024-01-22, 11:45 - 12:00

Objective: Bovine viral diarrhea virus (BVDV) costs the cattle industry $1.5-$2.5 billion dollars annually. BVDV crosses the placenta and induces early embryonic death, abortion, and the generation of weak, non-viable calves. Before 125 days of gestation, the fetus has not yet developed an adaptive immune response. As such, fetuses infected during this period become immunotolerant to the virus. Postnatally, these persistently infected (PI) calves often have congenital deformities and remain chronically immunosuppressed. Epigenomic data from PI fetal spleen indicates differential methylation of genes associated with the neural, skeletal, cardiac, and immune systems. When fetal infection occurs later than 150 days of gestation, the mounted adaptive immune response is more robust and includes the production of antigen specific antibodies capable of clearing the virus. Fetal transient infections (TI) produce underweight calves with impaired growth rates. At 4 months of age, TI calves have sub-normal proportions of T cells and analysis of the methylome indicates differential methylation of genes associated with the immune, metabolic, and reproductive systems. It was hypothesized that epigenetic alterations occur during fetal BVDV infection and persist not only to the early postnatal period, but also throughout life.

Methods: To test this hypothesis, BVDV naive, pregnant heifers were inoculated with non-cytopathic BVDV-2 or phosphate buffered saline on day 175 of gestation to generate TI and control calves, respectively. PI cattle were identified on a local, cooperating ranch. PBMCs were isolated from whole blood of TI, PI, and Control cattle and subjected to reduced representation bisulfite sequencing (RRBS) via Zymo Research at birth and/or 4 months of age.

Results: Epigenetic data collected on PI calves at 245 days of gestation and 4 months of age correspond to known pathologies of PI cattle within the skeletal, cardiac, metabolic, and immune systems. Of the 2,640 differentially methylated sites (DMSs) identified in fetal spleen at 245 days of gestation and the 4,921 DMSs identified in peripheral blood mononuclear cells (PBMCs) at 4 months of age, 670 genes were found to contain at least 1 DMS. Analysis of the methylome at birth in TI cattle revealed inherent differential methylation of genes associated with cell regulation, metabolism, anatomical development, and the immune system. Of the 2,326 DMSs identified at birth and the 4,015 DMSs identified at 4 months of age in the generated TI calves, 616 DMSs were found on genes common to both datasets. DMSs were more abundant in TI calves at 4 months of age, global cell regulation, metabolism, and the immune system remain consistently impacted.

Conclusions: Analysis of the methylome at birth demonstrates that TI calves have differentially methylated genes compared to their uninfected counterparts. The existence of DMSs prior to influence of the external environment indicates that fetal BVDV infection leads to the alteration of DNA methylation. Additional DMSs are evident at 4 months of age, suggesting the potential to influence both TI and PI calves into the postnatal period through fetal programming. This research was supported by USDA NIFA Grants: 2019-67015-29866, 2021-38420-34040, and 2023-67011-40513.

Financial Support: This research was supported by USDA NIFA Grants: 2019-67015-29866, 2021-38420-34040, and 2023-67011-40513.

Notes:

133 - Prevention and control of diseases in aquaculture species

Benjamin Beck[1], Allison Wise[1], Craig Shoemaker[1], Troy Bader[1]

[1]USDA-ARS Aquatic Animal Health Research Unit. benjamin.beck@usda.gov

Session: Animal Vaccinology Research Network Symposium, 2024-01-22, 2:00 - 2:45

While aquaculture represents the fastest growing segment of animal agriculture, the expansion of aquaculture globally has been severely hindered by disease; particularly diseases of bacterial origin. Currently in the US farmed catfish industry, an emerging bacterial pathogen termed virulent *Aeromonas hydrophila* (vAh) attributes nearly $35 million dollars in economic losses annually. This bacterial pathogen infects both channel and hybrid catfish inducing skin necrosis, internal and external hemorrhaging, and exophthalmia. Farmers can lose over 50% of a harvest yield in less than a week when infected, increasing the urgency for more effective preventative and/or control measures. In this talk, the author will discuss the development of a reliable and reproducible challenge model for this debilitating pathogen, offer insight into mechanisms governing the susceptibility of the catfish host, and highlight a series of efficacy studies featuring novel inactivated bacterin vaccines delivered via immersion and oral routes. Results from these trials are allowing for the development of more efficacious vaccine products and delivery on catfish farms, ultimately, preventing mass mortality due to vAh.

Notes:

134 - Progresses and challenges in the development of vaccines against intracellular intestinal parasites in poultry

Hyun S. Lillehoj[1]

[1]Animal Bioscience and Biotechnology Laboratory, Beltsville Agricultural Research Center, USDA-Agricultural Research Service. hyun.lillehoj@usda.gov

Session: Animal Vaccinology Research Network Symposium, 2024-01-22, 2:45 - 3:30

Coccidiosis is an avian intestinal disease caused by several distinct species of *Eimeria* parasites that damage the host's intestinal system, resulting in poor nutrition absorption, reduced growth, and often death. Coccidiosis is estimated to cost more than USD 14.5 billion annual losses globally and coccidiosis control using various anticoccidial chemicals, such as ionophores, and coccidiostats, has long been a mainstream strategy in modern poultry production. However, due to the restrictive use of antibiotics in animal agriculture, antibiotic alternative control strategies are being developed with much effort on developing alternative strategies, including vaccines and effective dietary strategies using phytochemicals, probiotics, prebiotics, or hyperimmune antibodies. Immunologic approaches including vaccination with recombinant vaccines, and other antibiotic alternatives to improve host innate immunity has been tried. However, a detailed understanding of the *Eimeria* lifecycle, intestinal immune response, and the intricate interaction of parasites with the gut microbiome is required for effective application of new immunotherapeutics including vaccines to the commercial practice. Therefore, this talk will provide the current knowledge on the host immune response to coccidiosis in poultry and discuss the efficacy of various *Eimeria* vaccine candidate antigens in protecting chickens against coccidiosis. Moreover, alternative countermeasures such as hyperimmune antibodies, antimicrobial peptides, phytochemicals and probiotics will be discussed.

Notes:

135 - High volume testing for foreign animal diseases using SmartChip real-time PCR system

Sirisha Parameswaran[1], Luis G. Giménez-Lirola[1], Phillip C. Gauger C. Gauger[1], Rahul K. Nelli[1]

[1]Veterinary Diagnostic Laboratory, Iowa State University. sirisha@iastate.edu

Session: Diagnostic Testing 2, 2024-01-22, 2:00 - 2:15

Objective: During outbreaks of foreign animal diseases (FAD) like African swine fever (ASF), classical swine fever (CSF), and foot and mouth disease (FMD), the priority is to diagnose infections early to prevent their rapid spread. While current 96-well-based real-time polymerase chain reactions (qPCR) are effective at identifying these pathogens, there is a need for a high-throughput qPCR system that can handle the massive volumes of testing required during a FAD outbreak. The SmartChip real-time PCR system (SCRPS) offers a solution with its advanced microfluidic qPCR assays in a 5,184 Nano-well chip that can accurately detect pathogens in nanoliter volumes. This study aims to validate the relative specificity and sensitivity of standard NAHLN-approved 96-well ASF, CSF, and FMD qPCR assays to SCRPS-based qPCR assays.

Methods: A total of 341 samples (276 negative cohort and 57 proficiency panel samples) were tested. Negative cohort samples were part of another NAHLN-funded study in which oral fluid (n=92) and processing fluid (n=184) samples were collected from different farm sites across the United States between April- May 2022 and previously confirmed negative at Iowa State University-Veterinary Diagnostic Laboratory (ISU-VDL). Non-infectious proficiency panels (PT, Reference, or training panels) dated between 2014-2020 were provided by the National Veterinary Services Laboratory - Foreign Animal Disease Diagnostic Laboratory (NVSL-FADDL). Magnetic bead-based nucleic acid extractions were performed using a MagMax kit and KingFisher Apex (ThermoFisher). Samples were handled or dispensed using Bravo Liquid handler (Agilent) and Multi-Sample Nano Dispenser (MSND; Takara) instruments. All RT-qPCR assays were TaqMan-based single plex FAM-labelled reactions specific for ASF, CSF, and FMD, with 4X TaqMan Fast 1-step master mix (ThermoFisher) and similar primer/probe concentrations and modified thermocycling conditions (50° for 5 min, 95°C for 20 sec, 95°C for 10 sec and 60°C for 45 sec for 45 cycles) as described in NVSL protocols. Each triplicate run included samples with Xeno as an exogenous internal control.

Results: The SCRPS qPCR assays provided consistent and reproducible triplicate results, with Ct values ranging from 20.0 to 36.0. As expected, all 276 negative cohort samples tested negative on SCRPS chips, which aligns with the 96-well qPCR assays. The Ct values of 57 non-infectious proficiency panels fell within the expected ranges provided by FADDL. Our findings indicate that the relative sensitivity and specificity are 100% and 99.9%, respectively, between SCRPS and 96-well qPCR assays.

Conclusions: Our research has successfully demonstrated the implementation of the FAD qPCR assay on microfluidic platforms. This first proof-of-concept study will pave the way for innovative opportunities in veterinary molecular diagnostics, including the ability to manage high-volume testing (>30,000 reactions/24 hours) and lower the costs associated with molecular testing at VDLs.

Financial Support: The authors would like to thank the National Animal Health Laboratory Network (NAHLN) for funding this project and the National Veterinary Services Laboratories Foreign Animal Disease Diagnostic Laboratory for providing reagents for this study.

Notes:

136 - Detection of SARS-CoV-2 and other canine and feline reparatory pathogens using multiplex qPCR/RT-qPCR

Come J Thieulent[1], Mariano Carossino[1], Laura Peak[2], Keith Strother[2], Ganwu Li[3], Wendy Wolfson[4], Udeni B R Balasuriya[1]

[1]Department of Pathobiological Sciences & Louisiana Animal Disease Diagnostic Laboratory, School of Veterinary Medicine, Louisiana State University, [2]Louisiana Animal Disease Diagnostic Laboratory, School of Veterinary Medicine, Louisiana State University, [3]Department of Veterinary Diagnostic and Production Animal Medicine, College of Veterinary Medicine, Iowa State University, [4]Department of Veterinary Clinical Sciences, School of Veterinary Medicine, Louisiana State University. cthieulent@lsu.edu

Session: Diagnostic Testing 2, 2024-01-22, 2:15 - 2:30

Objective: Canine infectious respiratory disease complex (CIRDC) and feline upper respiratory tract disease (URTD) are the primary causes of respiratory disease in companion animals and are associated with a wide array of viruses and bacteria acting as either individual etiologic agents or in combination, making etiologic diagnosis challenging. Additionally, SARS-CoV-2 has been reported to infect both dogs and cats. Therefore, the rapid detection and differentiation of SARS-CoV-2 from other common viral and bacterial agents in a single specimen is critical.

Methods: Two panels of one-step TaqMan® multiplex qPCR/RT-qPCR were developed and validated to identify CIRDC and feline URTD-associated agents along with SARS-CoV-2. The canine respiratory panel was designed to detect eight viral (canine adenovirus 2, canine distemper virus, canine herpesvirus 1, canine parainfluenza virus, canine pneumovirus, canine respiratory coronavirus, influenza A virus [H3N2, H3N8 and H1N1] and SARS-CoV-2) and four bacterial pathogens (*Bordetella bronchiseptica*, *Mycoplasma canis*, *M. cynos* and *Streptococcus equi* subsp. *zooepidemicus*). Similarly, the feline respiratory panel was designed to detect four viral (feline calicivirus, feline herpesvirus, Influenza A virus and SARS-CoV-2) and three bacterial agents (*Chlamydia felis*, *B. bronchiseptica* and *M. felis*). The analytical performance of each assay was evaluated using reference strains of each pathogen and plasmid DNA or *in vitro* transcribed RNA containing the target sequences. These panels were then tested on 76 and 63 clinical specimens collected from CIRDC-suspected dogs and URTD-suspected felines, respectively.

Results: All the multiplex assays demonstrated high specificity, analytical sensitivity, efficiency, and linearity. Among the clinical samples derived from dogs, *M. canis*, *M. cynos,* and canine respiratory coronavirus were the most frequently detected. The emerging canine pneumovirus was detected in four samples. Among the clinical samples from cats, *M. felis* was the most common agent detected, followed by feline herpesvirus type-1, *Chlamydia felis* and feline calicivirus. SARS-CoV-2 was detected in four canine and two feline samples. Co-infection was common among the tested specimens, with a rate of 29% and 59% in the canine and feline respiratory samples, respectively.

Conclusions: These two new panels of one-step TaqMan® multiplex qPCR/RT-qPCR are valuable and reliable for rapidly detecting and identifying canine and feline respiratory pathogens, along with SARS-CoV-2. The high frequency of co-infections highlights the need for simultaneous detection of multiple pathogens using such panels to implement proper treatment and/or prevention plans.

Financial Support: This study was funded by the Vet-LIRN COVID-19 Capacity Grant number 1U18FD007514 and supported by the FDA of the U.S. Department of HHS and by the NIH-USDA NIFA R01 Research Grant Program Dual Purpose with Dual Benefit (award number AWD-47990-1).

Notes:

137 - Multi-locus sequence typing of Mycoplasma ovipneumoniae using multiplex PCR and rapid Nanopore sequencing

I. Framst[1], N. Ricker[1], T.E. Besser[2], G. Maboni[3]

[1]Ontario Veterinary College, [2]Washington State University, [3]University of Georgia. iframst@uoguelph.ca

Session: Diagnostic Testing 2, 2024-01-22, 2:30 - 2:45

Objective: *Mycoplasma ovipneumoniae* is responsible for epizootic pneumonia, a factor in the large-scale population decline of Bighorn sheep. Multi-locus sequence typing (MLST) of *M. ovipneumoniae* is an important tool in epidemiologic studies tracking transmission of this bacterium between affected Bighorn populations and domestic sheep. *M. ovipneumoniae* MLST relies on multiple singleplex nested PCRs and Sanger sequencing. The current method is time consuming and sometimes fails to generate MLST sequences. Oxford Nanopore Technology (ONT) sequencing may offer improved reliability and decreased cost; however, ONT Rapid library preparation for multiplex amplicon sequencing has only been explored by one other group. The objective of this study is to develop and validate a workflow for strain typing of *M. ovipneumoniae* using multiplex PCR and Nanopore Rapid sequencing.

Methods: The accuracy and practicality of sequencing of four MLST genes by Sanger, Illumina and ONT were compared. Singleplex PCR products of four genes were sequenced by Sanger and Illumina, whereas a multiplex PCR was developed for ONT sequencing. Multiplex PCR products were sequenced using two different Nanopore library preparation approaches; 1) Rapid barcoding library presentation and 2) Native barcoding library preparation. Each library preparation was sequenced for 16 hours, then the flow cell was washed, and a new library was immediately loaded and sequenced for 16 hours. Reads were processed using a custom bioinformatic pipeline to deconvolute multiplexed amplicons and generate a polished consensus. Both the multiplex PCR and ONT workflow were optimized and validated using 72 clinical samples from bighorn sheep. Samples with less than 50x coverage for one or more loci were discarded and re-sequenced.

Results: Illumina sequencing recovered high quality sequences for three of the four loci. The full length (680 bp) *rpoB* amplicon was not recovered due to an insert size limitation of 550 bp. The optimized multiplex PCR produced four visible bands corresponding with expected size for the target loci. Rapid Nanopore barcoding library preparation recovered all MLST sequences, matching the corresponding Sanger reference sequence. Rapid barcoding also had the shortest total workflow time of 20 hours sample-to-sequence. Washing and reusing flow cells did not affect the quality of the reads, however total yield was halved.

Conclusions: Typing of *M. ovipneumoniae* was successful with multiplex PCR and Nanopore Rapid barcoding library preparation. Furthermore, washing and reusing flow cells reduced the cost per sample without decreasing the accuracy of the method. Although Nanopore does not provide documentation for amplicon sequencing using Rapid library preparation, we demonstrate that this method can produce highly accurate amplicon sequences which are suitable for MLST typing. This new workflow will allow improved typing of *M. ovipneumoniae* clinical samples and increased efficiency in diagnostic settings. To the best of our knowledge, this is the first workflow using Nanopore Rapid sequencing for MLST typing of any *Mycoplasma* species directly from clinical samples, and we speculate that this method could be applied to other MLST schemes for efficient culture-free bacterial typing.

Financial Support: Funding for this project was provided by the Wild Sheep Foundation.

Notes:

138 - *Mycobacterium bovis* infection among cattle herds in MI and NM: Application of novel pathogen specific biomarkers

Joseph R. Darish[1], Rinosh Mani[2], Scott Fitzgerald[2], Srinand Sreevatsan[1]

[1]Department of Pathobiology and Diagnostic Investigation, Michigan State University, [2]Veterinary Diagnostic Laboratory, Michigan State University. darishjo@msu.edu

Session: Diagnostic Testing 2, 2024-01-22, 2:45 - 3:00

Objective: Bovine Tuberculosis (bTB), caused by *Mycobacterium tuberculosis* variant bovis (MBO) is a contagious cattle disease that costs $3 billion in agricultural economic losses worldwide. Current diagnosis involves a caudal fold test (CFT) and confirmed with mycobacterial culture from lesions, if identified at necropsy. However, a CFT test lacks specificity and can generate false positive results, while confirmatory bacterial isolation tests are slow, delaying time to diagnosis and therefore mitigation of bTB in cattle herds. We hypothesized that the use of 3 validated MBO pathogen specific biomarkers, by Lamont et al (2014), will increase the sensitivity and specificity of bTB testing.

Methods: Whole-blood samples were collected from Michigan (n=119), New Mexico (n=41) and from a control herd (n=17). Subsequently the plasma was extracted for use in an indirect ELISA. Three pathogen specific peptides, validated for bovine TB diagnostics, were evaluated by an indirect ELISA. The three peptides are: Pks5 (cell wall biosynthesis), Mb2515c (LuxR transcription regulator), and Mb1895c (molybdenum binding protein).

Results: Of the 177 samples assayed 40 samples were CFT positive and of these four were confirmed by a *Mycobacterium bovis* culture of the lesions. Twenty one of the 177 samples were positive for Mb2515c, 17 by Mb1895c and 22 with Pks5. Fourteen of these samples had reacted with all three biomarkers and three out of four of the mycobacterial culture confirmed samples also tested positive on the biomarkers.

Conclusions: The biomarkers are exclusively present when *Mycobacterium bovis* is replicating and dividing in the host, unlike the currently applied diagnostics that depends on the recall of host immune responses to *M. bovis* and offers a promising avenue for unambiguous detection of bTB. This will also help eliminate false positives that arise due to exposures to environmental mycobacteria or to related pathogens like *Mycobacterium avium* subsp. *Paratuberculosis;* as well as cattle that have cleared the infection but continue to recall an immune response to TB antigens.

Financial Support: MDARD Funding Support: MDARD-AA-21-144; M-AAA Funding Support; Michigan State University for Biosafety Level 3 Access.

Notes:

139 - A novel, non-invasive, portable method for measuring airway resistance in horses

Christopher Simotas[1], Melissa Mazan[2], Thomas Coons[1], Kristin Wendell[1], Jill Minuto[2], Daniela Bedenice[2], Edward Deveney[3], Brian Barrows[1]

[1]Tufts University School of Engineering, [2]Tufts University School of Veterinary Medicine, [3]Bridgewater State University, Department of Physic and Photonics Engineering. melissa.mazan@tufts.edu

Session: Diagnostic Testing 1, 2024-01-21, 10:30 - 10:45

Objective: Equine asthma (EA) is second only to musculoskeletal injury as a cause of wastage in the equine industry, causing poor performance through impairment of oxygen exchange. EA is characterized by increases in respiratory resistance through tissue remodeling and bronchospasm, thus the ability to measure resistance as a diagnostic tool and to monitor response to treatment is of critical importance to management of this disease. Methods that can be used both in the field and in the specialty hospital for measurement of respiratory resistance are not currently available on the market, thus there is a critical need for development of such a tool. This study investigates respiratory resistance in horses measured with the interrupter technique (EquiRint/Rint), a portable test, in comparison with forced oscillatory mechanics (FOM/RRS) and esophageal balloon-pneumotachography (EBP/RL).

Methods: The Equi-Rint employs the method of rapid interruption of airflow to allow equilibration of alveolar and mask pressure along with pre-interruption measurement of flow to allow derivation of resistance (P/V'). A portable pneumatics-driven shutter and low-deadspace mask along with pneumotach, pressure transducers, and dedicated software comprise the essential components of the EquiRint. Short-term and diurnal measurements were made to determine reproducibility of Rint in normal horses. 12 EA horses were randomly assigned to EquiRint or FOM (RRS) for measurement of baseline and airway hyperresponsiveness (AHR) defined as 75% increase in R with <= 6 mg/ml of histamine. 8 normal horses were randomly assigned to baseline measurements with EquiRint or EB-P. Within-test variability for EquiRint was assessed by coefficient of variation (COV). Related samples T-test was used to compare baseline values and Chi-square analysis to compare diagnoses of airway hyperresponsiveness between tests.

Results: Rint in normal horses was 0.38 $\pm$ 0.08 cmH2O/l/s; short-term reproducibility was satisfactory, with COV of 5% within a 60-minute period, and 7% within a 24-hour period. Rint was higher than RRS in horses with moderate asthma (0.56 $\pm$ 0.08 cmH2O/l/s v. 0.49 $\pm$ 0.07) and in normal horses, baseline Rint was higher than RL (0.38 $\pm$ 0.08 cmH2O/l/s v. 0.29 $\pm$ 0.07); the differences were not significant. There was no difference in diagnosis of airway hyperresponsiveness (AHR) measured by EquiRint v. FOM.

Conclusions: EquiRint is portable, well-tolerated, and comparable to current methods for measurement of baseline and perturbed respiratory resistance in horses.

Financial Support: Boehringer Ingelheim Advancement in Equine Research Award.

Notes:

140 - Evaluation of new antigens for ELISA as an alternative method for the serodiagnosis of bovine leptospirosis

Camila Hamond[1], Karen LeCount[1], Tammy Anderson[1], Brenda R. Morningstar-Shaw[1], Linda K. Schlater[1], Elsio A. Wunder Jr[2]

[1]National Veterinary Services Laboratories, APHIS, U.S. Department of Agriculture, [2]Department of Pathobiology and Veterinary Science, College of Agriculture, Health and Natural Resources, University of Connecticut. camila.hamond@usda.gov

Objective: Bovine leptospirosis can cause abortions, stillbirths, reduce milk yield, and decreased daily weight gain, with a major economic impact. The serologic diagnosis of leptospirosis has relied on the microscopic agglutination test (MAT). However, this technique is complex, highly trained staff, required live cultures, and it cannot differentiate vaccinated from infected animals. Chronic infection can be detected in urine by isolation of the pathogen or PCR. *Leptospira* is a fastidious organism and shedding of pathogenic leptospires in urine can be intermittent, decreasing the sensitivity of those methods. Currently, there is no effective diagnostic assay for animal leptospirosis with high sensitivity and specificity. For that reason, ELISA have been developed as an alternative method to detect leptospiral antibodies using a single serum dilution. The aim of this study was to evaluate four highly conserved leptospiral proteins, previously identified as potential virulence factors and protective antigens, as an alternative method for the serodiagnosis of bovine leptospirosis.

Methods: The study was divided into two steps. Reference rabbit sera were tested first to verify if the four recombinant proteins, expressed based on the genome of *L. interrogans* serovar Copenhageni, were conserved enough to be identified by sera from representative strains of 13 different *Leptospira* species. In the second step, we tested a total of 77 bovine serum samples grouped as confirmed and non-confirmed cases, categorized into 5 subgroups: 1) MAT and *lipL32* qPCR positive (n=16); 2) MAT positive with titer ≥ 400 and *lipL32* qPCR negative (n=19); 3) MAT negative with *lipL32* qPCR positive (n=2); 4) MAT positive with titer < 400 and *lipL32* qPCR negative (n=11); and 5) MAT negative and *lipL32* qPCR negative (n=29). All experiments were repeated twice. Cut-off was determined as the mean optical density (OD) plus three standard deviations (SDs) of MAT and qPCR negative samples (group 5).

Results: An ELISA with each protein was performed. Neither well-to-well nor plate-to-plate variation exceeded 10%, confirming assay repeatability. All thirty-eight rabbit sera tested recognized the four proteins evaluated, indicating the ability of those proteins to induce a broad and conserved antibody response. For the bovine sera, our results showed that all cattle samples with qPCR positive have a high level of antibodies against all four proteins, including animals with negative serology but qPCR positive. Our results showed that all protein candidates were able to differentiate qPCR positive and high MAT titers with qPCR negative from group with low MAT titers that could be derived from vaccination.

Conclusions: These results demonstrate that animals with confirmed leptospiral infection based on MAT and/or qPCR induced a strong humoral immune response against all four proteins evaluated, indicating their expression during infection and a potential role in bovine leptospiral pathogenesis. Furthermore, our results showed that with ideal protein candidates, it is feasible to develop a broad serological assay that can identify chronically infected animals.

Notes:

141 - The role of animal migration in spreading environmentally transmitted parasites

Vanessa Ezenwa[1], Jason Donaldson[2], Houssein Kimaro[3], Basil Senso[4], Aiden Trentinus[4], T. Michael Anderson[5], Eileen Devaney[3], Gareth Hempson[3], J. Grant Hopcraft[3], Jennifer McIntyre[3], Thomas Morrison[3], Ricardo Holdo[2]

[1]Yale University, [2]University of Georgia, [3]University of Glasgow, [4]Tanzanian Centre for Research Cooperation, [5]Wake Forest University. vanessa.ezenwa@yale.edu

Session: One Health / Public Health 2, 2024-01-22, 2:00 - 2:15

Objective: Every year, billions of migratory animals traverse large distances, encountering a diverse suite of non-migratory (i.e., resident) species along their routes. Although animal migrations have been linked to the dissemination of several high-profile parasites and pathogens to resident species (e.g., Avian influenza virus, *Brucella abortus*, *Toxoplasma gondii*), we lack a systematic understanding of when during the migratory cycle cross-species transmission risk is greatest, or which resident species are most vulnerable to infection. Environmentally transmitted parasites and pathogens pose a particularly challenging problem because their ability to persist in the environment often decouples the presence of migrant hosts from the timing of actual peak cross-species transmission risk. To better understand how migratory species influence the dynamics of environmentally transmitted diseases in residents, we are using the classic Serengeti wildebeest migration as a model system to investigate cross-species transmission of fecal-oral transmitted gastrointestinal nematodes. Specific objectives were to: (i) quantify the effect of wildebeest presence and environmental factors on nematode abundance in the environment, and (ii) evaluate which of four different resident species (buffalo, grant's gazelle, hartebeest, topi) are most vulnerable to increased parasitism in response to migration.

Methods: Focusing on a set of permanent study sites in central Serengeti which vary in the degree to which they are used by migratory wildebeest as stopover sites, we quantified nematode egg burdens in resident herbivore species monthly over a 2-year period. We also simultaneously tracked changes in wildebeest presence and fecal inputs, environmental variables (e.g. soil temperature, relative humidity), and nematode larval abundance in pasture.

Results: Our results suggest that during migration events, a combination of wildebeest density and environmental factors drive variation in nematode larval abundance in the environment. We also find that resident species vary considerably in the degree to which they are vulnerable to migration-associated changes in nematode abundance.

Conclusions: Our study is helping to assemble a more complex picture of the conditions under which migration poses the greatest risks for cross-species disease transmission.

Financial Support: U.S. Department of Agriculture, National Institute for Food and Agriculture UK Biotechnology and Biological Sciences Research Council Grant.

Notes:

142 - Prevalence and genomic characterization of beta-lactamase-producing E. coli in migratory geese in West Texas, USA

Yamima Tasnim[1], Md. Kaisar Rahman[1], Babafela Awosile[1], Cherissa Abdul-Hamid[2]

[1]Texas Tech University School of Veterinary Medicine, [2]Zoonosis Control Program, Texas Department of State Health Services. ytasnim@ttu.edu

Session: One Health / Public Health 2, 2024-01-22, 2:15 - 2:30

Objective: Migratory geese are important birds that frequent recreational parks. They can serve as reservoirs of important zoonotic pathogens and antimicrobial-resistant bacteria that are of public health concern. Such one health interface at the recreational parks or community centers can create a perfect environment that favors transmitting important antimicrobial-resistant bacteria. The objective of this study was to determine the prevalence and the genomic characteristics of *beta-lactamase-producing Escherichia coli* isolated from the feces of migratory geese at one health interface in West Texas.

Methods: A cross-sectional study was conducted in 22 recreational parks in West Texas. For this study, we collected geese' fecal (n=165), water from the lakes (n=118), and soil (n=74) around the lakes within the recreational parks. We used Chromogenic agar to isolate extended-spectrum beta-lactamase (ESBL) producing *E. coli*. The whole genome sequencing method was used to determine the genomic characteristics of selected *E. coli* isolates.

Results: From 357 samples, 12.61% (95%CI: 9.34-16.50) were positive for ESBL-*E. coli*. From the whole genome sequencing of 29 isolates, 16 isolates harbored at least 1 beta-lactamase gene (*bla*$_{\text{CTX-M-1}}$, *bla*$_{\text{CTX-M-65}}$, *bla*$_{\text{CTX-M-27}}$, *bla*$_{\text{CTX-M-55}}$, *bla*$_{\text{CTX-M-32}}$, *bla*$_{\text{TEM-1A}}$, *bla*$_{\text{TEM-1B}}$) with 9 isolates from fecal, 2 isolates from soil, and 5 isolates from water samples. Most of the isolates carried genes conferring resistance to tetracyclines (*tet(A)*, *tet(B)*), aminoglycosides (*aac (3)-IIa, aph(6)-Id, aadA1, aph(3')-Ia, aadA1*), sulfonamides (*sul1, sul2*), amphenicol(*floR*), trimethoprim (*dfrA1, dfrA14, dfrA17*) and streptogramin B (MLSB) agent (*mph(A)*). 18 isolates showed chromosomal mutations in the promoter region G of the ampC beta-lactamase gene. We also detected sixteen incompatibility plasmid groups, with *IncF* being the most common. A total of 60 types of virulence genes were identified, including *fimH, csgA, hlyE, intA, fdeC, papC, intA, chuA, toxB, espA, pic,* and *kpsM*. These virulence genes are related to adherence, exotoxin, invasion, nutrition/metabolic factor, and effective delivery system respectively. *astA* virulence gene was identified from 1 isolation of feces, 2 isolates of soil, and 1 isolate of water samples which can cause severe gastrointestinal disease in animals. It is possible that the ill birds carried *Escherichia spp.* that harbored virulence-related genes, as these genes relate to disease-causing *E. coli* strains in people.

Conclusions: This study demonstrates the potential of migratory geese at recreational parks as reservoirs of resistant bacteria and fecal contamination of an environment by geese can create a microbial storm. Based on our findings, the migratory geese population may act as sentinels and sources of AMR contamination and potentially pathogenic *E. coli* for humans, and some may differ in their carriage of certain sequence types, resistance genes, virulence genes, and plasmids according to their environment. The detection of a multidrug-resistant *E. coli* strain resistant reinforces the importance of adequate hygiene practices for humans and pet animals after coming back from the recreational park.

Financial Support: Texas Tech University School of Veterinary Medicine, Amarillo TX, USA; Zoonosis Control Program, Texas Department of State Health Services, Lubbock, TX USA.

Notes:

143 - Genetic variation of fecal *E. coli* from shelter dogs, raccoons, and opossums by fimH sequence typing

Harshul Singh[1], Nicoletta Conti[2], Pratap Kafle[2], Reta Abdi[2]

[1]Department of Biological and Chemical Sciences, Theobald Science Center, New York Institute of Technology, [2]Department of Biomedical Sciences, College of Veterinary Medicine, Long Island University. reta.abdi@liu.edu

Session: One Health / Public Health 2, 2024-01-22, 2:30 - 2:45

Objective: Commensal and pathogenic *E. coli* adhere to host species and various tissues. This is accomplished by its FimH protein (adhesin) that is located at the tip of the fimbriae. FimH protein has two regions, namely, N-terminus part that binds to biotic/abiotic surfaces and C-terminus part (anchor FimH to fimbriae). Mutation in one or both domains increases *E. coli* virulence and helps to colonize new niche; hence, FimH sequence varies from strain-to-strain. Dog ownership, wildlife trade, and their health issues by *E. coli* are rising. However, genetic variability of FimH in *E. coli* of dogs and wildlife has not been studied. This study evaluated the genetic variability and phylogenetics of fecal *E. coli* (n=34 isolates) from dogs (n=26), raccoons (n=5), and opossums (n=3) in Long Island and Connecticut using FimH sequence typing method.

Methods: We extracted the DNA of *E. coli,* amplified their FimH gene by PCR and subsequently sequenced them, trimmed and edited the sequences by UGENE, performed multiple sequence alignment and phylogenetic tree by MEGA-X, and refined the shape of the phylogenetic tree by Evolview, We also translated the FimH DNA sequences to peptide sequences by Expasy and MEGA-X then determined the amino acid (AA) composition and their % by peptide 2.0 and Pepinfo. We determined the chemistry of FimH protein such as its acidic, basic, and hydrophobic positions by Pepstats, peptide 2.0, and STRAP,

Results: We obtained 728 bp FimH DNA fragments for all of 34 *E. coli* isolates after trimming and editing by UGENE. In these DNA fragments, we noticed nucleotide variations at 84 positions, having a total sum of 362 nucleotide differences. Of 362 total nucleotide mutations, transition mutation (64.4%) dominated transversion mutation (33.7%) and deletion (1.9%). The 34 isolates had 29 nodes (ancestors) phylogenetically, indicating the isolates were not each other's closest relatives. Translation of the 728 bp FimH sequences yielded a peptide length of 242 AAs for each isolate. All the 20 AAs participated in the FimH peptide formation, but the share of valine was higher (12%), 9.9% glycine, 9.9% serine, and 9.9% threonine, among others. The physicochemical property of the consensus FimH protein fragment was 47.9% neutral, 42.2% hydrophobic, 5.4% acidic, and 4.6% basic, which has implication for its downstream use for vaccine, adjuvant, or diagnostic. Of the 84 naturally mutated nucleotide positions, 33 positions (39.3%) led to a change in AA, which happened in 14 (41.2%) of 34 isolates. Of 33 positions, AA mutations occurred in the pilin domain (63.3% positions) than in the lectin-binding domain (36.4%). The 14 isolates replaced mainly their hydrophobic (e.g. Ala and Val) with neutral/charged residues (Asn, Arg, and Glu).

Conclusions: The 34 *E. coli* isolates from shelter dogs, raccoons, and opossums varied in nucleotides genetically (at 84 positions), phylogenetically (29 nodes/ancestors), and in proteomics (variable AAs at 33 positions). Dogs and wildlife carry distantly related *E. coli.* Overall, most *E. coli* from dogs, raccoons, and opossums vary in FimH, suggesting FimH's use as a diversity marker. The biological consequences of the FimH mutations need further study.

Financial Support: Long Island University financed this study.

Notes:

144 - Seroprevalence of *Toxoplasma gondii* in goats and associated risk factors in Northern California

Hibah Abuhamdieh[1], Meera C. Heller[2], Alda Pires[2], Rosie C. Busch[2], Karen Shapiro[3], Maia Laabs[4], Andrea E. Packham[5]

[1]Livestock Herd Health & Reproduction Resident, University of California, Davis, [2]Department of Population Health and Reproduction, School of Veterinary Medicine, University of California, Davis, [3]Department of Pathology, Microbiology & Immunology, University of California, Davis, [4]Swinging Udders Veterinary Services, [5]One Health Institute, University of California, Davis. habuhamdieh@ucdavis.edu

Session: One Health / Public Health 2, 2024-01-22, 2:45 - 3:00

ObjectiveS: *Toxoplasma gondii* (*T. gondii*) is a protozoal disease that is of global importance due to its zoonotic potential. Cats, the definitive host, shed infected oocysts in their feces. People are infected via ingestion of cat feces, ingestion of tissue cysts in undercooked meat of intermediate hosts such as sheep and goats, or exposure to fluids or tissues from aborting small ruminants. Small ruminant farming is a growing industry in the U.S, and goat meat is commonly sold directly to consumers or produced for home consumption. The goal of this study was to estimate the seroprevalence of *T. gondii* in goats in Northern California, and to identify risk factors associated with seroprevalence in goats. A secondary objective was to determine test agreement between a commercial ELISA test kit, and indirect fluorescent antibody test (IFAT).

Methods: A cross-sectional study was conducted, 649 goats from 29 farms throughout Northern California were enrolled, with adjunct surveys conducted at each farm. ELISA and IFAT were used to estimate the seroprevalence of *T. gondii* in those samples. A kappa agreement test was conducted to compare the ELISA results with the IFAT results at varying dilutions of the IFAT (1:160, 1:320, 1:320+). A mixed effects logistic regression was conducted to determine risk factors.

Results: A kappa agreement of 0.29 was found between the ELISA and IFAT using a cut-point of 1:320+. A kappa of 0.15 was found between the ELISA and IFAT using a cut-off dilution of 1:320, and finally a kappa of 0.025 was found between the ELISA and IFAT using a cut-off dilution of 1:160. The 1:320 IFAT results were used to evaluate farm-level risk factors. Goats used for land clearing, goats with access to pasture, and the presence of feral cats or dogs on farm increased the odds of goats being seropositive for *T. gondii*. Feeding kids pasteurized milk, and storing goat feed under a tarp, decreased these odds.

Conclusions: There was lack of agreement between the ELISA test kit and IFAT tests; . Therefore, it is undetermined which test accurately reported the true seroprevalence of *T. gondii* in goats. The IFAT was used to evaluate risk factors in the current study. The survey results identified recommendations and educational opportunities that can be made at a farm level, based on known routes of transmission, to reduce *T. gondii* transmission from goats to people, such as recommendations for cooking meat, storing goat feed and management of feral cats.

Financial Support: Thank you to the Foundation for Food & Agriculture Research (FFAR) & Center for Food Animal Health (CFAH) for helping fund this project.

Notes:

145 - Cluster data analysis in epidemiologic studies: Application of mixed effect model to predict pandemic COVID-19 outbreaks in areas with inadequate healthcare services

Golo Dabassa[1], Demelash Areda[2]

[1]Yabello Regional Veterinary Laboratory, Oromia Bureau of Agriculture, Ethiopia, [2]Ottawa University. demelash.biffa@gmail.com

Session: One Health / Public Health 2, 2024-01-22, 3:00 - 3:15

ObjectiveS: Mixed-effect models have emerged as a powerful tool in epidemiologic studies, particularly when dealing with clustered data. These models allow researchers to account for both fixed and random effects, acknowledging the hierarchical structure of data in clustered settings. This study aimed to apply advanced mixed-effect models tailored to the unique characteristics of clustered COVID19 data in regions with limited healthcare services.

Methods: This was part of the Ethiopian Ministry of Health's surveillance effort to investigate the spread of COVID19 in Southern Ethiopia, specifically the Borana region, where healthcare facilities are limited. COVID19 testing was initiated at the outset of the pandemic outbreak and conducted using molecular tests (PCR). A total of 10,154 individuals underwent COVID19 testing. To analyze the distribution of COVID19 test positivity, a mixed-effect logistic regression model was employed. This model was executed in R using the "glmer" function from the "lme4" package. The month of the year was considered a random effect variable to account for any correlation or clustering of test positivity within the same month. The fitness of the final model was assessed by comparing it to a null model using goodness-of-fit statistics. The Chi-Square statistic was employed to compare the goodness of fit between the null model and the final model.

Results: Model goodness of fit test indicated that the final model provides a significantly better fit to the data than the null model. Also, AIC and BIC values were lower for the final model indicating its superior fit compared to the null model. The odds of testing positive for COVID19 were higher (OR=1.041 [95% CI=1.001-1.352] among female individuals than male. There was a significant variation in COVID19 test positivity between age group. For every one-year increase in age, the odds of being positive for COVID19 increased by odds of 1.006610[95% CI=1.00- 1.2]. The odds of testing positive for COVID19 were higher among individuals in bus station (OR=5.38, 95%CI= 1.21-23.91] compared to being in school. New patients were less likely to test positive compared to follow-up cases (OR=0.38; 95%CI=0.202-0.75]. The odds of getting positive test results were twice times higher (OR=2.25; 95%CI=1.46- 3.47] among individuals with a history of contact with COVID19 patients than being positive test in routine community surveillance. The odds of testing positive is higher (OR=1.37, 95%CI= 1.01-1.84] if individual samples are tested by technicians with higher qualification compared to professionals with several years of experience.

Conclusions: This study contributes valuable insights into the dynamics of COVID19 in resource-constrained settings and underscores the importance of tailored epidemiological modeling to inform evidence-based public health responses.

Notes:

146 - The effects of hygiene standards pre- and post- COVID shutdown on fever and diarrhea incidence in a daycare

Jeremiah Cox[1], Matt Salerno[1], Jenessa Winston[1], Jiyoung Lee[1], Kelly Baker[2], Rebecca Garabed[1]

[1]Ohio State University, [2]University of Iowa. cox.1276@buckeyemail.osu.edu

Session: One Health / Public Health 2, 2024-01-22, 3:15 – 3:30

Objective: The COVID-19 pandemic acted as a natural experiment, providing the opportunity to analyze patterns of fever and diarrhea incidence throughout the year within a childcare program (CCP) before and after the government-mandated shutdown. Upon reopening, a university-run CCP implemented enhanced hygiene requirements such as masking, screening for fevers, and increased handwashing, allowing a unique opportunity to study the effect of enhanced measures against pre-pandemic standards.

Methods: Data were collected from a university-run CCP by searching email communications for disease exposure notices and phrases related to symptoms of interest. Emails were collected during two separate investigations from January 2018 to May 2021, with illnesses separated into pre- and post-shutdown periods delineated by the government-mandated shutdown occurring from April through May 2020. Multivariable zero-inflated Poisson models were separately used with fever and diarrhea to estimate incidence risk ratios for Poisson models and odds ratios for logistic models. The standard Poisson model evaluated the probability of fever or diarrhea as an outcome while controlling for how many infants were present on a given day, a binary variable for pre- and post-shutdown periods, an increasing number of days after the shutdown had ended, and which room the infants were in. The zero-inflated logistic model looked at seasons as an independent variable for the probability of an outbreak.

Results: Data collection found 109 instances of fever and 64 instances of diarrhea across the study period. Following implementation of new safety protocols, daily attendance decreased by 55.6% in the period following the shutdown. There was no significant difference found in incidence rates when compared between the different rooms at the CCP. The zero-inflated Poisson found limited evidence for seasonality in fever and diarrhea incidence rates, with spring having the highest rates of fever and diarrhea, followed by summer. However, there was significant evidence to suggest that as attendance increases there is a decreased risk of fever but an increased risk of diarrhea. The models also indicated lower fever and diarrhea incidence before the COVID-19 shutdown with an increase in fever incidence as time after shutdown increased.

Conclusions: The reduced fever incidence with increasing attendance that was found is likely due to increased screening by the facility. The study suggests evidence of a relationship between rising attendance and increasing diarrhea events and begins to quantify the impact of hygiene and screening on disease transmission in infants.

Financial Support: A special thank you to Ohio State University's Infectious Disease Institute.

Notes:

147 - Isolation and characterization of bovine coronavirus strains from dairy cows, dairy calves, and beef cattle

Yu Li[1], Mingde Liu[1], Jiayu Xu[1], Chohee Koo[1], Francesca Granberry[2], Samantha R. Locke[2], Roberto A. Palomares[2], Gregory Habing[1], Linda Saif[1], Leyi Wang[3], Qiuhong Wang[1]

[1]The Ohio State University, [2]University of Georgia, [3]University of Illinois. li.13226@osu.edu

Session: Virology 3, 2024-01-22, 2:00 - 2:15

Objective: Bovine coronaviruses (BCoV) are prevalent worldwide and cause enteric or/and respiratory diseases in cattle. However, the mechanisms determining the major infection site is unknown. Until now, it has been around half a century since the first BCoV strain was discovered. There are still big knowledge gaps for BCoV pathogenesis. In this study, we isolated BCoVs in HRT-18 cells. Our objective is to compare these new strains with historical stains to study the evolution of BCoVs during past two decades, focusing on mutations in the receptor-binding protein spike (S) and hemagglutinin esterase (HE), and the genetic differences in the S protein between the viruses originated from respiratory and enteric samples.

Methods: A total of 166 samples from dairy cows, dairy calves, and beef cattle were collected from Ohio and Georgia during the past three years. We have screened the samples using TaqMan real-time reverse transcription-PCR (RT-qPCR) assay based on the BCoV conserved M gene. Five BCoVs were isolated in human rectal tumour-18 (HRT-18) cells and BCoV replication in cells was confirmed by cytopathic effects (CPEs), electron microscopy (EM), and immunofluorescence assay (IFA) methods. The BCoV isolate's growth kinetics were performed at 9 time points (1 h post-inoculation (hpi), 12hpi, 24hpi, 36hpi, 48hpi, 60hpi, 72hpi, 84hpi, and 96hpi) and compared with an historical BCoV strain DBA. The genomes of currently circulating BCoV strains from bovine respiratory (n=1/10) and enteric samples (n=2/156) were sequenced using NGS and sanger sequencing combined method. Sequence alignments and phylogenetic analysis were performed using Clustal Omega and MEGA, respectively.

Results: The positive rate of BCoV in these farms were 14/89 (15.73%) of dairy calves, 1/39 (2.56%) of beef cattle, and 0/38 (0%) of dairy cows. We successfully isolated 5 BCoV strains in HRT-18 cells. The growth kinetics showed that the BCoV isolate BC7 reached the peak titer of 6 $\log_{10}$ plaque forming unit (PFU)/mL at 48 hpi. According to the phylogenetic tree, the BCoV isolates were clustered in GIIb group with the North American historical strains DB2. The new isolates shared 98% nucleotide identity with the prototype Mebus strain, with mutations alone the genome including HE, S, E, M, and N genes. We also compared the spike (S) proteins of one nasal sample and one fecal sample from the same farm. There was only one amino acid difference in the S protein (617T for the respiratory and 617I for the enteric sample).

Conclusions: During the past two decades, BCoVs have evolved with mutations in HE, S, E, M, and N genes. BCoV can cause respiratory and/or enteric disease, which is a good coronavirus model to study the mechanisms for CoVs to switch tropism between respiratory and enteric tissues. In the future, we will build an infectious clone to study the potential viral factors and utilize CRISPER-Cas9 to screen for host genes to explore the viral and host factors contributing to the tropism changes.

Financial Support: Salaries and research support were provided towards to Dr. Wang by state and federal funds appropriated to College of Food, Agricultural and Environmental Sciences (CFAES), The Ohio State University.

Notes:

148 - Host microRNAs finetunes Bovine coronavirus virus (BCoV) tissue tropism, pathogenesis, and immune regulation

Maged Hemida[1]

[1]Long Island Univeristy. maged.hemida@liu.edu

Session: Virology 3, 2024-01-22, 2:15 - 2:30

Objective: Bovine coronavirus (BCoV) is endemic in the UAS, which has a single genotype and causes pneumotropic infection in cattle. The mechanism of this dual tropism is still unclear. This hampered the development of novel diagnostic markers, assays, and vaccines against BCoV. MicroRNAs (miRNAs) are important gene regulators. Our hypothesis is host miRNAs play key roles in fine-tuning tissue tropism, pathogenesis, and immune regulation of many viruses. (1) To identify some miRNAs as markers for BCoV, (2) to study the roles of selected miRNAs in tissue tropism and pathogenesis of BCoV, and (3) to explore the roles of miRNAs in BCoV immune regulation/evasion.

Methods: We used two BCoV isolates (Ent/Resp) to infect two bovine cell lines (bovine endothelial cells and bovine nasal turbinate cells). We extracted the total RNAs from the BCoV-infected and sham non-infected cells and then subjected them to the next-generation sequencing (NGS) technology. We obtained miRNA and mRNA expression profiles for each group of cells. Confirmation of the miRNA and mRNA expression profiles of some selected miRNAs and mRNAs of certain key genes by using some specific miRNA and gene-specific oligonucleotides by the quantitative real-time PCR assay. Data and statistical analysis were carried out by comparing the results from each group.

Results: Seven miRNA candidates could be potential markers for BCoV infection. miRNA-1 is a specific marker for BCoV-Respiratory infection (upregulated in cells infected with the BCoV-Resp isolate (27.9-fold)). Three miRNAs (22-3p, 19a, and 22855a) were upregulated in the BCoV-Enteric-infected cells compared to the sham and the BCoV-Respiratory-infected cells and are therefore considered potential markers for BCoV-Enteric infection. Three miRNAs (497, 2887, and 15a/b) could act as general markers for BCoV infection because they are upregulated in cells infected with both isolates—miRNA-181 cluster target BCoV genome at three genetic locations (ORF1a, S2, and N). The ACE2 expression level is markedly downregulated in cells infected with either Enteric or Respiratory isolates of BCoV. miRNA-181 is upregulated and targets 3'UTR of the ACE2 gene (receptors for SARS-CoV-2). miRNA-16a is upregulated in cells infected with BCoV-Respiratory isolate. It has two target sites in the BCoV- S gene and other targets in the cleavage site of the bovine furin gene (downregulated in BCoV-infected cells). Also, miRNA-1, miRNA-22-3p, miRNA-15a/b, and miRNA-2887 target immune regulatory genes (MAPK1/CD164, IL6R, SOCS2/7, and TLR9) respectively.

Conclusions: miRNAs are key players in fine-tuning BCoV infection pathogenesis and could be useful genetic markers for BCoV infection. BCoV-based miRNA vaccines could be a novel approach to control BCoV infection in cattle herds.

Notes:

149 - Administration of Nrf2 agonists reduce bovine RSV replication in vitro and ameliorates RSV disease severity in mice

Fabian E Diaz[1], Macy K Moore[1], Jodi L McGill[1]

[1]Iowa State University. fediaz1@iastate.edu

Session: Virology 3, 2024-01-22, 2:30 - 2:45

Objective: Bovine respiratory disease (BRD) remains a leading cause of morbidity and mortality in the cattle industry, with bovine Respiratory syncytial Virus (bRSV) being a major etiological agent. Innovative interventions to reduce bRSV and BRD burden worldwide are required due to the increasing impact of antimicrobial resistance on animal and human health. Clinical and rodent studies suggest that the Nrf2 pathway protects against RSV infection by orchestrating several antioxidant mechanisms. In addition, Nrf2 agonists 4-octyl-itaconate (4-OI) and dimethyl fumarate (DMF) have recently shown antiviral and anti-inflammatory activity against SARS-CoV2 and Influenza A virus in vitro and in vivo. We have previously shown that 4-OI and DMF reduce inflammatory transcripts in response to bRSV and human RSV (hRSV) in respiratory epithelial cells, highlighting the potential of this pathway as a therapeutic target. Here, we evaluated whether Nrf2 agonists impact RSV replication in vitro and reduce RSV severity in a rodent model.

Methods: To evaluate if Nrf2 agonists impact RSV infectious titers, bovine turbinate (BT) cells and human lung epithelial cells (BEAS-2b) were pre-treated with either 4-OI (100, 200 μM) or DMF (50, 100 μM) for six hours, then infected at MOI 0.1 with bRSV (BTs) or hRSV (BEAS-2b), respectively. Infectious titers (TCID50/mL) were quantified in BT or HEp-2 cells after 48h. To test the efficacy of NRF2 agonists in preventing RSV disease, mice were treated i.n. with 4-OI (400 ug/mice) or DMF (80 ug/mice), or saline (vehicle) daily before infection and until necropsy, then infected with 50uL hRSV strain A/1997 (10^8 $TCID_{50}$/mL). Lungs were collected at 3dpi to quantify viral loads (as N-hRSV/β-actin copies), cytokines, and to evaluate histopathological changes.

Results: DMF at 100 and 4-OI at 200 μM reduced bovine and human RSV infectious titers ($p<0.01$) in BT and BEAS-2b cells, respectively. In mice, DMF at 80 and 4-OI at 400 ug reduced weight loss ($p=0.032$ and 0.0066, respectively) and lung viral loads ($p=0.04$ and 0.026, respectively). Both drugs also significantly reduced CCL5 ($p<0.0001$), KC ($p<0.05$ & <0.01), IFN-β transcripts ($p<0.05$ and $=0.05$), and IL-6 secretion ($p<0.05$ & <0.01) in the lungs. Preliminary pathology analyses indicate that treatments also reduced infiltration by lymphoid and myeloid cells. We are currently increasing the sample size for pathology analyses and looking into the mechanisms of action of these agonists.

Conclusions: Our results indicate that DMF and 4-OI display antiviral activity against RSV in vitro and in vivo and reduce the severity of RSV disease in a murine model when used daily by reducing inflammatory responses and viral replication. In summary, the anti-inflammatory and antiviral activity of Nrf2 agonists observed here support further investigation on their efficacy against bRSV and BRD in small and large ruminant species.

Financial Support: USDA Capacity: Animal Health and Disease Research Program

Notes:

150 - Bovine herpesvirus 1 replication and gene expression is activated by Sp1 and glucocorticoid receptor

Clinton Jones[1], Fouad El-Mayet[1]

[1]Oklahoma State University. clint.jones10@okstate.edu

Session: Virology 3, 2024-01-22, 2:45 - 3:00

Objective: Bovine herpesvirus 1 (BoHV-1) is a significant viral pathogen that causes respiratory tract disorders and suppresses immune responses in cattle, which can culminate in life-threatening bacterial pneumonia. Following acute infection, BoHV-1 establishes lifelong latency in sensory neurons in trigeminal ganglia (TG) and pharyngeal tonsils. Calves latently infected with BoHV-1 consistently reactivate from latency after receiving a single intravenous injection of the synthetic corticosteroid dexamethasone, which mimics the effect of stress. A major focus in my laboratory is to define the mechanism by which stress triggers bovine herpesvirus 1 (BoHV-1) replication and reactivation from latency. The objective of this study was to examine the effect that two cellular transcription factors, specificity protein 1 (Sp1) and glucocorticoid receptor (GR), had on BoHV-1 replication and gene expression.

Methods: Infection of bovine cells with BoHV-1 and analyzing the effect of a cellular transcription factor Sp1 silencing RNAs (siRNAs) was used to test whether Sp1 regulates viral replication. Mithramycin, an inhibitor of Sp1, was also tested for its ability to regulate BoHV-1 replication. Western blot studies were performed to examine the effects that viral replication had on Sp1 protein levels and to test whether glucocorticoid receptor (GR) and Sp1 interact. Transient transfection studies were also performed to examine the effect GR and Sp1 had on the immediate early transcription unit 1 (IEtu1) promoter. The IEtu1 promoter drives expression of two key viral transcriptional regulators, infected cell protein 0 (bICP0) and bICP4. Finally, co-immunoprecipitation studies tested whether GR and Sp1 form a stable complex.

Results: These studies revealed the cellular transcription factor Sp1 is a stress induced transcription factor in TG neurons and pharyngeal tonsil during early stages of reactivation from latency. Silencing Sp1 protein expression with siRNA or treatment with Mithramycin significantly reduced BoHV-1 replication in cultured cells. BoHV-1 infection of permissive cells increased Sp1 steady-state protein levels, and Sp1 was primarily localized to the nucleus when compared to uninfected cells. Transient transfection studies revealed GR and Sp1 cooperatively transactivated the BoHV-1-IEtu1 promoter. However, mutating the two GR response elements (GREs) in the IEtu1 promoter ablated GR and Sp1 mediated transactivation.

Conclusions: These studies revealed a stable complex comprised of GR and Sp1 cooperatively transactivated the IEtu1 promoter and stimulated productive infection. We predict interactions between Sp1 and GR activate viral gene expression and replication during stress-induced BoHV-1 reactivation from latency. Additional studies designed to test whether Sp1 also activates other BoHV-1 promoters are in progress.

Financial Support: This research was supported by grants from the USDA-NIFA 2018-06668 and 2021-67015, National Institute of Neurological Disorders and Stroke of the National Institutes of Health under Award Number R01NS111167, and funds from the Sitlington Endowment.

Notes:

151 - Chromatin modifications of the Bovine Alphaherpesvirus 1 genome during reactivation from latency

Jeffery B. Ostler[1], Clinton Jones[1]

[1]Veterinary Pathobiology, College of Veterinary Medicine, Oklahoma State University. jostler@okstate.edu

Session: Virology 3, 2024-01-22, 3:00 - 3:15

Objective: Bovine Alphaherpesvirus 1 (BoHV-1) is a major cattle pathogen that contributes significantly to Bovine Respiratory Disease Complex (BRDC), which results in multibillion-dollar losses annually in the US. Controlling BoHV-1 infection is complicated by the persistent lifecycle of virus infection. Following acute infection of the ocular, respiratory, or reproductive tissues, the virus establishes a lifelong latent infection in the sensory ganglia marked by periodic reactivation leading to recurrent acute disease. Periods of physiological or environmental stress increase the rate of reactivation and enable spread to susceptible individuals. Our goal is to dissect the mechanisms by which stress drives BoHV-1 reactivation from latency in order to better design therapeutics and vaccines to ultimately reduce the disease burden in cattle. This project specifically will identify host transcription factors that associate with the virus genome during the early stages of reactivation and characterize changes in virus chromatinization. Of particular interest is the Glucocorticoid Receptor (GR), a key stress-induced transcription factor that we have shown activates several key Immediate Early genes critical to establishing BoHV-1 productive infection. Critically, GR is activated by the synthetic corticosteroid dexamethasone (DEX), which reliably induces reactivation in latently infected calves.

Methods: Naïve calves were infected with BoHV-1 and allowed to establish latency. To induce reactivation, calves were injected with DEX by I.V., and again injected with DEX 2 and 4 days later by I.M., at which point the calves were euthanized and Trigeminal Ganglia (TG) harvested. As a control, TG from latently infected animals were also harvested. Chromatin Immunoprecipitation (ChIP) was performed using the harvested TG to identify transcription factors and histone modifications associated with the virus chromatin.

Results: We found that following DEX treatment, the GR associated with the BoHV-1 genome significantly relative to latently infected animals. Furthermore, during latency, H3K9me^3, a repressive chromatin marking, but not H3K9acetyl, an active chromatin marking, was found associated with the BoHV-1 genome. Following DEX treatment there was no change to H3K9me^3, but we detected a significant increase in H3K9acetyl markings associated with the BoHV-1 genome.

Conclusions: During DEX-induced reactivation from latency, GR associates with the BoHV-1 genome. GR is a pioneer transcription factor, capable of interacting with heterochromatin to induce gene expression. At the same time as GR associates with the virus genome, the chromatin is remodeled from primarily repressive markings (H3K9me^3), to include active chromatin markings (H3K9acetyl). Notably, H3K9me^3 markings are still detected on virus chromatin during reactivation. This is expected, as not all latent genomes experience reactivation together. Furthermore, our experiments were not able to distinguish whether both marks were found on the same individual genome, but rather a profile of the population of virus in a single TG.

Financial Support: This project was supported by Agriculture and Food Research Initiative Competitive Grant no. 2023-67015-39738 from the USDA National Institute of Food and Agriculture.

Notes:

152 - Evaluating temperature effects on bluetongue virus serotype 10 and 17 coinfection in *Culicoides sonorensis*

Molly Carpenter[1], Jennifer H. Kopanke[2], Case Rodgers[1], Justin S. Lee[3], Barbara Graham[1], Tyler Sherman[1], Kirsten Reed[1], Mark Stenglein[1], Christie E. Mayo[1]

[1]Colorado State Universtiy, [2]Oregon Health & Science University, [3]Centers for Disease Control and Prevention. molly.carpenter@colostate.edu

Session: Virology 3, 2024-01-22, 3:15 - 3:30

Objective: Bluetongue virus (BTV) is a segmented, double-stranded RNA virus transmitted by *Culicoides* biting midges. Infection of domestic and wild ruminants with BTV can result in devastating disease and significant economic losses. In concert with climate change, BTV outbreaks have been characterized by an expanding geographical range and incursions of novel serotypes into endemic regions. While factors driving BTV's expansion are poorly understood, reassortment between virus strains may enhance BTV's ability to spread to new regions. However, an understanding of the effect of temperature on reassortment is lacking. The objectives of this study include comparing how ambient temperatures of 20°C, 25°C, or 30°C affected *Culicoides* survival, virogenesis, and potential reassortment in *Culicoides sonorensis* coinfected with BTV serotype 10 and 17.

Methods: To establish single-virus and coinfections, midges were fed a blood meal containing ~10^5 TCID50/ml of BTV-10, BTV-17, or BTV-10+17. Midges were maintained at 20°C, 25°C, or 30°C. Midge survival was assessed by daily counts of survivors from each temperature and infection group in dedicated survival cartons of 50 midges and performed in duplicate. Pools of midges (n = 5) were collected every other day for pan BTV and COX1 (housekeeping gene) qRT-PCR. A curve was fit to the DCt values (pan BTV Ct - COX Ct) for each group and linear portions evaluated by pairwise comparisons with *P* values adjusted with Tukey's method. Pools of coinfected midges (n=10) collected on days 3, 7, 11, 15, and 19 were processed for BTV plaque-isolation. The complete genotypes of isolated plaques were determined using next-generation sequencing.

Results: Midges maintained at 30°C and 25°C produced plaques earlier (day 3) than midges held at 20°C (day 7). However, midges maintained at 20°C had the longest survival time, followed by midges held at 25°C, and then 30°C. Most plaques from coinfected midges had genotypes that aligned with BTV-17. However, a pool of midges held at 20°C produced mixed genotypes plaques at day 19 post infection and a pool of midges held at 30°C had most plaques align with BTV-10 at day 7 post infection.

Conclusions: Warmer temperatures may promote earlier virogenesis; however, there is a tradeoff with decreased survival of the midge. Overall, plaques with BTV-17 genotype dominated, but BTV-10 RNA was detected in some of the plaques suggesting that parental strain fitness and super infection exclusion may play a role in successful reassortment events. Bluetongue virus reassortment patterns and their biological consequences will add an important dimension to the modeling of viral expansion and evolution in the context of climate change.

Financial Support: Funding was provided by USDA-NIFA AFRI GRANT Number 2019-67015-28982 as part of the joint USDA-NSF-NIH_BBSRC-BSF Ecology and Evolution of Infectious Diseases Program, NIH/NCATS Colorado CTSA Grant Number TL1 TR002533TL1, NIH Ruth L. Kirschestein National Research Service Award Training Program T32.

Notes:

153 - Molecular epidemiology of antimicrobial resistant pathogens in distinct broiler farms in Southeastern US

Jessica Parzygnat[1], Lyndy Harden[1], Dawn Hull[2], Muhammed Muyyarikkandy[1], Sid Thakur[1]

[1]North Carolina State University College of Veterinary Medicine, Department of Population Health and Pathobiology, [2]Walter Reed Army Institute of Research. jlparzyg@ncsu.edu

Session: Epidemiology 3, 2024-01-22, 2:00 - 2:15

Objective: Broiler chicken is the top consumed meat in the United States and can be produced in both commercial and backyard farm environments. Chickens are a known reservoir for antimicrobial resistant pathogens even in an antibiotic-free farm environment. Backyard poultry has greatly increased in popularity in the United States, yet there is a lack of research regarding the prevalence of these pathogens and their antimicrobial resistant profiles in backyard broiler settings. This issue is heightened by the CDC's reported *Salmonella* outbreaks linked to backyard chickens occurring yearly since 2019 that has made thousands of people sick. This study aims to determine differences in prevalence and phenotypic antimicrobial resistance profiles of high concern pathogens (*Salmonella, Campylobacter,* and Extended-spectrum beta-lactamase *E. coli*) in backyard and commercial broiler farms. In addition, our goal is to promote mindful practices for producer and consumer safety. Long term, this study aims to lay groundwork for assessing how differences in distinct broiler farm environments and management practices may affect persistence of antimicrobial resistant pathogens.

Methods: This study encompasses a longitudinal systems-based approach to better understand the difference between the two farm types across the production time frame. Ten backyard and ten commercial farms were visited at three time points across production. At each visit 26 samples were collected (fecal (n=10), soil (n=5), litter/compost (n=5), feeder/waterer swab (n=6)) and processed in the lab for *Salmonella, Campylobacter,* and ESBL *E. coli.* Phenotypic resistance of *Salmonella, Campylobacter,* and ESBL *E. coli* isolates was conducted through the broth microdilution method. The minimum inhibitory concentration was determined and NARMS/CLSI breakpoints were applied to determine susceptible, intermediate, dose-dependent sensitive, and resistant isolates.

Results: Overall, *Salmonella* was prevalent among 19.10% and 52.18% of backyard and commercial farms, respectively. *Campylobacter* was found in 22.05% of backyard samples and 12.18% of commercial samples. ESBL *E. coli* was found in 12.95% backyard and 0.77% commercial farm samples. For commercial *Salmonella,* 33% of isolates were resistant to nalidixic acid and intermediate to ciprofloxacin, which are two antimicrobials that are considered first-line in treating *Salmonella* infections. Backyard farms saw a lower percentage with a little less than 1% of isolates resistant to nalidixic acid and intermediate to ciprofloxacin. Ciprofloxacin is also known for being an antimicrobial used for treating *Campylobacter* infections, in which we found 25.7% of backyard isolates and 63.2% of commercial isolates were resistant. ESBL *E. coli* results revealed a proportion of susceptible-dose dependent (Backyard: 48.5%; Commercial: 100%) and resistant (Backyard: 16.8%) isolates to 4th generation cephalosporin, cefepime.

Conclusions: The results of this study indicate a need for further research to ensure production and food safety especially considering increased popularity of backyard production and the impact broiler meat has in the United States. Understanding this is important from a One Health perspective, given the results of this study have implications for human, animal, and environmental health alike. Future steps include evaluating whole genome sequences from the isolates to determine genotypic resistance profiles.

Financial Support: U.S. Department of Agriculture, National Institute of Food and Agriculture, Sustainable Agricultural Systems Grant (USDA NIFA SAS Grant).

Notes:

154 - Prediction of piglet throughput after live virus exposure in breeding herds undergoing PRRS elimination

Eliana S. Paladino[1], Alyssa Betlach[2], Maria Pieters[3], Cesar Corzo[3], Aaron Rendahl[1], Randall Singer[1]

[1]Department of Veterinary and Biomedical Sciences, College of Veterinary Medicine, University of Minnesota, [2]Swine Vet Center, [3]Department of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota. epaladin@umn.edu

Session: Epidemiology 3, 2024-01-22, 2:15 - 2:30

Objective: Swine production systems across the USA commonly implement different measures to control or eliminate the PRRS virus. Elimination through herd closure and whole herd exposure utilizing the recently introduced live resident virus to expose all sows is one option. The primary goal of a live virus inoculation (LVI) is to consistently wean virus-free pigs in the shortest time possible post-break. However, concerns regarding the production impact of this practice remain. The objective of this study was to quantify production losses and predict the number of weaned pigs following the implementation of this strategy.

Methods: Twelve farrow-to-wean farms belonging to one production company were enrolled in the study. Farms experienced PRRS outbreaks between 2021-2022 and implemented a load-close-expose approach via LVI. Production records from enrolled herds were obtained and a linear mixed model was fitted to explore the role of covariates on the average number of weaned piglets, as a function of gestation week, after exposing pregnant sows to the protocol. Predictors included were: i) previous PRRS herd status, ii) interval (time between outbreak and LVI), iii) herd size, iv) parity, and v) interactions with pregnancy week at LVI. Estimated marginal means were calculated and reported alongside 95% confidence intervals.

Results: Records from 28,331 pregnant sows exposed to LVI were analyzed. Statistically significant predictors included PRRS status, parity, and interactions of both interval and parity with pregnancy week. Overall, the average number of weaned piglets was higher in females of lower parity orders, the ones exposed early in gestation, and also in farms with previous exposure to PRRS. Sows at 16-weeks gestation at the moment of LVI exposure weaned on average 5.45 piglets (95%CI:4.01,6.90), whereas those at the first week weaned 11.62 (95%CI:11,3,11.94). Sows at 10-weeks, weaned 6.82 (95%CI:5.45,8.19).

Conclusions: LVI has been demonstrated as an effective strategy to aid virus elimination sooner than other interventions, although its implementation may initially increase losses in both late-term gestation and older females, resulting in reduced productivity. Results from the current study are in agreement with the literature in that the most severe losses were observed in late gestation sows at the time of LVI. This can be attributed to the immunocompetence development of the fetus at 70 days of gestation, which increases the susceptibility to congenital PRRS. In the short term, farms with longer intervals between outbreak and LVI presented higher averages of weaned piglets. Moreover, our results suggest that previous herd immunity plays a role in mitigating losses. For the next steps, the long-term effect of LVI on productivity will be assessed, as a function of gestation period stratified by the aforementioned predictors of interest.

Financial Support: College of Veterinary Medicine of the University of Minnesota.

Notes:

155 - Microbiomes and resistomes of poultry farms indicate potential reservoirs and transmission of antimicrobial resistance

Alinne L. R. Santana-Pereira[1], Pankaj Gaokar[1], Kenneth Macklin[2], Laura Huber[1]

[1]Dept. Pathobiology, CMV - Auburn University, [2]Dept. Poultry Science, Mississippi State University. alp0051@auburn.edu

Session: Epidemiology 3, 2024-01-22, 2:30 - 2:45

Objective: The rise of antimicrobial resistance (AMR) is now considered one of the major threats to human and animal health. The main contributing factor is the overuse of antibiotics in animal-food production, which accounts for most of the antimicrobial use (AMU). Poultry production is an important source of AMR since it represents 50% of the meat consumed globally and relies on high intensification, increasing AMU. The effects of AMU in the microbiome and potential AMR transmission within and across farms, and potentially to food products, are key to implementing appropriate biosafety practices. This study reconstructed the microbiomes from litter, soil, and environmental fecal samples from poultry farms in southeastern US to compare community structures, AMR load and potential transmission/colonization events within and across farms.

Methods: Pullet, breeder and broiler farms from the same company were sampled. Litter, soil and feces, both surrounding the poultry houses, were collected for each one of the farms. Samples were shotgun sequenced in triplicates. For each sample the microbial community was reconstructed via read mapping to reference markers. 16S gene was used for broad community reconstruction and MetaPhLAn4 was used to generate species level classifications. Then, the reads for each sample were assembled into contigs. ARGs were mined from each sample to create a profile of endogenous ARGs. Then, reads were mapped to the ARGs to estimate ARG abundance within the community generating an ARG profile. Profiles were then compared within and across farms.

Results: The farm microbiomes were composed of 20 phyla and 1349 species. In all farms, across all sample types (litter, feces, and soil) the microbiomes were dominated by the phyla Firmicutes, Actinobacteria, Proteobacteria and Bacteroidetes, albeit with distinct patterns for each farm type and sample type. Fecal samples were too heterogeneous, as expected, and cannot be directly compared to the other representatives. Broiler farms had the highest homogeneity between samples, while being the least diverse. They were dominated by Firmicutes (70%), especially families Staphylococcaceae and Lactobacillaceae. The former is enriched in the litter samples of all farms compared to soil, while both increase in abundance in the broiler farm. Pullet litter is also distinct, being dominated by actinobacteria and firmicutes (>90%). The soil samples of both Pullet and Breeder farms are the most diverse samples, having unique profiles. ARG mining revealed resistance to 22 different antibiotic types. Most genes were related to macrolide and tetracycline resistance. Moreover, when corrected for microbiome size, litter samples carried a higher number of ARGs than soil, despite soil harboring endogenous ARGs.

Conclusions: There are significant differences between the microbiomes of farm types, however some microorganisms enriched in pullet litter are carried across the chain and can be vectors of resistance. There are indications of heavy colonization between litter and soil in broiler farms which is conducive to ARG transmission. All microbiomes are enriched in ARGs associated with antibiotics commonly used in poultry farms. The litter microbiome is the most enriched in ARGs and a potential source of ARG load, introducing AMR outside the poultry houses.

Notes:

156 - Genomic analysis of the emerging pathogen *Helcococcus ovis* reveals new species: *Candidatus Helcococcus bovis*

Federico Cunha[1], Kristi Jones[1], Segundo Casaro[1], Rafael Bisinotto[1], Mary Brown[1], Subhashinie Kariyawasam[1], Yuting Zhai[1], Kwangcheol Jeong[1], Klibs Galvao[1]

[1]University of Florida. fcunha@ufl.edu

Session: Epidemiology 3, 2024-01-22, 2:45 - 3:00

Objective: *Helcococcus ovis* (*H. ovis*) is an emerging bacterial pathogen that causes opportunistic respiratory, mammary, and uterine infections across mammalian hosts. Comparative genomic analyses have identified a cryptic clade of *H. ovis* and suggested the potential for a novel strain or species within the genus *Helcococcus*. The objective of this study was to explore the prevalence of cryptic *Helcococcus sp.* strains in the uterus of dairy cows, and conduct a comparative genomic analysis to establish a taxonomic classification for *Helcococcus* sp. isolates.

Methods: Uterine swabs were collected from healthy lactating Holstein cows with and without metritis in North Central Florida. Swab contents were cultivated on *Helcococcus* elective agar (HEA) and selected colonies were speciated via 16S rRNA gene sequence analysis. Whole genome sequencing was carried out on 31 isolates using both Illumina short-read sequencing and Oxford Nanopore long-read sequencing. ProgressiveMauve was used to align and visualize genomes. Phylogenetic and average nucleotide identity (ANI) analyses were carried out with the BV-BRC codon tree pipeline and the JSpecies web server. Putative virulence factors (VFs) were identified using BLASTP against the Virulence Factor Database (VFDB). EggNOG v5.0 and InterProScan were used to predict protein function and classify protein domains. Biochemical and antimicrobial susceptibility testing of 7 strains was carried out on the VITEK2 platform.

Results: Phylogenetic and ANI analyses revealed 4 isolates (KG38, KG95, KG105, and KG167) form a distinct clade within the *Helcococcus* genus which has an average nucleotide identity of 87.6% when compared to *H. ovis* strains. Although these isolates' colony morphology is indistinguishable from that of *H. ovis,* they grow more slowly on HEA (96 vs. 48 hours). The Vitek 2 system only produced low discrimination and unknown organism results and was not able to differentiate between strains of separate clades based on their biochemical profile. Antimicrobial resistance to tetracyclines was high among tested isolates across clades, which is explained by the high prevalence of *tetA*, *tetB*, *tetT,* and *tetM* genes across strains. Strains within this newly described clade lack four putative VFs that are typically found in medium and high-virulence *H. ovis* isolates. These VFs are associated with functions such as adherence, immune evasion, and cell wall synthesis. Within this clade, KG38 was previously shown to have attenuated virulence when compared to *H. ovis* isolates and to contain a terminal nonsense sequence variation in the *znuC* coding sequence (286G>T) changing a glutamic acid to a stop codon (E97X). This sequence variation, that leaves the zinT-associated ZnuABC operon in KG38 without a functional cytoplasmic ATPase component, is not shared by the rest of the strains within the new clade.

Conclusions: Together these results demonstrate that KG38, KG95, KG105, and KG167 represent a novel bacterial species for which we propose the name "*Candidatus Helcococcus bovis*" sp. nov. Genomic analyses suggest zinc sequestration capacity is a virulence determinant for these isolates. The development of new rapid biochemical testing methods is warranted for the rapid identification of both *H. ovis* and "*Candidatus Helcococcus bovis"* sp. nov. in clinical settings.

Financial Support: This project was supported by the USDA-NIFA-CRIS (Accession No. 1002880), USDA-NIFA-AFRI (Accession No. 1026802) and CVM Research Competitive Award (Grant FLA-VME- 00131662).

Notes:

157 - (-)-Epigallocatechin-3-gallate does not affect *Lawsonia intracellularis* infection of McCoy cells or swine enteroids

Caitlin Klaeui[1], Yvonne Peng[1], Connie Gebhart[2], Milena Saqui-Salces[1]

[1]Department of Animal Science, University of Minnesota, [2]College of Veterinary Medicine, University of Minnesota. klaeu003@umn.edu

Session: Epidemiology 3, 2024-01-22, 3:00 - 3:15

Objective: *Lawsonia intracellularis* is an obligate intracellular bacterium causing proliferative enteropathy (PE) in pigs. PE causes diarrhea and weight loss and control primarily relies on antibiotic treatments. Green tea extracts exhibit antimicrobial properties against bacteria including *Pseudomonas aeruginosa, Staphylococcus aureus,* and *Escherichia coli*. These properties are attributed partly to (-)-epigallocatechin-3-gallate (EGCG), a catechin in green tea extracts known to contribute to beneficial health effects of green tea. The objective of this *in vitro* study was to evaluate EGCG safety on intestinal epithelial cells and its potential application in inhibiting *L. intracellularis* infection.

Methods: To evaluate EGCG safety, enteroids, swine ileum-derived organoid structures (mini-guts) were seeded into 96-well plates. Enteroids were treated with 0, 100, or 500 nM EGCG in Human IntestiCult Organoid Growth Medium (n = 3 wells / treatment / time) and incubated at 37°C in 5% CO_2. Media (without EGCG) was replaced every 48 hours. A CellTiter-Glo 3D viability assay was conducted at 3, 5, 7, 10, and 14 days post treatment. Luminescence was analyzed by two-way ANOVA and Tukey's test. EGCG did not affect enteroid viability ($P = 0.882$). To evaluate EGCG effects on *L. intracellularis* infection of enteroids, 16-well chamber slides were seeded with enteroids. *L. intracellularis* (PHE MN-100) was cultured in mouse fibroblast (McCoy) cells and suspended in organoid media. Enteroids were infected with media containing 10^7 organisms / mL or uninfected. Slides were incubated at 37°C in a microaerophilic environment produced with 80% nitrogen, 10% hydrogen, and 10% CO_2 meeting *L. intracellularis* culture requirements. Three days post-infection (dpi), organoid media containing 0, 100, or 500 nM EGCG was added to wells (n = 3 wells / treatment / time). Media was collected 48 hours post-infection and replaced without EGCG every 48 hours. Slides were formalin-fixed and immunostained for *L. intracellularis* at 5- and 7-dpi. Bacteria in media were quantified by qPCR. Differences in organisms / mL among treatments were evaluated by two-way ANOVA and Tukey's test.

Results: *L. intracellularis* organisms / mL were not different except at 3-dpi when media from enteroids treated with 500 nM EGCG contained lesser organisms / mL ($P = 0.021$) than those untreated. To evaluate cytotoxic effects of EGCG on *L. intracellularis* in infected McCoy cells, 10^7 organisms / mL was suspended in Dulbecco's Modified Eagle's Medium with 7% fetal bovine serum and incubated two hours with EGCG exceeding 1 µM / mL or media alone. Media was added to McCoy cells in 16-well chamber slides, incubated at 37°C in a microaerophilic environment, and collected and replaced with media containing EGCG exceeding 1 µM / mL or untreated media at 1-dpi and untreated media at 3-dpi. Formalin-fixed slides were immunostained for *L. intracellularis* at 3-dpi. EGCG treated McCoy cell viability was reduced 50% by 3-dpi while untreated McCoy remained viable, regardless of infection. The ratio of organisms in media to inoculum was lesser in treated cells than untreated at 3-dpi ($P = 0.0348$) but not 1-dpi ($P = 0.773$).

Conclusions: At the dosages described, EGCG was toxic for McCoy cells but not for enteroids. EGCG treatment did not reduce or eliminate *L. intracellularis in vitro*.

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

Notes:

158 - An Experimental Field Trial Investigating the Use of Bacteriophage and Manure Slurry Applications in Beef Cattle Feedlot Pens for *Salmonella* Mitigation

Colette A. Nickodem[1], Ashley N. Arnold[2], Matthew R. Beck[3], K. Jack Bush[4], Kerri B. Gehring[5], Jason J. Gill[5,6], Tram Le[5,6], Jarret A. Proctor[5], John T. Richeson[7], H. Morgan Scott[8], Jason K. Smith[9], T. Matthew Taylor[5], Javier Vinasco[8], Keri N. Norman[1]

[1]Department of Veterinary Integrative Biosciences, Texas A&M University, [2]Texas A&M Veterinary Diagnostic Laboratory, Texas A&M University, [3]United States Department of Agriculture-Agriculture Research Service, [4]Texas A&M AgriLife Research, Texas A&M University System, [5]Department of Animal Science, Texas A&M University, [6]Center for Phage Technology, Texas A&M University, [7]Department of Agricultural Sciences, West Texas A&M University, [8]Department of Veterinary Pathobiology, Texas A&M University, [9]Texas A&M AgriLife Extension, Department of Animal Science, Texas A&M University. cnickodem@wisc.edu

Session: Epidemiology 3, 2024-01-22, 3:15 - 3:30

Objectives: Most post-harvest interventions that address *Salmonella* in beef products are applied directly to the carcass surface and are ineffective at reducing *Salmonella* that are harbored in cattle lymph nodes. Bacteriophages are viruses that are ubiquitous in the environment, including agricultural facilities, that only infect and destroy bacterial cells. Phage treatments have been used for several post-harvest and pre-harvest pathogen mitigation applications in the agricultural industry. The objectives for this experiment were to determine whether natural bacteriophage populations or laboratory-curated bacteriophage cocktails could reduce *Salmonella* prevalence in the feedlot pen environment and subsequently on cattle hides and in cattle lymph nodes. Lymph nodes may become incorporated into ground beef products, which can contribute to beef-related foodborne illness, and therefore, these treatments have the potential to address food safety concerns.

Methods: A 2 x 2, unbalanced experiment was conducted to determine the effectiveness of pre-harvest treatments applied to the pen surface for *Salmonella* mitigation in cattle. Treatments included a manure slurry intended to mimic pen run-off (n=4 pens), a bacteriophage cocktail (n=4), a combination of both treatments (n=5), and a control group (n=5) that received no treatment. Environment samples for 18 feedlot pens and fecal grabs, hide swabs, and subiliac lymph nodes from 178 cattle were collected across 4 consecutive weeks. All samples were selectively enriched for *Salmonella* and isolates were sequenced to identified serovars and antimicrobial resistant genes. A multilevel mixed effects logistic regression model was used to evaluate the impact of treatment on *Salmonella* prevalence by sample type. Predictive margins for *Salmonella* prevalence using a 3-way full factorial term were generated to create margins plots for each sample type across the study period. A multiple correspondence analysis (MCA) was used to assess associations between treatment, day, and sample type for *Salmonella* serovars.

Results: After the initiation of treatment, the average *Salmonella* prevalence in feces, brisket, and rump samples was lower in all treatment groups compared to the control group. According to the multilevel mixed effects logistic regression model, the combination treatment was most effective at reducing *Salmonella*, and the prevalence was significantly lower compared with the control group for rump swabs on Days 14 and 21. The treatment impact on *Salmonella* prevalence in the lymph nodes could not be determined due to low prevalence. The post-treatment serovar composition was similar for the phage cocktail treatment group and control group, with Montevideo (44.3%, 31/70; 68.6% 107/156) and Virginia (22.9%, 16/70; 22.8%, 38/167) most frequently identified. However, the MCA revealed that the combination treatment group had a distinctly different composition mainly consisting of serovar Muenster (43.2%, 32/74).

Conclusions: The *Salmonella* reduction on cattle hides suggests that bacteriophage or water treatments applied to the feedlot pen surface may reduce *Salmonella* populations on cattle during the pre-harvest period, resulting in reduced contamination during slaughter and processing. Both bacteriophage treatments are safe and easy to use, making them promising *Salmonella* mitigation techniques that could be implemented in the pre-harvest setting. Future studies testing the increased frequency and duration of phage applications will help with optimization of treatment parameters.

Notes:

159 - Feeding choices owners make: early findings from the Dog Aging Project

Janice O'Brien[1, 2], Audrey Ruple[1, 2], Katie Tolbert[2, 3]

[1]Virginia-Maryland College of Veterinary Medicine, [2]Dog Aging Project, [3]Texas A&M School of Veterinary Medicine. janiceobrien@vt.edu

Session: General Health & Physiology 2, 2024-01-22, 2:00 - 2:15

Objective: Nutrition research in dogs so far has focused on colony dogs maintained in a protected environment, and on small cohorts of dogs that are patients of a veterinary specialist (e.g. nutritionist, internal medicine). The Dog Aging Project cohort of over 45,000 dogs in the United States presents a unique opportunity to study nutrition in pets at the population level. These early findings report the first two years of Dog Aging Project nutrition survey results.

Methods: 32,405 owner-reported surveys collected through the Dog Aging Project cohort study were analyzed using descriptive methods for the relevant nutrition questions, as the survey also covers lifestyle and environmental exposures. The relative percentages of primary and secondary diet components in the cohort, as well as some qualitative research on the survey questions and how to improve the survey questions to be clearer for all pet owners.

Results: Commercially prepared kibble is the most common primary component of food at 81% of the total population. The next most common foods are canned food (4%), home cooked (4%), and refrigerated/frozen raw (4%). Organic diets were reported at 19%, while grain free diets were reported at 39%. When owners changed diets, the most common component was kibble (62%), while the next most common food types changed to were home cooked (12%), canned (7%), and refrigerated/frozen raw (7%). The relative proportions of non-kibble foods were larger in the change group. Reasons that owners changed foods were reported as: brand change (26%), stopping grain-free (17%), health condition-related (16%), allergy (11%), and life stage change (11%).

Conclusions: Our findings regarding the dominance of kibble as the primary food choice are consistent with previous surveys. The reasons that owners change foods is interesting, especially the relative proportion of owners switching away from grain free diets. Given the large proportion of the cohort that is reportedly consuming a grain-free diet and the FDA-reported risks of cardiac disease as linked to grain-free diets in dogs, these findings are simultaneously encouraging and worrying. A large percentage of owners feed or previously fed grain-free diets, but 17% of owners that switched diets were doing so as a result of the FDA-announcements regarding those diets, which shows that government agency announcements are perceived as trustworthy by pet owners.

Notes:

160 - What is normal? Establishing a baseline of gut health throughout the gastrointestinal tract of feedlot cattle

J. Daniel Young[1], Lee J. Pinnell[2], Cory Wolfe[2], Matthew A. Scott[2], Ty E. Lawrence[1], Joao P. Cavasin[3], John T. Richeson[1], Paul S. Morley[2]

[1]Department of Agricultural Sciences, West Texas A&M University, [2]VERO Program, Texas A&M University, [3]College of Veterinary Medicine and Biomedical Sciences, Texas A&M University. jdyoung3@buffs.wtamu.edu

Session: General Health & Physiology 2, 2024-01-22, 2:15 - 2:30

Objective: The gut barrier is a complex environment comprised of microbes, microbial metabolites, host-generated bioactive molecules, epithelial cells, and the lamina propria containing immune cells, blood vessels, and lymphatic networks. A wealth of literature in humans and murine disease models has associated changes in gut barrier function with a wide variety of health issues. Despite the high prevalence of diseases incredibly similar to those associated with gut barrier dysfunction in humans, there is limited research characterizing the gut barrier in the gastrointestinal tract (GIT) of feedlot cattle. The objective of this study was to characterize the composition of gut barrier features (e.g., microbiome, epithelial structures, tight-junction proteins, and gene expression for tight-junction proteins and inflammatory cytokines) in feedlot cattle.

Methods: Twenty-one steers from 21 feedlot locations throughout the Texas Panhandle region were harvested at the meat science laboratory at West Texas A&M University. Cattle were representative of populations within the region (live weight = 647 kg ± 45.92, marbling score = 513 ± 110, yield grade 3.61 ± 0.73). Fecal samples were collected from each animal, as were tissue samples, luminal contents, and epithelial tissue from the rumen, jejunum, and colon immediately post-evisceration. Tissue samples were fixed and prepared for histology to evaluate tissue morphology and immunohistochemistry (IHC) to evaluate protein expression for tight-junction proteins (Claudin 1, Claudin 2, Occludin, E-cadherin, ZO-1). Additionally, RT-qPCR for mRNA was used to quantify the gene expression of these same tight-junction proteins. The diversity and composition of luminal and mucosal microbial communities were characterized using 16S rRNA gene sequencing.

Results: This study represents one of the broadest investigations of gut barrier composition in feedlot steers. Small intestinal fluid was less rich and diverse ($P < 0.001$) compared to the other samples. Despite different origins and environmental influences, the ordination of microbial communities measured by NMDS was not different ($P \geq 0.05$) by animal but community structures were different ($P < 0.001$) across sample site. The relative abundance in the rumen was less ($P < 0.001$) for Firmicutes but greater ($P < 0.001$) for Bacteroidetes compared to the small and large intestine. However, the families composing these phyla were also different across sample site. Additionally, the small intestine had the greatest ($P < 0.001$) relative abundance of Actinobacteria compared to the other sections of the GIT. Overall, the between animal variation observed in microbial community structure and tight-junction gene expression illustrates the complexity of the GIT.

Conclusions: This is one of the first studies to use a broad array of molecular techniques (i.e., 16S rRNA gene sequencing, histology, IHC, mRNA, qPCR) for assessing components of the gut barrier in feedlot cattle. Ordination of the microbiome did not differ between animals; however, there were large differences in the microbial community structure between locations within the GIT. These data provide a valuable contribution to future research exploring a new paradigm for factors affecting health and disease in cattle by providing a baseline of normal barrier function to compare interventions and serve as a non-diseased comparison.

Financial Support: Texas A&M University

Notes:

161 - Effects of intranasal Zn and vitamin A treatments on respiratory disease challenged beef steers

Emma Rients[1], Carlos Franco[1], Stephanie Hansen[1], Jodi McGill[1]

[1]Iowa State University. erients@iastate.edu

Session: General Health & Physiology 2, 2024-01-22, 2:30 - 2:45

Objective: One major cost of bovine respiratory disease (BRD) is loss of production during illness. Finding strategies to increase resiliency to disease can help increase production during recovery. Zinc is a critical trace mineral involved in many processes of the immune system, including innate and adaptive cell signaling (Wessels et al., 2017). Vitamin A (VA) also plays many roles in the immune system, including key roles in mucosal immunity (Huang et al., 2018). This study investigated the effects of intranasal (IN) Zn and VA treatments on BRD challenged beef steers.

Methods: Angus crossbred steers (n = 48; 333 ± 4.2 kg), split into two groups (n = 24) were used in a 14 d BRD challenge study. On d -1, steers were trucked for 6 hours. Steers were challenged on d 0 via aerosol inoculation with 10^4 $TCID_{50}$ BRSV followed by $5x10^8$ CFU of *Mannheimia haemolytica* on d 5. On d 4, steers received IN treatments of Zn (ZnO nanoparticles), VA, Zn and VA (VA+Zn), or control with no IN treatment. For trace mineral and VA analysis, liver biopsies were collected on d -9 and 20 and plasma was collected on d 0, 4 (trace mineral only), 5, 7, and 14. RNA was isolated from bronchoalveolar lavage (BAL) cells (d 0, 7) for gene expression related to inflammation, VA and trace mineral metabolism. Statistics were analyzed using the mixed procedure of SAS 9.4 with fixed effects of treatment and group. Contrast statements were utilized to determine Zn and VA effects on gene expression.

Results: Intranasal treatments did not affect viral shedding throughout the study ($P \geq 0.59$). There were no Group x TRT x day interactions for plasma trace minerals and VA concentrations ($P \geq 0.18$). IN VA prevented the inflection-induced decline in plasma VA noted on d 5, after which all treatments were similar (TRT × Day $P < 0.01$). Within day there were few differences in plasma Fe concentrations between treatments except for d 14 where VA+Zn had increased plasma Fe concentrations compared to all other treatments (TRT × Day $P = 0.04$). There were no TRT × day interactions for plasma Zn and Cu (TRT × Day $P \geq 0.50$). After infection, liver VA concentrations were decreased in ZN compared to VA with CON and VA+ZN not different from VA or ZN ($P = 0.05$). ZN tended to have lesser expression of IL-10 in BAL cells on d 7 ($P = 0.07$). There were no other effects of Zn or VA for the genes analyzed in this study ($P \geq 0.12$).

Conclusions: Disease progression and IN Zn and VA treatments can affect the plasma concentrations of trace minerals and VA. Further investigation into the effects of Zn and VA IN treatments may be warranted to increase resiliency to disease challenge.

Financial Support: This project was supported by Agriculture and Food Research Initiative Competitive Grant no. 2020-06540 from the USDA National Institute of Food and Agriculture.

Notes:

162 - Oleic acid enhances lipid accumulation and improves mitochondrial function in bovine adipocytes

Ursula Abou-Rjeileh[1, 2], Adam L. Lock[1, 3], G. Andres Contreras[1, 2]

[1]Michigan State University, [2]Large Animal and Clinical Sciences, [3]Animal Science. abourje2@msu.edu

Session: General Health & Physiology 2, 2024-01-22, 2:45 - 3:00

Objective: Determine the effect of oleic acid (OA) on lipogenesis and mitochondrial function in bovine adipocytes.

Methods: Pre-adipocytes were isolated from subcutaneous adipose tissue explants (n=9, non-lactating, non-gestating dairy cows) and induced to differentiate. Mature adipocytes were cultured with standard differentiation media (CON) supplemented with palmitic acid (PA) or OA at 100, 200, and 300µM, or mixed PA:OA at 60:40, 50:50, and 40:60 ratios at 300µM for 7d. All fatty acids (FA) were solubilized in albumin (10% BSA). Intracellular lipid droplets were quantified using Adipored assay (RFU/ng DNA). Expression of lipogenic and mitochondrial gene networks was evaluated using RT-qPCR. Protein was quantified by capillary electrophoresis. The statistical model included the random effect of cow and fixed effect of treatment.

Results: Compared with CON, the 300PA, 200OA, 300OA, 60:40, 50:50, and 40:60 treatments enhanced lipid accumulation (P<0.01). 300OA and 40:60 stimulated lipid uptake and adipogenesis through increasing the expression of *PPARγ* compared to all other treatments (P<0.01). Moreover, compared with 300PA, 300OA and 40:60 tended to increase expression of insulin-regulated glucose transporter *GLUT4* (P=0.06). Compared with CON and 200PA, 300OA and 50:50 tended to increase lipid droplet associated protein PLIN5 content (P=0.10). Within the mitochondria, 300PA tended to decrease the expression of FA transport protein system (*CAC*, *CPT1*, *CPT2*), complex I protein (*NDUFS1*), *SIRT1*, and *PGC1α* (0.05≤P≤0.07) compared with CON, 300OA and 40:60 treatments.

Conclusions: Our results show that OA, in combination with PA, restores mitochondrial biogenesis and improves oxidative phosphorylation. This provides mechanistic evidence for the use of OA in dairy cow diets during the periparturient period to enhance lipid accumulation and limit lipolysis. Hence, improving energy balance will ultimately minimize health disorders and improve production of early postpartum cows.

Financial Support: USDA grants (2019-67015-33386, 2021-67015-29443); Michigan Alliance for Animal Agriculture (AA18-028).

Notes:

163 - Effect of Lactobacillus acidophilus postbiotic on improving nursery pig performance and mitigating weaning stress.

Adam Rogers[1], Andrea Binnebose[1], Ali Naqvi[1]

[1]Cargill Inc. adam_rogers2@cargill.com

Session: General Health & Physiology 2, 2024-01-22, 3:00 - 3:15

Objective: To determine the impact of *Lactobacillus acidophilus* postbiotic on commercial nursery performance over a twelve-month period.

Methods: A year-long study was conducted with a midwestern pork producer to evaluate the effects of an in-feed supplementation of *Lactobacillus acidophilus* postbiotic. Performance was measured for final weight, average daily gain (ADG), feed conversion rate (FCR), and mortality.

Results: Overall, *Lactobacillus acidophilus* fermentation product (LAFP) demonstrated beneficial effects on the production of weaning age pigs in this study, particularly FCR where a main effect trend was observed across the entire trial.

Conclusions: Under the conditions of this study: Treatment with LAFP increased performance by improved feed conversion ratio and final weight and/or reduced feed consumed. Mortality improved or stayed the same across all treatment groups. Treatment with LAFP modulated the changes in cytokine profiles experienced through the nursery period.

Notes:

164 - Intestinal microbiome confers strong colonization resistance against necrotic enteritis in chicken

Jing Liu[1], Melanie Ann Whitmore[1], Isabel Tobin[1], Markus Kim[1], Zijun Zhao[1], Jiaqing Guo[1], Michael Kaiser[2], Susan Lamont[2], Raj Kulkarni[3], Hans Cheng[3], Guolong Zhang[1]

[1]Department of Animal and Food Sciences, Oklahoma State University, [2]Department of Animal Science, Iowa State University, [3]Endemic Poultry Viral Diseases Research Unit, U.S. National Poultry Research Center. jing.liu12@okstate.edu

Session: General Health & Physiology 2, 2024-01-22, 3:15 - 3:30

Objective: Necrotic enteritis (NE) poses a significant economic burden on the global poultry industry, resulting in approximately $6 billion in annual losses. The withdrawal of in-feed antibiotics from livestock production in the U.S. and the growing demand for antibiotic-free animal products have led to an increase in NE incidence, highlighting the urgent need for effective antibiotic alternatives. Manipulating the gut microbiota has emerged as a promising approach to enhance animal health and productivity. We hypothesize that inbred chicken lines resistant to NE harbor specific gut microbes that offer superior colonization resistance. The objectives of this study are to investigate the difference in the gut microbiome between NE-resistant and NE-susceptible chicken lines and to further demonstrate that the microbiota from resistant chicken lines provides better protection against NE than those from susceptible lines.

Methods: To assess the difference in the gut microbiome among different chicken lines, we induced NE in two NE-resistant chicken lines (Fayoumi M5.1 and ADOL Line 6), two susceptible lines (Ghs6 and ADOL Line 7), and Cobb broilers through sequential challenges with *Eimeria maxima* and *Clostridium perfringens*. Animal survival and intestinal lesions were recorded, and the intestinal microbiota composition was investigated using 16S rRNA gene sequencing. To further explore whether the microbiomes of NE-resistant chickens offer better NE protection than those of susceptible lines, we performed cecal microbiota transplantation with the five chicken lines, followed by NE induction and evaluation of the disease outcome.

Results: We confirmed that Fayoumi M5.1 and ADOL Line 6 chickens are more resistant to NE than Ghs6, ADOL Line 7, and Cobb chickens, as evidenced by the survival rate and intestinal lesion score in three independent trials. Nearly 100% of Fayoumi M5.1 and ADOL Line 6 chickens survived NE, while 25-50% of Cobb and two susceptible chicken lines died and experienced severe intestinal lesions. The microbiotas in the ileum and cecum of the five chicken lines were different from each other under both healthy and NE conditions. Furthermore, the cecal microbiota of Fayoumi M5.1 chickens provided impressive 100% protection from NE with either no or mild intestinal lesions, while approximately 40% of chickens died from severe intestinal lesions without microbiota transplantation. Interestingly, the cecal microbiota from two susceptible lines of chickens, Ghs6 and Cobb, also conferred significant protection of naïve Cobb chickens from NE, although with reduced efficacy.

Conclusions: Our findings strongly suggest that the gut microbiota plays a crucial role in colonization resistance to NE, and NE-resistant chickens harbor unique bacteria that provide better NE resistance than susceptible chicken lines. Therefore, the gut microbiota from NE-resistant chickens holds promise as a potential avenue to combat NE and other enteric diseases.

Financial Support: USDA-NIFA grant 2022-67016-37208

Notes:

165 - Vaccines - critical components of effective veterinary antimicrobial stewardship

Glenn F. Browning[1]

[1]Melbourne Veterinary School, The University of Melbourne. glenfb@unimelb.edu.au

Session: Animal Vaccinology Research Network Symposium, 2024-01-22, 4:15 - 5:00

Unnecessary use of antimicrobial drugs can result in selection of multi-resistant organisms without conferring any benefit to the animals to which they are administered. The aim of antimicrobial stewardship is to counter this risk by optimizing antimicrobial treatment by ensuring that it is only used when it will have a beneficial effect on animal health. Much of the focus on addressing the threat of antimicrobial resistance in agriculture has been focused on imposing restrictions on the administration of antimicrobials to animals. However, our experience, particularly in the poultry industry, has shown that one of the most effective ways to reduce antimicrobial use is to develop efficacious vaccines against critical bacterial pathogens, particularly those that can be challenging to diagnose. While the impact of many bacterial diseases of poultry has been eliminated in commercial poultry by all-in-all-out production systems and high levels of farm biosecurity, in some situations, and particularly in low- and middle-income countries, these diseases continue to be an ongoing problem. The development of live attenuated *M. gallisepticum* and *M. synoviae* vaccines and their widespread adoption by the Australian industry resulted in a dramatic reduction in the use of tylosin in poultry, and these vaccines are now available in many countries, although their use has been restricted to high value breeder birds. Our observations in low- and middle-income countries in recent years have suggested that broader availability of these vaccines, coupled with improved bacteriological diagnostic capacity, is likely to reduce not only the use of antimicrobials with activity against mycoplasmas, but also those that are used to treat colibacillosis. Furthermore, improved diagnosis of viral diseases of poultry in the field, and more widespread implementation of the extensive vaccination programs commonly used in wealthy countries, is likely to result in a significant reduction in rates of antimicrobial treatment, and particularly in the use of high importance antimicrobials, because of the high prevalence of empirical treatment of diseased poultry in some low- and middle-income countries. Similar beneficial effects are likely to result from development of improved vaccines for key bacterial diseases of pigs and cattle.

Notes:

166 - Does vaccination prevent antimicrobial resistance?

Paul S. Morley[1]

[1]VERO Program, Texas A&M University. pmorley@cvm.tamu.edu

Session: Animal Vaccinology Research Network Symposium, 2024-01-22, 5:00 - 5:45

Antimicrobial resistance (AMR) is commonly presented as one of the most important issues endangering public health globally, and international health organizations (WHO, CDC, FAO) attribute misuse of antimicrobial drugs in people and animals as one of the most important factors driving a public health crisis. Amid this crisis, vaccination is advocated as a pivotal strategy to combat AMR. The relationship between vaccination and antimicrobial resistance (AMR) is complex and multifaceted. Vaccination primarily aims to prevent infectious diseases thereby reducing the need for antibiotic treatment. However, the direct impact of vaccination on antimicrobial resistance is not straightforward. Vaccines can indirectly influence AMR by preventing infections that would otherwise require antibiotic intervention. This reduction in antibiotic use may contribute to lowering the selective pressure on bacteria to develop resistance. Additionally, some vaccines targeting bacteria in which resistance is commonly encountered may indirectly impact antibiotic resistance by reducing the prevalence of resistant strains. Animals are commonly vaccinated against agents causing diseases most associated with antimicrobial treatments. Despite frequent inclusion in "core" preventive medicine programs, these vaccines are often recognized as having limited efficacy. For instance, systematic reviews reveal that cattle vaccines targeting agents associated with bovine respiratory disease (BRD) exert negligible impact on disease occurrence. While these findings raise fundamental issues about vaccine efficacy, the outcomes are undoubtedly affected by the practical limitations of when vaccines are typically administered in production settings. Use of state-of-the-art molecular biology tools is revolutionizing our understanding of health and disease pathogenesis. Previous approaches for vaccination invariably focus on specific agents as pathogens, rather than considering their interconnectedness to other microbiota in a larger microbial community. Leveraging newly discovered microbial-host relationships associated with health and disease presents potentially novel strategies for vaccines to alleviate the reliance on antimicrobial drugs for disease treatment and prevention. While vaccination may play a role in preventing infectious diseases and reducing the need for antibiotics, its direct impact on antimicrobial resistance is nuanced.

Notes:

167 - Efficacy of tulathromycin for treatment of respiratory disease in goats: microbiological data

Amanda J. Kreuder[1], Ronald W. Griffith[1], Kristen Hayman[1], Michelle Buckley[1], Alyona Michael[1], Rachel Friedrich[1], Patrick Gorden[1], Paul J. Plummer[1], Holden Hooyer[1], Nicole Sterzinger[1], Rance Musco[1], Heather Peterson[1], Jen Payne[1], Chelsea Harris[1], Emma Mannery[1], Abby Kraemer[1], Lee Yantis[1], Adam Steffensmeier[1]

[1]Iowa State University. akreuder@iastate.edu

Session: Antimicrobial Use & Resistance 2, 2024-01-22, 4:15 - 4:30

Objective: Respiratory infections represent a significant disease burden on goat production in the United States. Our overall goal is to complete the research necessary for successful application for FDA label approval of the use of tulathromycin for treatment of respiratory disease in non-lactating goats through the Minor Use Minor Species (MUMS) program. A key component of this work is to report the current species distribution and antimicrobial susceptibility of bacterial respiratory pathogens currently encountered in the U.S. goat population.

Methods: Two blinded randomized controlled trials of naturally occurring respiratory disease in non-lactating meat and dairy breed goats sourced from multiple states were completed. A total of 197 goats (98 goats and 99 goats, respectively) met enrollment criteria, were randomly treated with either tulathromycin or a placebo (equal distribution) and had nasopharyngeal swabs collected at enrollment. Of those, 111 died or were euthanized as treatment failures and had lung swabs collected at necropsy. Routine culture followed by broth microdilution antimicrobial susceptibility testing was performed to evaluate the presence of pathogenic bacteria within the Pasteurellaceae family and assess antimicrobial resistance in this population.

Results: From the pre-treatment nasal swabs, 48.2% (95/197) were positive for *Mannheimia haemolytica*, 33.0% (65/197) for *Pasteurella multocida,* and 2.0% (4/197) for *Bibersteinia trehalosi.* Minimal antimicrobial resistance was noted in these isolates. From the lung swabs collected from treatment failures, 31.5% (35/111) were positive for *M. haemolytica*, 66.0% (59/111) for *P. multocida,* and 3.6% (4/11) for *B. trehalosi*. Increased minimum inhibitory concentrations (MICs) to macrolides (gamithromycin and tulathromycin, but not tildipirosin), tetracyclines, and beta-lactams (ampicillin, penicillin) were noted in some *M. haemolytica* lung isolates (<20%), particularly during the second study.

Conclusions: An atypical pattern of increased MICs to only two of three macrolides tested was identified in *M. haemolytica* isolates that warrants further investigation.

Financial Support: This project was supported by Agriculture and Food Research Initiative Competitive Grant no. 2021-67015-34421 from the USDA National Institute of Food and Agriculture. The investigational drug was provided by Zoetis.

Notes:

168 - Comparison of two methods for collecting antimicrobial usage data on large dairy farms in Ohio and California.

R. Portillo-Gonzalez[1], A. Garzon-Audor[2], R. V. V. Pereira[2], Ting-Yu Cheng[1], Greg Habing[1]

[1]The Ohio Sate University, [2]University of California, Davis. portillo-gonzalez.1@osu.edu

Session: Antimicrobial Use & Resistance 2, 2024-01-22, 4:30 - 4:45

Objective: Quantification of on-farm antimicrobial use (AMU) is a critical step in the fight against antimicrobial resistance. Prior studies have used empty drug containers (EDC) to quantify AMU; however, the method is labor-intensive and time-consuming. Therefore, this study aimed to compare it against farm treatment records (FTR) and estimate the agreement level in on-farm antimicrobial treatment incidence (TI).

Methods: The study was part of a quasi-experimental field trial, but only thirteen farms enrolled in Ohio and California were used for this component of the study. On-farm AMU was quantified for six months in three different 30-day intervals by assessing the on-farm treatment records and by counting the number of used antibiotic packages discarded in containers provided by the research team. Descriptive analysis was performed to calculate TI by collection method, class, and route of administration. Lin's concordance correlation coefficient (CCC) and intraclass correlation (ICC) were obtained to evaluate the strength of agreement and the reliability of the collection methods, respectively.

Results: The highest mean TI by antimicrobial class and route of administration were obtained from the cephalosporin group (7.3 and 6.8) and lactating-cow therapy (4.9 and 5.8) for FTR and EDC, respectively. Lin's CCC was 0.60 (95% CI: 0.46-0.71), showing moderate agreement between the collection methods. The ICC was 93.7 for EDC and 93.0 for FTR indicating a large variation in on-farm AMU between farms rather than within farms.

Conclusions: Regardless of the collection method used, AMU varied significantly among the enrolled farms. Additionally, the estimated mean TI between these collection methods was significantly associated with a moderate agreement in collecting on-farm AMU. Future efforts should be oriented to standardize on-farm antimicrobial collection methods to facilitate their application and improve data accuracy.

Financial Support: This study was done in collaboration with the University of California, Davis. The authors acknowledge the United States Department of Agriculture (USDA) for supporting this study through grant # 2018-68003-27466.

Notes:

169 - Influence of antimicrobial metaphylaxis on health, performance, antimicrobial use, and resistance in stocker calves

Brent Credille[1], Roy Berghaus[1], Ella Jane Miller[1], Allison Credille[1], Nora Schrag[2], Hemant Naikare[3]

[1]Food Animal Health and Managment Program, University of Georgia, [2]Livestock Veterinary Resources, LLC, [3]Tifton Veterinary Diagnostic and Investigational Laboratory, University of Georgia. bc24@uga.edu

Session: Antimicrobial Use & Resistance 2, 2024-01-22, 4:45 - 5:00

Objective: The objective of this study was to evaluate the impact of metaphylaxis on health, performance, and selection of antimicrobial resistant bacteria in the upper airway. A secondary objective was to measure contextual antimicrobial use (AMU) in high-risk beef stocker calves that received metaphylaxis, and those that did not**.**

Methods: Calves (n=155) were randomly assigned to receive either saline (1.1ml/100 lbs SQ) or tulathromycin (2.5 mg/kg SQ) at the time of arrival processing. Deep nasopharyngeal swabs (DNP) were collected from each calf at arrival and day 14. Calves were monitored for the development of BRD for 42 days. Body weights were obtained at arrival, day 14, 28, and 42. Contextual antimicrobial use (AMU) was calculated using dose and mass-based metrics.

Results: Calves given tulathromycin had significantly higher average daily gain (ADG, 0.96 ± 0.07 kg vs 0.82 ± 0.07 kg; P = 0.034) and lower risk of BRD than controls (17% vs 40%; P = 0.008]. Proportions of calves with BRD pathogens identified on DNP culture at arrival was similar between treatment groups [tulathromycin (17%) vs saline (17%); P = 0.94]. Proportions of calves that had BRD pathogens identified at day 14 was significantly lower for calves receiving tulathromycin compared to controls (15% vs 60%, P < 0.001). Overall, 81% of *P. multocida* isolates and 47% of *M. haemolytica* isolates were pansusceptible. When measured as regimens per head in, AMU in calves receiving tulathromycin was significantly higher than calves receiving saline (P = 0.01).

Conclusions: Metaphylaxis with tulathromycin has positive impacts on health and performance of high-risk beef stocker calves, does not contribute to the selection of resistant bacterial isolates in the nasopharynx of treated cattle, and can increase total antimicrobial use of medicated cattle.

Financial Support: The authors would like to thank the Georgia Commodity Commission for Beef and Georgia Farm Bureau Federation for their support of this research.

Notes:

170 - Clinical outcomes of feedlot calves with antibiotic resistant and non-resistant respiratory disease bacteria

Deana Veal Hardee[1, 2], David G. Renter[1], Lonty K. Bryant[2]

[1]Center for Outcomes Research and Epidemiology, Kansas State University, [2]Merck Animal Health. deana.hardee@merck.com

Session: Antimicrobial Use & Resistance 2, 2024-01-22, 5:00 - 5:15

Objective: The objective of the study was to evaluate associations among results from antibiotic susceptibility testing (AST) of bacterial pathogens *Mannheimia haemolytica* and *Pasteurella multocida* and clinical outcomes for bovine respiratory disease (BRD).

Methods: Heifers (N=1032) at high-risk for developing BRD were received at a commercial feedlot in central Oklahoma in fall of 2021. At arrival to the feedlot (study day 0), calves received standard processing including metaphylaxis with tildipirosin. Following metaphylaxis, clinical BRD cases were treated with florfenicol with flunixin, enrofloxacin, and oxytetracycline for first through third treatments if necessary. Deep nasopharyngeal swabs (DNS) were collected from BRD treated calves and all calves at day 0, at 10-14 days on feed, at reimplant (mean = 85 days), and at terminal sort (mean = 140 days). Swabs were transported in Amie's gel agar and delivered the next day to an accredited diagnostic laboratory for culture and sensitivity. Interpretive criteria were breakpoints established by the Clinical and Laboratory Standards Institute. Results were collapsed into two categories: resistant and not resistant (susceptible + intermediate). Clinical outcomes were observed for the entire feeding period and treatment success defined as a BRD case that did not die or need further treatment for BRD. Fisher's exact tests were applied to determine if there were statistically significant differences ($p \leq 0.05$) in health outcomes between calves with resistant and non-resistant isolates.

Results: Overall BRD mortality was 8.81% (91/1032) and morbidity was 36.53% (377/1032). Prevalence from DNS collected at day 0 and first BRD treatment respectively were: 6.30 (95% CI: 4.89-7.96) and 16.67% (95% CI: 13.02-20.85) for *M. haemolytica* and 16.38% (95% CI: 14.17-18.78) and 19.62% (95% CI: 15.71-24.03) for *P. multocida*. There was no evidence for associations between tildipirosin resistance at day 0 and subsequent BRD morbidity, retreatment, or mortality. For BRD cases sampled at first treatment, subsequent mortality was higher for calves with *M. haemolytica* resistant to enrofloxacin versus calves with non-resistant isolates (56.3% vs. 13.0%; p = 0.001), but in the feedlot treatment protocol, enrofloxacin was only given to calves requiring a second BRD treatment. There was no evidence for other associations between AST results from tildipirosin, florfenicol, or tetracycline and clinical outcomes.

Conclusions: Our results demonstrate that there are limited associations between BRD clinical outcomes and AST results. Antibiotic resistance is complex and understanding the role it plays in clinical failures of BRD is not well understood. These data demonstrated the challenge in linking phenotypic antibiotic resistance with health events.

Financial Support: Merck Animal Health

Notes:

171 - Characterization of the oral pharmacokinetics of tylosin and its effect on the rumen microbiome

Jennifer Halleran[1], Julio Perez Mojica[1], Ivonne Martinez[1], Madelyn Schwartz[1], Laura Neumann[1], Linda Dillenbeck[2], Danielle Mzyk[1], Derek Foster[1]

[1]North Carolina State University, [2]Colorado State University. jlhaller@ncsu.edu

Session: Antimicrobial Use & Resistance 2, 2024-01-22, 5:15 - 5:30

Objective: Human exposure to antimicrobial resistant (AMR) bacteria is multifactorial, but one potential source for AMR development is the use of in-feed antimicrobials in food animals. In the beef cattle industry, liver abscesses (LA) are a significant economic concern with prevalence rates ranging from 12-32%. To reduce the incidence of LA, in-feed antimicrobials are routinely administered, the most common being tylosin, a macrolide. While tylosin is used strictly in veterinary medicine, macrolides are considered critically important antibiotics in humans. Prolonged administration of tylosin is associated with increases in the prevalence of macrolide resistant microorganisms in cattle; tylosin has a 0 day meat withdrawal interval and is commonly fed throughout the whole finishing period. There is a need to develop an appropriate duration of therapy of tylosin. The primary objective of this study was to characterize the pharmacokinetics of tylosin in the portal circulation (suspected target site for tylosin) and to understand the effect tylosin administration has on the rumen microbiota. Our hypothesis states that tylosin concentrations will be higher in the portal circulation when compared to systemic circulation. In addition, we hypothesize that the administration of tylosin will alter the rumen microbiome, causing a decrease in abundance of bacterial communities responsible for LA.

Methods: Six Holstein-Jersey cross steers (6-7 months old) were used in this study. Prior to dosing, the steers had an intravenous jugular catheter placed, and under light sedation, an ultrasound guided portal vein catheter was placed. The steers were then fed the FDA approved dose of tylosin (90mg/head) once a day for three days. Peripheral and portal blood were collected at routine intervals throughout the study period. Rumen Fluid was collected prior to dosing, and once a day throughout the study period. Tylosin concentration analysis was conducted on the peripheral blood, portal blood and rumen fluid using liquid chromatography-mass spectroscopy. The rumen fluid also underwent bacterial DNA extraction using ZymoBiomics DNA miniprep Kit and is being submitted for amplicon sequencing analysis.

Results: All steers tolerated the portal vein catheters well, with all catheters still functioning at the end of the study period. The steers completely consumed the feed with tylosin at each feeding, but there was no tylosin detected in the peripheral or portal blood in any steer at any time point. However, tylosin was detected in the rumen fluid up until 96 hours after the initial dose. The results for the microbiome analysis are still pending.

Conclusions: Based on these findings, it appears that the true mechanism of action of tylosin in the prevention of LA is modulation of the rumen microbiome as it does not reach therapeutic concentrations in the portal circulation. Recognizing this target site for tylosin provides crucial insight into developing prudent use to minimize incidence of LA while potentially mitigating AMR.

Financial Support: Start Up

Notes:

172 - Measuring intake of chlortetracycline-containing mineral offered free-choice to beef cows on pasture

W. Isaac Jumper[1], Madeline G. McKnight[2], Kelsey Harvey[3], Juan F. Cordero[3], Jason R. Russell[4], David R. Smith[1]

[1]Department of Pathobiology and Population Medicine, College of Veterinary Medicine, Mississippi State University, [2]Department of Animal and Dairy Sciences, Mississippi State University, [3]North Mississippi Research and Extension Center-Prairie Research Unit, [4]Zinpro Corporation. isaac.jumper@msstate.edu

Session: Antimicrobial Use & Resistance 2, 2024-01-22, 5:30 - 5:45

Objective: The objective of this study was to describe the consumption of chlortetracycline (CTC)-containing mineral offered free-choice to beef cows on pasture.

Methods: Fall-calving (i.e., August-November) Angus-Charolais cross commercial cows (n=103) housed on native grass pasture were divided evenly into three groups. Each group had access to a SmartFeed (C-Lock Inc.) portable, self-contained unit with two feed bins suspended on load cells and equipped with radio frequency identification (RFID) readers. Access to each feed bin was restricted to one cow at a time, and RFID readers recorded cow identification, feeding time, and mass consumed per feeding event. After feeding dried distillers' grain (DDG) in the feeders for a 27-day adaptation period, the DDG was replaced by a commercial granular mineral containing CTC at a concentration of 6.17 g/kg. Mineral intake data was collected for 46 days, from 7/5/2022 to 8/19/2022. Generalized linear regression was used to test associations between cow age and both total amount of mineral consumed and frequency of feeder visits. The runs test for serial randomness was used to evaluate clustering of feeding and non-feeding events. Statistical significance was set *a priori* at alpha=0.05.

Results: Overall average mineral consumption was 0.04 kg/cow/day. The average cow consumed 1.88 kg mineral total over 46 days. During the 46-day trial period, 101/103 cows failed to consume enough mineral to receive the daily label-recommended CTC dose of 1.1 mg/kg bodyweight/day. The average length of time between feeder visits was six days. On average, 27% of cows visited the mineral feeder and consumed each day. As cow age increased, mineral feeder visits became less frequent ($P < 0.001$) and mineral consumption (kg/hd) decreased as well ($P < 0.001$). However, during the adaption period, no association was found between cow age and feeder visit frequency ($P = 0.104$), or amount consumed ($P < 0.77$). Feeding and non-feeding events during the 46-day trial period were not randomly distributed ($P < 0.001$) but occurred in clusters over time during the trial period.

Conclusions: Offering CTC-medicated mineral in a free-choice manner does not ensure that cows on pasture consume the label-recommended daily CTC dose of 1.1 mg/kg bodyweight.

Financial Support: A contribution of the Beef Cattle Population Health and Reproduction Program at Mississippi State University.

Notes:

173 - A network evaluation of human movement data across multiple swine farm system

Tara Prezioso[1], Alicia Boakes[2], Jeff Wrathall[2], W. Jonas Reger[3], Suman Idan Bhowmick[1], Rebecca Lee Smith[1]

[1]University of Illinois Champaign/Urbana Dept of Pathobiology, [2]Farm Health Guardian, [3]Independent. tarap3@illinois.edu

Session: Biosecurity & Infection Control 2, 2024-01-22, 4:15 - 4:30

Objective: High volume global trade increases countries' susceptibility to foreign animal diseases. United States agriculture contributes about 13% to the country's GDP (gross domestic product), including over $26 billon total hog sales in 2017 and over $8.1 billion in pork product exports contributing to about 12% of the world's pork production. An international trade embargo due to a high-risk disease would result in the loss of billions in direct sales, more in related industries. The swine industry is vulnerable to the rapid spread of disease due to systemic structural issues. While animal movement networks have been used to identify disease spread risks and design response plans, human movement between farms has not been accounted for. A variety of farm visits could be responsible for infectious disease spread, including feed trucks, service vehicles, management, and veterinarians.

Hypothesis: A swine farm network including human movement will identify risk structures not present in animal movement networks alone.

Methods: Farm visit data was collected from a private database over the period from April 1st, 2022 to April 27th, 2022, representing three swine management companies. The data include property and property group IDs, location, and user/truck IDs, all of which were anonymized. Other variables include the property type, vehicle type, entry type, and dates of visits. Observations without a property ID, user/truck ID, or at least two farm visits were removed from the data. A static directed animal and human movement network was created with this database using the igraph package in R; separate sub-networks were created using only animal movement, human movement and individual truck types. The network statistics for each subnetwork were compared against each other and an average of 1000 randomized networks with the same number of nodes and edges.

Results: The final full static network included 455 properties, 320001 edges, 33 different property types, 9 vehicle types, and 12 different entry types. Measures of network structure included the longest path length (5), the overall, in and out degree distributions, and betweenness distribution. Measures of interconnectivity included the eigenvector centrality distribution, the undirected density (0.109), the average path length (2.102), transitivity (215842 triads present), the global transitivity (0.4362), and the local transitivity for each farm. Identification of network substructures include the number and sizes of cliques, property type assortativity (0.193), the property group assortativity (0.933), the degree assortativity (-0.220)), and the reciprocity (0.713).

Conclusions: The static version of the full network appears to be very well connected, more so than expected and higher than the randomized expected connectiveness. However, this may change as the network is divided into subgraphs and dynamically studied. Monitoring human movement is just as or more important than monitoring animal movement alone.

Financial Support: I would like to acknowledge the University of Illinois Research Board #RB23057.

Notes:

174 - Reinforcement learning for agent-based modeling of swine producer biosecurity adoption

Jackson Dean[1], Eric Clark[1], Kevin Andrew[1], Scott Turnbull[1], Richmond Baye[1], Samuel F. Rosenblatt[1], Johnbosco Osuagwu[1], Asim Zia[1], Scott Merrill[1], Julie Smith[1], Laurent Hebert-Dufresne[1], Nick Cheney[1]

[1]University of Vermont. jackson.dean@uvm.edu

Session: Biosecurity & Infection Control 2, 2024-01-22, 4:30 - 4:45

Objective: Animal disease costs the livestock industry billions of dollars annually. Strategic biosecurity investments by producers can significantly reduce these costs. However, identifying the most effective investment strategies and the influence of government policy remains an open problem. Agent-Based Modeling (ABM) is a promising approach for studying disease spread, assessing biosecurity investment strategies, and the effectiveness of government policies. In our ABM, we propose that using reinforcement learning (RL) allows for more dynamic agent responses to policy changes compared to static, hand-designed policies. We explored this hypothesis in a hog producer network ABM under development.

Methods: We implemented the Deep Q-Learning reinforcement learning algorithm in Python and integrated into our ABM, developed using FLAME GPU. Agents decided every week of simulation time whether to invest in biosecurity or allow their protection to degrade. We calibrated our ABM, which is still in development, to loosely model Porcine Reproductive and Respiratory Syndrome (PRRS).

Before adding RL, the agents decided based on three hand-designed, fixed-strategies derived from data collected from human participants who played a biosecurity-investment game. These strategies dictated investment decisions based on the prevalence of infections within the producers' veterinary networks. In the "risk-averse" scenario, agents consistently invested in biosecurity, independent of infection rates. The "risk-opportunist" scenario presented a more calculated approach, with agents investing when infection rates were high, and remaining conservative otherwise. Conversely, in the "risk-seeking" scenario, agents were unlikely to invest regardless of infection rates, exhibiting a high tolerance for risk.

After adding RL, we tested three biosecurity cost scenarios (free, medium-cost, high-cost) and averaged twenty runs of each scenario for the final results. The hand-designed policies were replaced with neural networks trained with Deep Q-Learning. The RL agents' observations included the number of infected farms in their veterinary network, along with information about budget and local biosecurity levels. We used a reward function based on total farm budget, which decreased when agents invested in biosecurity or were infected and increased when they sold pigs.

Results: When biosecurity was free, RL agents always invested in biosecurity, similar to the agents in the hand-designed "risk-averse" scenario. In the medium-cost biosecurity condition, RL agents learned to invest in biosecurity when risk was high but not when risk was minimum, similar to the hand-designed "risk-opportunist" behavior observed in some human decision-makers. When biosecurity was expensive, RL agents were very unlikely to invest in biosecurity, similar to the agents in the "risk-seeking" scenario.

Conclusions: This proof-of-concept methods study demonstrates the potential of reinforcement learning to enhance dynamic agent behavior within biosecurity ABMs in response to policy changes. Future work will investigate more complex observation and action spaces, different policies, as well as compare more directly with data from other sources. We aim for future versions of this ABM with reinforcement learning to inform real-world policy and biosecurity investment strategies.

Financial Support: This work was funded by USDA-NIFA AFRI grant # 2021-67015-35236 as part of the joint USDA-NSF-NIH-UKRI-BSF-NSFC Ecology and Evolution of Infectious Diseases program. Computations were performed on the Vermont Advanced Computing Core supported in part by NSF award No. OAC-1827314.

Notes:

175 - Increasing the speed of large-scale agent-based models of porcine disease transfer using computational acceleration

Kevin Andrew[1], Samuel F. Rosenblatt[1], Katey Forsyth[1], Eric Clark[1], Scott Turnbull[1], Julia M. Smith[1], Asim Zia[1]

[1]Univeristy of Vermont. kandrew6@uvm.edu

Session: Biosecurity & Infection Control 2, 2024-01-22, 4:45 - 5:00

Objective: The complexity and interconnectedness of the animal supply chain system make the impact of system aspects on contagion outbreaks difficult to measure and predict. Agent-based models provide a means to examine these impacts with high precision, specificity, and system realism, including highly heterogeneous agent behaviors and protocols depending on risk preference and company biosecurity protocols.

Methods: We developed a large-scale, complex agent-based model of the swine industry in the United States. We model interactions between hog producers, processors, feed mills, and veterinarians, including contagion modeling, economic modeling, and human behavior modeling. We analyze how behavior and decisions concerning the adoption of biosecurity practices at their facilities may impact the spread of porcine disease throughout the country.

Results: Implemented using the FLAMEGPU2 GPU-accelerated agent-based modeling library with CUDA C++, which takes advantage of recent developments in software parallelization using GPU computing, our simulations were significantly faster than previous models implemented using AnyLogic/Java using standard CPU processors while increasing the complexity of the modeled system.

Conclusions: This study aims to create a dynamic model of the relationship between humans, swine, and disease in the United States. As this model is developed further, we will simulate various scenarios and introduce African Swine Fever (ASF) to predict its early stages by calibrating the model with data from multiple sources. Using this model, we will test the effects of system constraints, human behavior, and corporate action on the frequency of porcine reproductive and respiratory syndrome (PRRS) outbreaks and the risk of a major outbreak of ASF. Ultimately, the results of this study can inform policymakers, corporate interests, and individual farmers on incentive structures and best practices to reduce outbreaks with minimal legislative intervention and profit loss. Additionally, by implementing our model as an open-source library, we hope to provide transparency in model implementation that allows the underlying techniques to be better understood and adapted than the current state-of-the-art models used by the United States Department of Agriculture and other researchers.

Financial Support: This work was funded by USDA-NIFA AFRI grant # 2021-67015-35236 as part of the joint USDA-NSF-NIH-UKRI-BSF-NSFC Ecology and Evolution of Infectious Diseases program.

Notes:

176 - Maximizing epidemics with spatial and categorical assortativities in modular geometric contagion networks

Samuel F Rosenblatt[1, 2], Laurent Hébert-Dufresne[1, 2]

[1]Vermont Complex Systems Center, [2]University of Vermont. sfrosenb@uvm.edu

Session: Biosecurity & Infection Control 2, 2024-01-22, 5:00 - 5:15

Objective: Some of the most influential results in the study of networks show how important a few random long-range links can be on the connectivity of otherwise local networks. In reality, these long-range links are rarely random and instead stem from alternate connection mechanisms. In swine-production networks, connections are dominated by two mechanisms which produce their own type of locality: geographic proximity (farms interacting with others close-by) and in-community preference (farms interacting with others in the same company). In this study we explore epidemic dynamics on networks with varying proportions of these mechanisms, which are meant to abstractly represent livestock production premises and their various interactions such as livestock and feed shipments.

Methods: We propose a mixture model which combines soft random geometric networks and a community-specific configuration model via constructing pairs of networks with equivalent degree sequences but distinct edge creation mechanisms, and selecting edges at random from each with proportional probability summing to 1. We conduct computational, markovian, event-driven simulations of Susceptible-Infectious-Recovered dynamics (SIR) on the networks constructed using our model to study epidemic outbreaks under different scenarios, including the impact of heterogeneous biosecurity protocol norms across companies, represented in our model via varying levels of susceptibility correlated with company affiliation.

Results: The simulations reveal that outbreaks are small when networks are highly assortative in terms of either spatial location or category. However, outbreaks quickly escalate in scale by an order of magnitude when mixing mechanisms create "shortcuts" in the system. Particularly, when there are numerous companies, the most severe outbreaks occur when both types of assortativity are equally important. On the other hand, with fewer companies, outbreaks are maximized when categorical assortativity is relatively more dominant than spatial proximity.

Conclusions: We then apply hypotheses generated from these abstracted experiments to the specific setting of large, complex, agent-based model of the swine production network in the United States. The research proposes structural interventions that could be implemented through laws, incentives, or corporate actions to reduce outbreaks of Porcine Reproductive and Respiratory Syndrome and reduce the risk of African Swine Fever at minimal economic cost.

Financial Support: This work was funded by USDA-NIFA AFRI grant # 2021-67015-35236 as part of the joint USDA-NSF-NIH-UKRI-BSF-NSFC Ecology and Evolution of Infectious Diseases program.

Notes:

177 - The North American Animal Disease Spread Model (NAADSM) as a decision-making tool for FMD outbreaks in New England

Johnbosco U. Osuagwu[1], Julia M. Smith[1], Aaron Reeves[2]

[1]Department of Animal and Veterinary Sciences, UVM, [2]Center for Applied Public Health Research (APHR), RTI International. josuagwu@uvm.edu

Session: Biosecurity & Infection Control 2, 2024-01-22, 5:15 - 5:30

Objective: Foot-and-Mouth-Disease (FMD) is a virus that affects cloven-hoofed animals that spreads through bodily secretions and excretions (breath, saliva, mucus, milk and feces) of infected animals. The UK FMD outbreak in 2001 lasted for 221 days and accrued €5 billion in direct losses. With the US being about 40 times larger in landmass than the UK, one can only imagine the huge losses to be estimated should such a US outbreak occur. Users of the North American Animal Disease Spread Model (NAADSM), a stochastic, spatial, flexible computer program, can develop simulated models of highly contagious diseases like FMD. Therefore, we aim to use it to evaluate outbreak scenarios and potential control strategies for FMD in New England dairy farms.

Methods: Data regarding location coordinates and categories of dairy farm operations (1,921 farms) across the New England states were obtained from the USDA National Agriculture Statistics Service (NASS) and other government sources. In situations where co-ordinate and dairy herd sizes were unavailable from these data sources, co-ordinates were generated from their postcodes using a Google extension called Geocode®, while random dairy herd sizes to fit the farm categories were generated using Microsoft Excel (Version 2209 Build 16.0.15629.20200) 64-bit. Disease modeling was then performed using NAADSM® (version 3.3.2). 30 farms were excluded from this analysis because of the inability to generate coordinates for them. 5 simulation runs were performed in NAADSM till the end of an FMD outbreak scenario. Results were summarised into five sections: (a) Disease transmission status (b) Dairy herd disease status (c) Control zone output (d) Disease detection capability and (e) Disease control status.

Results: 71% of FMD virus transmission was through direct contact, followed by indirect contact (22%). 95% of all iterations had an actual epidemic curve whose outbreak lasted for 110 days and affected about 500 (26%) dairy farms, reaching a peak of 12 newly infected units/day in just 26 days. However, with fallible disease detection modeled, the apparent epidemic curve had its outbreak last for 123 days, peaking at 35 days. During this outbreak, about 200 dairy herds were in high-risk zones and 450 herds in moderate-risk zones. Direct traces (both forward and backward) had over 90% success rates in detecting infected herds and were more capable of detecting infected herds than indirect traces or clinical detection. The FMD outbreak control strategy saw 786 (over 40%) production units destroyed and a ring vaccination of 134 units by the outbreak's end.

Conclusions: NAADSM can enhance the readiness of the dairy industry of the New England milkshed in the face of an FMD outbreak through the decisions it helps inform in terms of FMD virus transmission dynamics, its spread through animal-to-animal contact as well as via contact with contaminated personnel or equipment, the effect of local spread via airborne dissemination, and plausible FMD outcomes following the use of contact tracing, diagnostic testing, animal movement control, animal destruction and vaccination as control strategies.

Financial Support: This work was supported by the US Department of Agriculture National Institute of Food and Agriculture, Agricultural Biosecurity program, under Award No. 2022-69014-37041.

Notes:

178 - Expert consensus study regarding disease control strategies to prevent calf mortality in beef herds

V. Margarita Sanguinetti[1], Cindy Adams[1], John Campbell[2], Sylvia Checkley[1], M. Claire Windeyer[1]

[1]University of Calgary, Faculty of Veterinary Medicine, [2]University of Saskatchewan, Western College of Veterinary Medicine. virginia.sanguinetti@ucalgary.ca

Session: Biosecurity & Infection Control 2, 2024-01-22, 5:30 - 5:45

Objective: To determine which disease control strategies used to prevent calf mortality should be included in a tool to facilitate shared decision-making between producers and veterinarians according to expert consensus.

Methods: This study used a Modified Delphi approach to collect expert opinions and was approved by the University of Calgary Conjoint Faculties Research Ethics Board. The method involved two rounds of questionnaires and a set of workshops where consensus was targeted. Recruited experts had a Doctor in Veterinary Medicine degree and knowledge of the western Canadian beef industry. Experts were asked to weigh the relative importance of effectiveness, ease of implementation, and economic feasibility. Then, they scored these items individually for colostrum, breeding and calving, vaccination, biosecurity, and antibiotic administration using a 6-point scale. A score of 0 indicated the strategy was considered effective, easy to implement, or economically feasible 'not at all for any herd', 1 was 'not at all for most herds', 2 was 'somewhat for some herds', 3 was 'very much for some herds', 4 was 'very much for most herds', and 5 was 'always for all herds.' Overall scores were calculated considering the relative weights and the individual scores for each strategy. After each questionnaire, a feedback report showing the scores was sent to the experts. Strategies with median overall scores of ≥2.50 during the second questionnaire were discussed during the workshop sessions, held online (Zoom Cloud Meetings, Zoom Video Communications., Inc. San Jose, California). Experts were required to vote on which strategies should be included in an on-farm decision tool intended to guide discussions between producers and veterinarians. Voting results were shown to the experts and discussed.

Results: Twelve experts (7 veterinary practitioners and 5 academics) were enrolled. Twenty-five and 28 strategies during the first (Q1) and second (Q2) questionnaires, respectively, were scored as ≥2.50. The strategies considered as useful 'always for all herds' were: administering clostridial vaccines in calves (Q1: 4.66/5.00, Q2: 4.75/5.00) and feeding colostrum or colostrum replacer to the calf using a nipple bottle (Q1: 4.53/5, Q2: 4.50/5) or oesophageal tube (Q1: 4.55/5.00, Q2: 4.50/5.00). Additionally, 12 strategies were scored as useful 'very much for most herds, 13 as useful 'very much for some herds', and 4 as 'somewhat for some herds.' After a thorough discussion at the workshops, participants agreed that all strategies scoring ≥2.50 were important enough to be discussed with the producer, and therefore, should be included in a decision tool.

Conclusions: These findings are important to support evidence-informed management and to develop guidelines that target complex problems like calf mortality in beef herds. Results will be used with the findings of a systematic review and a benchmarking study of calfhood disease control strategies implemented in Canada to develop a precision calf health decision tool. This tool will facilitate discussions between producers and veterinarians and enable producers to apply the best strategies to minimize calf mortality on their operations.

Notes:

179 - The effect of co-infection of infectious bronchitis virus on virulence of AIV H9N2 in birds: An in-silico analysis

Mohammed A. AboElkhair[1], Mohamed E. Hasan[2], Ahmed Mousa[3], Ibrahim Moharam[4], Hesham Sultan[4], Yashpal Malik[5], Moustafa A. Sakr[6]

[1]Department of Virology, Faculty of Veterinary Medicine, University of Sadat City, [2]Bioinformatics Department, Genetic Engineering and Biotechnology Research Institute (GEBRI), University of Sadat City, [3]Department of Biochemistry and Nutrition, Faculty of Veterinary Medicine, University of Sadat City, [4]Department of Bird and Rabbit Diseases, Faculty of Veterinary Medicine, University of Sadat City, [5]Indian Veterinary Research Institute (IVRI), [6]Molecular Diagnostics and Therapeutics Department, Genetic Engineering and Biotechnology Research Institute (GEBRI), University of Sadat City, Egypt. mohamed.abouelkhair@vet.usc.edu.eg

Session: Virology 4, 2024-01-22, 4:15 - 4:30

Objective: In the last few decades, frequent incidences of avian influenza (AI) H9N2 outbreaks have caused high mortality in poultry farms resulting in colossal economic losses in several countries. In Egypt, the co-infection of H9N2 with the infectious bronchitis virus (IBV) has been observed extensively during these outbreaks. However, the pathogenicity of H9N2 in these outbreaks remained controversial. The current study reports isolation and characterization of the H9N2 virus recovered from a concurrent IBV infected broiler chicken flock in Egypt during 2011.

Methods: The genomic RNA was subjected to RT-PCR amplification followed by sequencing and analysis. The deduced amino acid sequences of the eight segments of the current study H9N2 isolate were compared with those of Egyptian H9N2 viruses isolated from healthy and diseased chicken flocks from 2011 to 2013.

Results: In the phylogenetic analysis, the current study isolate was found to be closely related to the other Egyptian H9N2 viruses. Notably, no particular molecular characteristic difference was noticed among all the Egyptian H9N2 isolates from apparently healthy, diseased or co-infected with IBV chicken flocks. Nevertheless, in-silico analysis, we noted modulation of stability and motifs structure of Hemagglutinin (HA) antigen among the co-infecting H9N2 AI and the IBV and isolates from the diseased flocks.

Conclusions: The findings suggest that the putative factor for enhancement of the H9N2 pathogenicity could be co-infection with other respiratory pathogens such as IBV that might change the HA stability and function.

Financial Support: Egyptian Government and Obihiro University of Agriculture and Veterinary Medicine, Japan.

Notes:

180 - Transmission of a human-origin H3N2 in pigs increases fitness of HA and NA to the swine host

Matias Cardenas[1], Brittany Seibert[1], Brianna Cowan[1], Ana Luiza Soares Fraiha[1, 2], L. Claire Gay[1], Flavio Cargnin Faccin[1], C. Joaquin Caceres[1], Amy L. Vincent Baker[3], Tavis K. Anderson[3], Daniel R. Perez[1], Daniela Rajao[1]

[1]University of Georgia, [2]Universidade Federal de Minas Gerais, [3]National Animal Disease Center, ARS-USDA. daniela.rajao@uga.edu

Session: Virology 4, 2024-01-22, 4:30 - 4:45

Objective: Influenza A virus (FLUAV) infects a wide range of hosts and interspecies transmission is possible. Transmission of human-origin FLUAVs to pigs is frequently reported; however, only a fraction of them become established in pigs. The objective of this study was to understand the evolutionary processes that lead to the adaptation of human-origin hemagglutinin (HA) and neuraminidase (NA) to pigs.

Methods: We previously constructed a reassortant virus (pVIC11) containing the HA and NA gene segments from the H3N2 human strain A/Victoria/361/2011 (VIC11) and internal gene segments of an endemic swine strain (pOH/04) that resulted in improved transmission in pigs after acquiring a mutation in the HA (A138S). This mutant virus (pVIC11_A138S) was used in a serial transmission experiment in pigs in comparison to the swine-adapted (pOH/04) and human (pVIC11) parental viruses. Viral titers were determined by qRT-PCR in nasal swabs and BALF. Positive samples were sequenced using next generation sequencing (NGS) for variant analysis. Major variants were analyzed in a series of in vitro assays.

Results: The A138S HA mutation increased affinity for a2,6 receptors in vitro. It also enhanced affinity for swine lower respiratory tract epithelium and alveolar macrophages in vivo to similar levels as pOH/04, contrasting with the original pVIC11 that failed to replicate in the lungs. After two serial passages in swine, the virus gained a second mutation in the NA segment (D113A). This mutation, located in the low-affinity calcium-binding site, improved viral particle release, and increased replication and aerosol infection in MDCK and PK15 cells when low calcium concentrations were added to the media, but only when the A138S mutation was also present. Substitution D113A also increased NA enzymatic activity and thermostability when no calcium was present in the reaction buffer, showing a similar behavior as pOH/04 NA. Infection kinetics in A549 and differentiated human airway epithelial cells (HAE) showed no differences in growth between the human-origin mutant viruses and the precursor pVIC11, suggesting that these early adaptative changes might not affect viral fitness in humans.

Conclusions: Overall, our study indicates that adaptation of human viruses to the swine host involves an increased affinity for the lower respiratory tract and a2,6 receptors, and selection for NA proteins less sensitive to calcium concentrations, suggesting a novel role of calcium in the host range of FLUAV.

Financial Support: This project was supported by Agriculture and Food Research Initiative Competitive Grant no. 2020-67015-31563 /project accession no. 1022827 from the USDA National Institute of Food and Agriculture.

Notes:

181 - Nucleoprotein reassortment enhances transmissibility of H3 C-IVA clade influenza A virus in swine

Megan Neveau Thomas[1], Brianna Cowan[2], C. Joaquin Caceres[2], Daniela S. Rajao[2], Daniel R. Perez[2], Phillip C. Gauger[1], Amy L. Vincent Baker[3], Tavis K. Anderson[3]

[1]Dept. of Veterinary Microbiology and Preventive Medicine, College of Veterinary Medicine, Iowa State University, [2]Dept. of Population Health, College of Veterinary Medicine, University of Georgia, [3]Virus and Prion Research Unit, National Animal Disease Center, USDA-ARS. mneveau@iastate.edu

Session: Virology 4, 2024-01-22, 4:45 - 5:00

Objective: The detection of H3 C-IVA (1990.4.A) clade influenza A viruses (IAV) in U.S. swine significantly increased in 2019. The C-IVA IAV were not antigenically distinct from previous within-clade strains but reassorted to acquire an H1N1pdm09 (pdm) lineage nucleoprotein (NP) gene, replacing a TRIG lineage NP. We hypothesized that acquiring the pdm lineage NP conferred a selective advantage over prior circulating H3 viruses with a TRIG lineage NP.

Methods: To investigate the role of the NP reassortment in transmission, we identified two contemporary C-IVA H3N2 representative strains (NC/19 and MN/18) with different evolutionary origins of the NP gene, pdmNP or trigNP. A reverse genetics system was used to generate wildtype (wt) strains and to swap the pdm and TRIG lineage NP genes. This resulted in four viruses: wt-NC/19-pdmNP, rg-NC/19-trigNP, wt-MN/18-trigNP, rg-MN/18-pdmNP. We conducted an *in vivo* pathogenesis and transmission study in 65 conventional nursery-aged pigs.

Results: All four viruses successfully infected the 10 primary pigs and transmitted to the 5 indirect contact pigs per group. Animals infected via contact with rg-MN/18-pdmNP shed virus two days sooner (day 3 vs. 5 post contact) than those infected with wt-MN/18-trigNP. In contrast, the inverse did not occur for wt-NC/19-pdmNP and rg-NC/19-trigNP.

Conclusions: These data suggest that reassortment to acquire a pdmNP gene improved transmission efficiency in the C-IVA H3N2, but this is likely a multigenic trait. Replacing an NP gene alone may not completely diminish transmission of an otherwise successful wildtype virus. This study demonstrates the utility of whole genome sequencing in detecting novel reassortments, as these novel gene combinations may result in more transmissible viruses. Additionally, reassortment and associated evolutionary change may impact the expansion and contraction of IAV with specific HA clades. Thus, targeting expanding reassortants with dominant HA/NA will improve prediction of strains to include in vaccines.

Financial Support: This work was supported by the: Iowa State University (ISU) Presidential Interdisciplinary Research Initiative; ISU Veterinary Diagnostic Laboratory; USDA Agricultural Research Service (ARS); NIAID; USDA ARS Research Participation Program of ORISE; and SCINet project of the USDA ARS.

Notes:

182 - Defining antagonism hierarchy of porcine epidemic diarrhea virus for live vaccines design

Xufang Deng[1], Alexandra Buckley[2], Kelly Lager[2]

[1]Oklahoma State University, [2]National Animal Disease Center, USDA-ARS. xufang.deng@okstate.edu

Session: Virology 4, 2024-01-22, 5:00 - 5:15

Objective: Porcine epidemic diarrhea virus (PEDV) is a swine enteric coronavirus that causes severe diarrhea, dehydration, and death in neonatal pigs with a 90-100% mortality. Currently available vaccines provide only partial protection due to their inability to elicit robust lactogenic immunity in dams that protect neonatal pigs. Without access to a safe, effective vaccine, PEDV continually spreads with an average of 10-15% positivity and poses a significant threat to the pork industry. The overarching goal of this project is to develop live-attenuated vaccine prototypes with improved immunogenicity. Coronaviruses are infamous for their ability to suppress the host's innate immune responses via encoding multiple immune antagonists. Our and others' previous work characterized a panel of antagonistic molecules of PEDV. We hypothesize that the suppression of innate immunity by viral antagonists can lead to enhanced viral infection, tissue damage, and compromised adaptive immunity. The specific objectives of this work include I) identifying and characterizing key immune antagonists of PEDV, and II) identifying optimal mutation combinations of immune antagonists that maintain minimal pathogenicity but induce strong antibody responses in pigs.

Methods: Using reverse genetic platforms, we generated recombinant PEDVs that express single or combinations of mutated antagonist candidates and assessed viral replication in Vero cells. We also evaluated viral propagation and cellular innate immune responses to infections in porcine cell cultures. Viral pathogenicity and immunogenicity were assessed with pig models by scoring clinical signs, survival rate, fecal viral load, and antibody titers.

Results: We identified three authentic immune antagonists (Nsp1, 15, 16) of PEDV. Recombinant PEDV mutants carrying either each mutated gene alone or in a combination of three (mutNsp1/15/16) had similar replication kinetics in immune-deficient Vero cells but exhibited a marked replicative defect in immune-competent porcine kidney cell line PK1. Infection of PK1 with these mutant viruses produced higher levels of IFNs/ISG compared to the control cells infected with wild-type PEDV. These results suggest that the replicative defect is likely due to the elevated innate antiviral responses stimulated by the mutant viruses. Notably, the triple mutant (mutNsp1/15/16) had the most significant replicative attenuation and the highest IFN responses among the tested viruses, implying a combinatory/synergistic effect of multiple antagonists. Further piglet experiments with a dose of 500 $TCID_{50}$ revealed that mutNsp1/15/16 were highly attenuated in piglets with healthy intestinal tissue, low fecal viral load, and no clinical disease. Antibody test results showed that mutNsp1/15/16 infection could mount 4-fold lower antibody titers compared to wild-type virus infection after a single, low-dose infection.

Conclusions: Collectively, our study determined that Nsp1, 15, and 16 were authentic immune antagonists that inhibit the host's innate antiviral response and contribute to the enteric pathogenesis of PEDV. Our work also demonstrated that mutating multiple immune antagonists did not compromise viral propagation in immune-deficient cells that could be used for producing vaccine seed stocks but the resultant virus could be profoundly attenuated in pigs, which provided a practical approach to generate live-attenuated vaccine prototypes.

Financial Support: This project was supported by Agriculture and Food Research Initiative Competitive Grant no. 2023-67015-39096 from the USDA National Institute of Food and Agriculture.

Notes:

183 - Development of long-read targeted-whole genome sequencing for African and classical swine fever viruses.

Chester D. McDowell[1], Taeyong Kwon[1], Patricia Assato[1], Emily Mantlo[1], Jessie D. Trujillo[1], Natasha Gaudreault[1], Jayme A. Souza-Neto[1], Leonardo C. Caserta[2], Roman M. Pogranichniy[1], Diego D. Diel[2], Juergen A. Richt[1]

[1]Department of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, Kansas State University, [2]Department of Population Medicine and Diagnostic Sciences, Cornell University College of Veterinary Medicine. cdmcdow@vet.k-state.edu

Session: Virology 4, 2024-01-22, 5:15 - 5:30

Objective: African swine fever virus (ASFV) and classical swine fever virus (CSFV) are important transboundary animal diseases (TADs) affecting swine. ASFV is a large DNA virus with a genome size of 170-190 kilobases (kB) belonging to the family *Asfarviridae,* genus *Asfivirus*. CSFV is an RNA virus with a genome size of approximately 12 kB belonging to the family *Flaviviridae*, genus *Pestivirus*. Outbreaks involving either one of these viruses can result in massive culling of swine and export restrictions of pork products, leading to significant economic losses to the pork industry in affected countries. Current detection methods during an outbreak provide minimal genetic information on the circulating virus strains and genotypes. Due to the increasing availability and reduced cost of next generation sequencing (NGS), it is vital to have NGS protocols in place for the rapid identification and genetic characterization of ASFV and CSFV for the implementation of effective control measures.

Methods: In this study, panels of primers spanning the genomes of ASFV or CSFV were independently developed to generate approximately 10kB (ASFV) or 6kB (CSFV) amplicons. Primer panels consisting of 19 primer pairs for ASFV and 2 primer pairs for CSFV, provide whole genome amplification of each pathogen. These primer pairs were further optimized for batch primer pooling and thermocycling conditions. Following the optimization of primer pools, a total of 5 primer pools/reactions were used for ASFV and 2 primer pairs/reactions for CSFV. The ASFV primer pools were tested on viral DNA extracted from blood collected from pigs experimentally infected with ASFV genotype II viruses. The CSFV primer panel was tested on viral RNA extracted from 9 different strains of CSFV representing the 3 known CSFV genotypes and 21 clinical samples collected from pigs experimentally infected with 2 different genotype 1 strains. ASFV and CSFV amplicons from optimized PCR reactions were subsequently sequenced on the Oxford Nanopore MinION platform.

Results: The targeted protocols for amplification and sequencing of these viruses resulted in an average coverage greater than 1000X for ASFV with 99% of the genome covered, and 10,000X to 20,000X for CSFV with 97% to 99% of the genomes covered. The ASFV targeted whole genome sequencing protocol has been optimized for genotype II ASFVs which have been involved in recent outbreaks; optimization for other genotypes is in progress. The CSFV targeted whole genome sequencing protocol has potential universal applications for the detection of all currently known CSFV genotypes.

Conclusions: These targeted amplification and sequencing protocols will be important tools to assist in early pathogen detection and genomic characterization which may guide in the implementation of effective control measures should an outbreak of these high consequence swine viruses occur within the United States.

Financial Support: USDA APHIS award (agreement no. AP20VSD&B000C020), USDA-NSTP Fellowship, NBAF Transition Funding.

Notes:

184 - Characterizing infectious bursal disease virus (IBDV) reassortment and antigenic drift in the USA

Andrew Broadbent[1], Sofia Egana-Labrin[1], Andrew Brodrick[1], Milos Markis[2], Shankar Mondal[3]

[1]University of Maryland, [2]Aviserve LLC, [3]Maryland Department of Agriculture. ajbroad@umd.edu

Session: Virology 4, 2024-01-22, 5:30 - 5:45

Objective: Infectious bursal disease virus (IBDV) is a major challenge to the productivity of the US poultry industry. Novel antigenic drift variants and reassortant strains threaten the efficacy of vaccines, and it is therefore imperative to have current epidemiological data on what IBDV strains are circulating in the US, to maintain optimal control efforts. The Delmarva peninsula is a major poultry producing region, however the last characterization of circulating IBDV strains was 16 years ago. There is, therefore, an urgent need to increase IBDV surveillance in Delmarva, and evaluate the pathogenicity, immunosuppressive potential, and ability of strains to break through maternal antibody titers. Moreover, defining the mutations in IBDV that contribute the most to immune escape is vital for optimal disease control, as is developing tools to improve our understanding of how reassortant viruses emerge in nature, so we can better mitigate it. The objectives of this project are to: 1. Identify strains of IBDV circulating in Delmarva and evaluate their pathogenicity, immunosuppressive potential, and ability to break through maternal antibodies, 2. Define which amino acids contribute to immune escape, and 3. Develop a method to quantify IBDV reassortment frequency that can be used to model drivers and constraints.

Methods: 84 bursal samples were obtained from broiler farms in Delmarva between 2019 and 2023, and subject to reverse-transcription polymerase chain reaction (RT-PCR) to amplify the IBDV hypervariable region (HVR), encoded by Segment A, and the polymerase, encoded by Segment B. Sanger sequencing was conducted on positive samples, and the sequences of Segment A were analyzed for the presence of antigenic drift mutations, while the sequences of Segment B were used to evaluate if reassortment had occurred.

Results: We observed no evidence of very virulent strains circulating in Delmarva, or evidence of co-infection with multiple strains. We did, however, identify HVR mutations that were consistent with antigenic drift. In particular, four mutations appeared to have increased in frequency in the viral population since 2020, and may be important in driving immune escape.

Conclusions: Our observations are based on a limited number of samples, and ongoing efforts are aimed at increasing sampling. Future work aims to evaluate the pathogenicity, immunosuppressive potential, and the ability of the strains to break through maternal antibodies, as well as defining which amino acids contribute the most to immune escape, and developing a method to quantify IBDV reassortment frequency.

Financial Support: This research was supported by a U.S. Department of Agriculture, National Institute of Food and Agriculture, Agriculture and Food Research Initiative grant (2022-08113) and start-up funds from the University of Maryland.

Notes:

185 - Combining multiple sources of movement data strengthens traceability and disease surveillance

Sara C. Sequeira[1], Greg Habing[1], Andreia G. Arruda[1]

[1]Dep. Veterinary Preventive Medicine, The Ohio State University. correiasequeira.1@osu.edu

Session: Epidemiology 4, 2024-01-22, 4:15 - 4:30

Objective: Recent concerns with food safety in the United States (U.S.) have highlighted the importance of traceability systems in the food animal production chain. However, adoption of these systems in the U.S. presents various challenges. Interstate Certificates of Veterinary Inspection (ICVIs) are currently required for interstate animal movements. Regardless, it may be exempted under certain agreements, for instance Owner Shipper Statements (OSSs) which are not often considered in research. Dairy-beef calf production and distribution networks are understudied, yet important for understanding dynamics of disease transmission between animal and human populations. This study aimed to use Ohio-based movement records to describe calf networks in the U.S. and explore how OSSs impact the structure of calf networks built using ICVIs. We hypothesized that networks built exclusively using ICVIs will differ from those combined with OSSs.

Methods: Calf movement records to and from Ohio, accessible at the Ohio Department of Agriculture, were obtained through ICVIs and OSSs from June 2021 to June 2022, and analyzed using R. To explore and compare movement patterns, network analysis was performed individually for an ICVI-based network and a network combining both document types, using the R packages *igraph* and *visNetwork.* Zip codes were considered nodes and calf movements links. Whole-network (e.g., density, component ratio, etc.) and node-level (e.g., degree, eigenvector centrality, etc.) parameters were calculated for each network. Mann-Whitney U tests were performed to evaluate whether parameters differed statistically ($P<0.05$, 95% CI) by network type.

Results: The frequency of animal movements recorded through OSS (n=766, 49.8%) and ICVIs (n=772, 50.2%) was similar. Most animal movements included mixed sex (60.0%), dairy breeds (81.6%) and animals up to one week old (74.1%). Movements recorded through OSSs showed a larger median number of animals per movement (60; IQR 23-105) compared to ICVIs (49; IQR 16-80), reaching up to 696 calves per load. Failing to consider OSSs resulted in the absence of 40.3% of zip codes in the study. ICVI-based networks involved fewer zip codes across states, whereas combined networks exhibited a larger, denser and more cohesive network. The two analyzed networks revealed contrasting results regarding degree centrality, especially for out-going geographical regions or zip codes ($P< 0.01$), suggesting a discrepancy in their potential to influence dynamics of disease transmission. Lower eigenvector scores in the combined networks indicated a more integrated structure, with fewer influence of individual nodes over the full network ($P=0.01$).

Conclusions: Results suggest heterogeneous patterns of calf movements, depending on the source of records. OSSs are a representative proportion of the calf network in the state of Ohio. There were major differences in the movement structure when incorporating OSSs compared to when using ICVIs exclusively. This study emphasizes the importance of incorporating multiple sources of movement data for the development of targeted disease surveillance strategies.

Financial Support: This study was funded by the USDA (Grant No. 2022-6801536628), under the Agriculture and Food Research Initiative (AFRI) of the National Institute of Food and Agriculture (NIFA).

Notes:

186 - Prevalence of EHV-1 and EHV-4 in trigeminal ganglia and retropharyngeal lymph nodes in a random horse population

Edward Olajide[1], Amjad Khan[1], Jennifer G. Janes[1], Rebecca E. Ruby[1], Lutz Goehring[1]

[1]Department of Veterinary Science, University of Kentucky. Edward.Olajide@uky.edu

Session: Epidemiology 4, 2024-01-22, 4:30 - 4:45

Objective: Equid alphaherpesvirus 1 (EHV-1) and 4 (EHV-4) are pathogens of horses world-wide. In addition, EHV-1 is known for infection complications abortion and myeloencephalopathy. These viruses are known to establish latency in trigeminal ganglia and in respiratory tract associated lymphatics following primary exposure. It is essential to understand the mechanisms of latency and reactivation. The objective of this study was to assess the prevalence of EHV-1 and EHV-4 in latency locations trigeminal ganglion (TG), retropharyngeal lymph node (retLN), and tonsillary tissue ['pharyngeal roof'(PhR)] of horses randomly selected from a population.

Methods: A total of 89 horses [7 - 12 months of age (n= 12); >1 - 25years (n=77)] were included. Tissue samples were collected from horses that had been brought to the University of Kentucky Veterinary Diagnostic Laboratory for a necropsy exam and time of death/euthanasia was <24 hours. Tissues were preserved in formalin and then embedded in paraffin blocks. Total DNA was extracted. Subsequently, quantitative PCR analysis was conducted to quantify the genomes of EHV-1 and EHV-4. Statistical analysis was performed using IBM SPSS statistical software 26.0.0, and the Chi-square test was used to investigate age related association and distribution pattern.

Results: Thirty out of 88 horses tested positive for the glycoprotein B (gB) gene of EHV-1 in TG (34.1%), while 72.4% (63 out of 87) tested positive for EHV-4 (gB). Only 12 out of 89 (13.5%) were found to be infected with EHV-1, and 9.1% (8 out of 88) were infected with EHV-4 in the retLN. In the PhR, 22 out of 89 (24.7%) tested positive for EHV-1 while 9 out of 89 (10.1%) were infected with EHV-4. Notably, dual infection in horses was observed with both viruses in the retLN, PhR, and TG (1, 3, and 24 respectively). Furthermore, EHV-1 occurrence increased significantly with age in TG (P=0.004) and PhR (P=0.038), but not in retLN (P=0.099). Conversely, EHV-4 Prevalence in retLN was significant (P=0.008) among younger individuals while there was no age influence in TG (P=0.132) and PhR (P=0.192).

Conclusions: EHV4 prevalence appears to have an earlier clinical relevance than EHV-1 infection, as the age group <1 year was void of EHV-1 genome but was found frequently positive for EHV-4. The earlier assumption regarding latency in EHV-1 suggested it occurs early in life, but the current study has revealed that a certain age threshold must be reached. Conversely, for EHV-4, the study affirms that infection commences early in life without any age-related distinctions. An intriguing discovery is the notably low prevalence in the retropharyngeal lymph nodes (retLN) and tonsillary tissue among yearlings infected with EHV-1, and in young adult and adult horses infected with EHV-4. These further underscores that yearlings and foals are more commonly affected by EHV-4. Additionally, it is noteworthy that EHV-1 and EHV-4 genome copies are more abundant in the trigeminal ganglia compared to the other sites. Genome numbers are considered modest for all samples regardless of virus type with tonsillary tissue being the exception.

Financial Support: This study was supported by Gluck Equine Research Foundation.

Notes:

187 - Tracking the prevalence and antibiotic resistance of *Salmonella* isolated from a Wisconsin dairy farm over a year

Courtney Deblois[1, 2], Andrew Tu[3], Garret Suen[1], Andrew Scheftgen[3]

[1]Department of Bacteriology, University of Wisconsin-Madison, [2]Microbiology Doctoral Training Program, Department of Bacteriology, University of Wisconsin-Madison, [3]University of Wisconsin – Madison. cdeblois@wisc.edu

Session: Epidemiology 4, 2024-01-22, 4:45 - 5:00

Objective: Salmonellosis is a major disease of dairy cattle that has a significant adverse impact on animal health and productivity. *Salmonella* can colonize cattle asymptomatically and is shed through the feces into the farm environment. This asymptomatic nature can make it difficult to both identify and detect *Salmonella* in dairy herds. The serogroups of *Salmonella* associated with asymptomatic carriage, and their survival in the environment over time, has yet to be described. The objective of this study is to monitor the prevalence of *Salmonella* on a dairy farm in Wisconsin by sampling both dairy cattle and their environment biweekly over the course of a year. Isolations of *Salmonella* from collected samples were subjected to both serogrouping and antibiotic resistance profiles.

Methods: Fecal samples were collected biweekly over a 12-month period (n=25 collection points). At each sampling, fecal samples from the cow pens (i.e. the environment; n=8) and fecal samples obtained directly from the rectum of healthy lactating dairy cattle (n=12) were obtained. Fecal samples (n = 499 in total) were subjected to a *Salmonella* isolation protocol, serogrouped by agglutination, and tested for antibiotic resistance against 8 different antibiotics via disc diffusion.

Results: We found high prevalence of *Salmonella*, with an average of 90% of the cattle being carriers year-round. Percent recovery from environmental samples correlated with temperature and ranged from 40-90%. *Salmonella* serogroups C and K were found most abundantly on the farm, with serogroup C dominating the environment and K dominating direct fecal samples. Antibiotic resistance was found to be highest for neomycin and sulfadimethoxine, averaging 43% and 86% of the isolates showing signs of resistance to these two antibiotics, respectively. A total of 1-2 isolates were found to be sporadically resistant to other antibiotics tested; however, there were no correlations of antibiotic resistance between time, location, sample type, or serogroup.

Conclusions: The prevalence of *Salmonella* found in this study is higher than what is previously documented, but is likely due to the subclinical nature of the serogroups isolated (C and K). The majority of environmental isolates belonged to group C while group K dominated the direct fecal samples, suggesting that these serogroups may have niche specificity for their respective locations. Overall, antibiotic resistance was low with the exception of neomycin and sulfadimethoxine. Whole-genome sequencing of our isolates is currently being performed to determine genomic stability as a potential means for niche specificity.

Financial Support: This research was supported by the U.S. Department of Agriculture (USDA) National Institute of Food and Agriculture Predoctoral Fellowship grant no. 2023-67011-40521 supporting C.L.S. and a USDA National Institute of Food and Agriculture HATCH grant WIS04039 to GS.

Notes:

188 - Prevalence of Salmonella Dublin in mesenteric lymph nodes of special-fed veal calves from the US and Canada

Samantha Locke[1], Poonam Vinayamohan[1], Alejandra Arevalo-Mayorga[1], Victoria Schaffner[1], Alex Dunmyre[1], Jamie Ridolfo[1], Greg Habing[1]

[1]The Ohio State University College of Veterinary Medicine. locke.91@osu.edu

Session: Epidemiology 4, 2024-01-22, 5:00 - 5:15

Objective: *Salmonella* serovar Dublin is known to cause severe, invasive disease in both cattle and humans, constituting a public health threat. Cattle are considered the primary reservoir of *S.* Dublin, with calves particularly susceptible to infection and transmission. Previous sampling of special-fed veal calves has documented lymph node carriage of *S.* Dublin at harvest and may represent a public health concern, yet the geographic distribution of *S.* Dublin in special-fed veal production is not well understood. The objective of this study was to assess the prevalence of S. Dublin at harvest from special-fed veal calves raised in the United States and Canada. We hypothesized that *S.* Dublin lymph node carriage would be associated with the geographic origin of calves.

Methods: Twenty-two cohorts of special-fed veal calves originating from the United States (n=15) and Canada (n=9) were sampled. For two cohorts, calves from the US and Canada were housed in the same holding pen prior to sampling. Mesenteric lymph nodes were collected from 30 calves per cohort, for a total of 660 calves sampled. Samples were cultured following a standardized protocol for *Salmonella* isolation, and recovered *Salmonella* isolates were then serogrouped. Serogroup D1 isolates, to which Dublin belongs, were submitted for whole genome sequencing. Source information down to the state level was collected for calves grown in the U.S., while province was available for Canadian calves. Fisher's exact tests were used to assess the association between carriage of Dublin and location.

Results: Overall prevalence of *Salmonella* in mesenteric lymph nodes was 45.9% (303/660). All cohorts had at least one sample positive for *Salmonella*, with cohort-level prevalence of *Salmonella* spp. ranging from 3-100%. Cohort-level prevalence of *S.* Dublin ranged from 0-70%. *Salmonella* Dublin carriage was significantly associated with location (p-value < 0.02). Dublin was recovered from over half of sampled cohorts, with three Canadian and ten US cohorts positive. Multiple *Salmonella* serogroups were recovered from 10.9% (33/303) lymph nodes. Serogroup D1 was most frequently recovered from samples (29%), followed by serogroup B (23.8%) and E or G (22.8%).

Conclusions: Special-fed veal calves are a reservoir for *Salmonella* Dublin, and Dublin carriage is associated with geographic origin of calves. In order to design and implement effective preharvest interventions, future research should evaluate the extent of *S.* Dublin endemicity in veal calf production, as well as the timing of Dublin infection.

Financial Support: This research is funded by the USDA National Institute of Food and Agriculture grant no. 2022-68015-36628ca.

Notes:

189 - Effect of *Salmonella Dublin* latent-carrier dry cow vaccination on bacterial shedding and intrauterine transmission

R. Castro-Vargas[1], F. Cullens-Nobis[2], R. Mani[1], J. Roberts[3], A. Abuelo[1, 2]

[1]College of Veterinary Medicine, Michigan State University, [2]Michigan State University Extension, [3]Boehringer Ingelheim Animal Health. castrov8@msu.edu

Session: Epidemiology 4, 2024-01-22, 5:15 - 5:30

Objective: *Salmonella* Dublin latent-carrier cows represent a high risk for the transmission of infection to newborn calves via intra-uterine transmission and shedding of bacteria in feces and colostrum at calving. Vaccination of these latent-carriers dams during late gestation boosts the immunity against *S.* Dublin. This could reduce the activation of the dormant bacterium during the periparturient immune dysfunction period, thereby reducing the risk of early-life infection in the offspring. Thus, the objective of this study was to evaluate the extent to which vaccinating *S*. Dublin latent-carrier cows at dry-off with a commercial live bacterial vaccine (Entervene-D, Boehringer Ingelheim) reduces bacterial shedding at calving and intrauterine infection to calves.

Methods: To identify latent-carriers, we screened 1,084 cows in 4 Michigan commercial dairy farms with a history of *S*. Dublin. Cows were defined as latent-carriers when they showed three consecutive positive milk antibody ELISA tests conducted every two months. Subsequently, 148 latent-carriers were randomly allocated to the vaccine or control group. Vaccine cows received the commercial vaccine s.c. at dry-off and a booster two weeks later. Control cows received saline s.c. at the same times. At calving, we collected fecal and colostrum samples from the dam and a pre-colostral serum sample from the calf. Bacterial shedding was evaluated in feces and colostrum both qualitatively (Yes/No) and quantitatively through the bacterial enrichment culture method (ISO 6579-1:2017) and qPCR quantification of gene *vagC* copy numbers, respectively. Intrauterine transmission was defined when a calf was positive for serum antibody ELISA at birth. Results were evaluated via logistic regression for qualitative shedding and intrauterine transmission. A t-test was used to compare the number of *S*. Dublin copies estimated via qPCR.

Results: Vaccination decreased the likelihood of calves being born with *S*. Dublin antibodies (Relative Risk [95%CI]) = 0.19 [0.04 - 0.84]). However, no *S.* Dublin-positive isolates were identified through either bacteriological culture or qPCR in feces or colostrum.

Conclusions: Vaccination of *S.* Dublin latent-carrier cows at dry-off reduced intrauterine transmission to calves. This strategy could contribute to decreasing the transmission of *S.* Dublin in dairy farms. Additionally, the absence of *S.* Dublin positive fecal and colostrum samples warrants further evaluation of the traditional methods for identification of latent-carriers or *S.* Dublin isolation, as well as the role of latent-carriers in infecting newborn calves in the maternity area at birth.

Financial Support: Boehringer Ingelheim Animal Health USA; U.S. Department of Agriculture, NIFA grant # 2022-68008-36354.

Notes:

190 - Quantifying the impact of metaphylaxis on the abundance of key members of the respiratory microbiome in cattle

Molly McClurg[1], Alison Olsen-Gerlach[2], Matthew Scott[1], John Richeson[3], Lee Pinnell[1], Paul S. Morley[1], Robert Robert Valeris- Chacin[1]

[1]VERO Program, Texas A&M University, [2]Texas A&M School of Veterinary Medicine and Biomedical Sciences, [3]West Texas A&M University. mcclurgmc@tamu.edu

Session: Epidemiology 4, 2024-01-22, 5:30 - 5:45

Objective: Bovine Respiratory Disease (BRD) leads to substantial economic losses in the beef industry. A common control strategy is the use of antimicrobial drugs (AMD) in cattle upon facility arrival (metaphylaxis). However, limited knowledge exists on the impact of metaphylaxis on the bacterial populations in cattle over time. This study aimed to quantify the effect of metaphylaxis on the abundance of BRD-related bacteria in post-weaned beef cattle.

Methods: Cattle (N=105) were randomly stratified by weight into six treatment groups and a negative control group (n=15). The six treatment groups were given their respective metaphylaxis on Day 0: tulathromycin (TU), florfenicol (FL), oxytetracycline (OX), tildipirosin (TI), ceftiofur (CE), enrofloxacin (EN). Each treatment pen consisted of ten treated animals and five animals that were not treated that served as sentinel animals. Nasopharyngeal swabs were taken at six time points (Days 0, 3, 7, 14, 21, and 56). DNA was extracted from the swabs using QIAamp PowerSoil Pro Kit (Qiagen). The absolute abundance of *Mannheimia haemolytica, Mycoplasma bovis, Histophilus somni,* and *Pasteurella multocida* was quantified using digital qPCR in a microfluidic platform (QuantStudio Absolute Q digital PCR system, Applied Biosystems). Generalized linear mixed models (binomial distribution, complementary log-log link, animal ID as random intercept, and $P<0.05$) were used to compare the pathogens' absolute abundance between treatment groups and time points.

Results: Currently, we have results for three out of the six treatment groups (TU, FL, OX), and expect to have results for the last 3 treatment groups (TI, CE, EN) by the end of August 2023. TU, FL, and OX significantly reduced the *M. haemolytica* abundance in comparison to NC on days 21 and 56. A significant reduction of *M. bovis* abundance on days 7, 14, and 21 was detected only in TU. Despite an initial decrease, *P. multocida* abundance on day 56 in the OX group was significantly higher than in the other groups. Overall, *H. somni* abundance was very low in all groups and therefore, no meaningful inference could be drawn. Additionally, TU reduced the prevalence of *M. bovis*, *M. haemolytica*, and *P. multocida* by day 56.

Conclusions: The abundance of BRD-related bacteria was significantly lowered with TU in the long term when compared to FL and OX.

Financial Support: Texas A&M School of Veterinary Medicine, FFAR Veterinary Summer Fellowship, Texas Cattle Feeders Association, and Texas A&M AgriLife Research.

Notes:

191 - Does the fecal microbiome-resistome profile differ between samples collected sequentially from the same pig?

Tara Gaire[1], Jared Young[1], Mark Schwartz[1], Randall Singer[2], Maria Pieters[1], Noelle Noyes[1]

[1]Department of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota, [2]Department of Veterinary Biomedical Sciences, College of Veterinary Medicine, University of Minnesota. tgaire@umn.edu

Session: Metagenomics, 2024-01-22, 4:15 - 4:30

Objective: Variation in microbiome data poses the challenge of determining whetherdifferences between sequence features measurements are caused by biological differences, orby technical factors that occur during sampling collection and processing. Multiple sequentialsampling of the same body site is common in microbiome research, particularly whenresearchers plan to analyze samples using multiple workflows. For example, multiple fecalswabs from an individual animal may be collected at one sampling event, with each swabsubsequently being used for different workflows (i.e., culture versus molecular workflows). It isunknown whether such back-to-back, sequentially collected samples yield similar resistome-microbiome profiles; or whether sample order matters. The primary objective of this study is tounderstand the variability in fecal microbiome-resistome data derived from back-to-back, sequentially-collected replicates from the same animal. A secondary objective is to determinewhether comparable resistome-microbiome profiles are obtained using two library preparationapproaches, one of which is significantly more cost-effective than the other.

Methods: In a single commercial swine facility, a total of 32 pre-weaned piglets (~20 days old) were selected from five pens. Two sequential fecal swab samples (termed "A", i.e., first sample, and "B", second sample) were collected from individual piglets within each pen, at the sametime on the same day (N= 64 samples). The sampler's gloves were changed between collectionof each sample (including between the two samples from each pig). Each swab was placed intoan individual sterile Whirl-Pak and stored at -80°C. Metagenomic DNA was extracted from eachsample using the MagAttarct PowerSoil Pro DNA kit with KingFisher MagMax robotics. TheseDNA samples will be subjected to shotgun metagenomic library preparation using two librarypreparation workflows, one that includes the full volume of transposase and sample ("full") andthe other using a quarter-volume of each ("quarter"). Libraries will be sequenced to an expecteddepth of ~17M reads per sample. After obtaining the sequencing data, we will performbioinformatic analysis using the AMR++ v3 pipeline to identify ARGs and microbial taxa in eachlibrary. Resistome and microbiome profiles will be compared by replicate (A versus B) and bylibrary preparation workflow (full versus quarter), while accounting for clustering of pigs by pen, and repeated measures within pig and sample.

Results: Our data suggest that the average number of raw reads, the percentage of reads aligned with the resistome, and the normalized ARG counts were similar between samples obtained from full and quarter-reaction workflows. Similarly, we observed minimal variation in the overall ARG and microbial compositions, as well as the average ARG and microbial richness values, regardless of whether they were obtained from the full or quarter-reaction workflows or from samples A and B.

Conclusions: Our study indicates that the lower-cost, quarter-volume library preparation is a cost-effective alternative to full-volume, providing comparable detection of resistome and microbiome diversity in piglet feces, with consistent results between replicates (A versus B samples). We expect that these findings will inform the design of microbiome intervention studies in production pig populations.

Financial Support: This work is supported by Agriculture and Food Research Initiative grant no. 2021-68015-33499 from the USDA National Institute of Food and Agriculture.

Notes:

192 - Detection of multiple porcine viruses from oral fluid samples through TELSVirus workflow

Mariana Meneguzzi[1], Jonathan Bravo[2], Christina Boucher[2], Montserrat Torremorell[1], Noelle Noyes[1]

[1]Department of Veterinary Population Medicine, University of Minnesota, [2]Computer & Information Science & Engineering Department, University of Florida. meneg009@umn.edu

Session: Metagenomics, 2024-01-22, 4:30 - 4:45

Objective: Viral co-infections have become a common health challenge in swine farms worldwide, contributing to aggravated disease outcomes. Whole-genome sequencing (WGS) of viral genomes can improve our understanding of viral co-circulation dynamics in swine herds. Although the outcomes from WGS seem promising, the current methods for WGS are not able to detect all possible viruses in a sample, while also capturing the viruses' genetic diversity. As a result, our ability to study viral dynamics as well as the detection and characterization of potential emerging pathogens is limited. Therefore, our goal was to develop a workflow named "TELSVirus", or "Target-Enriched Long-read Sequencing of Virus" to more readily characterize the diversity of viral genomes in a sample.

Methods: The TELSVirus workflow combines a bait-capture method with long-read, real-time sequencing technology by Oxford Nanopore and an ensemble bioinformatics pipeline for data analysis. The bait-capture method can selectively capture specific genomes from a complex sample. For that, we bioinformatically designed a panel of probes (baits) that selectively targets 44 swine viruses. The probes were designed to cover 100% of all complete genomes for the 44 viruses comprising a total of 16,069 reference genomes. The captured viral sequences are then further enriched and sequenced. Six oral fluid samples collected from growing pigs were subjected to the TELSVirus workflow. RNA was extracted by QIAamp® Viral RNA Mini Kit, followed by complementary DNA synthesis. Probe hybridization and enrichment were performed using the SureSelect XT HS2 DNA System (Agilent Technologies). Subsequently, library preparation (SQK-PB004) was performed prior to loading the samples in the minION flow cell.

Results: Our data show the detection of multiple viruses in the oral fluid samples. Among all the samples, bocavirus, atypical porcine pestivirus, toroviruses, and porcine astrovirus 2 and 4 were the most detected viruses resulting in high horizontal coverage of individual genomes of between 70% and 100%. Aside from the cited viruses, other viruses such as porcine reproductive and respiratory syndrome virus, teschovirus A, porcine circovirus 2, and porcine sapelovirus were detected, although with lower horizontal coverage. The mean depth of coverage ranged from 0 to 2566X, with the highest coverage depth for porcine bocaviruses and toroviruses.

Conclusions: In conclusion, the TELSVirus workflow was able to detect multiple viruses from field samples. Generated results will aid in providing a better understanding of genomic diversity of under-characterized viruses, thus improving knowledge on the ecology and epidemiology of viral co-infections. Future work aims to apply the TELSVirus workflow to 200 oral fluid samples from Midwestern U.S. herds suspected of harboring multiple co-circulating viruses. Additionally, haplotype calling will be performed to further characterize the viruses' genetic diversity, which can aid in understanding viruses' evolution, epidemiology, transmission dynamics, and monitoring the emergence of new viral variants. Also, performance data from each farm will be included in the analysis to assess the impact of overall genetic diversity on production performance parameters.

Financial Support: This work is supported by Pipestone MorriSTONE Research Award, Education and Extension Technology Transfer Program, Minnesota Agricultural Research, USDA Hatch Capacity Grant Funding, National Pork Board, NIH National Institute of Allergy and Infectious Disease (NIAID), and Swine Health Information Center.

Notes:

193 - The effect of storage on bacterial viability in equine fecal microbial transfer fluid

O. Jacob[1], J. Parker [1], V. Stanton[2], E. Yopp[1], J. Parsons[1], A. Page [1], B. Davis[3], M. Flythe[3], E. Adam[1]

[1]Department of Veterinary Science, Gluck Equine Research Center, University of Kentucky, [2]Department of Statistics, University of Kentucky, [3]USDA-FAPRU, University of Kentucky. ocja224@g.uky.edu

Session: Metagenomics, 2024-01-22, 4:45 - 5:00

Objective: Antibiotic drugs are essential tools in human and veterinary medicine to prevent disease. However, they can disturb gastrointestinal microbiota and can cause antibiotic-associated diarrhea. Fecal microbial transfer (FMT) has been used to combat the deleterious effects of antibiotics on the gastrointestinal tract microbiota. In humans there is considerable evidence to support its use in cases of chronic antibiotic associated diarrhea. It is also a technique frequently used in equine clinical practice in horses with diarrhea. The preparation of fresh FMT liquid in clinical equine practice is time consuming and cumbersome. As such, the purpose of this study was to understand whether diluent type or storage conditions of FMT liquid affect bacterial viability in FMT liquid. This was performed by enumerating cellulolytic bacteria, amino acid and peptide degrading bacteria (APB), and *Lactobacillus* spp. under various storage conditions using two diluents. The results of this study can direct and inform veterinary clinicians by avoiding FMT liquid storage conditions that have deleterious effects on these important populations of bacteria.

Methods: Fresh feces were collected from healthy adult horses at the time of natural defecation and immediately processed. These apparently normal, healthy horses had not received antibiotic drugs during the 6 months prior to sampling and were maintained on pasture at University of Kentucky's Research Farm. FMT liquid was prepared using 500 g of fresh feces either hand mixed or blended with 1L of either 37°C tap water or sterile 0.9% saline solution. FMT liquid was stored under three-time conditions (0h, 8h, 24h) and four temperature conditions (-20°C, 4°C, room temperature, 37°C) prior to inoculation of media. Each set of conditions were tested with 8 biological replicates. Bacterial enumerations were performed in serial 10-fold dilutions using appropriate liquid or solid media. Each horse acted as its own control and differences in $\log_{10}$ bacterial enumerations were expressed as the difference from the time zero sample.

Results: Cellulolytic and *Lactobacillus* spp. bacterial numbers are well maintained after 8h at 4°C when compared to other storage conditions of the FMT liquid. There were no statistically significant differences in bacterial viability numbers detected between the two diluents under these study conditions.

Conclusions: FMT liquid bacterial viability did not appear to be affected by diluent type or whether it was blended or hand mixed. The storage of FMT liquid for 8 hours at 4°C may be suitable for clinical use if fresh FMT liquid is not available. However, further work is required to understand how the bacterial populations may be affected by storage.

Financial Support: This research was sponsored by the Summer Undergraduate Research Fellowship at University of Kentucky.

Notes:

194 - The impact of kit, environmental and sampling contamination on the observed microbiome of bovine milk

Yale Deng[1], Christopher J. Dean[1], Thomas C. Wehri[2], Felipe Pena-Mosca[1], Tui Ray[1], Brain A. Crooker[2], Sandra S. Godden[1], Luciano S. Caixeta[1], Noelle R. Noyes[1]

[1]Department of Veterinary Population Medicine, University of Minnesota, [2]Department of Animal Science, University of Minnesota. deng0291@umn.edu

Session: Metagenomics, 2024-01-22, 5:00 - 5:15

Objective: Obtaining a non-contaminated sample of bovine milk is challenging due to the nature of the sampling environment and the route by which milk is typically extracted from the mammary gland. Furthermore, the very low bacterial biomass of bovine milk exacerbates the impacts of contaminant sequences in downstream analyses, which can lead to severe biases. The objective of this study is to fill the critical knowledge gap, namely, does non-mastitis bovine milk even contain a native microbiome?

Methods: In this study, we sampled external (teat apex) and internal (teat canal) mammary epithelium and stripped and cisternal milk, used numerous negative controls (sampling blanks, extraction blanks and library blanks) to identify potential sources of contamination, and used two algorithms (*decontam* and *Sourcetracker*) to mathematically remove this contamination from our sample microbiomes and to track potential movement of microbes among our samples. Milk samples were also subjected to microbiological culture to identify the correlation with the outcome of 16S rRNA sequencing.

Results: Our results suggest that the vast majority (i.e., >75%) of the sequence data generated from bovine milk and mammary epithelium samples represents contaminating DNA. The contaminants in milk samples were primarily sourced from the DNA extraction kits and the internal (canal) and external (apex) skin of the teat, while the teat canal and apex samples were mainly contaminated during the sampling process. After decontamination, the milk microbiome displayed a more dispersed, less diverse and compositionally distinct bacterial profile compared to the teat canal and apex, though similar microbial compositions were observed between cisternal and stripped milk samples, as well as between teat apex and canal samples. *Staphylococcus* and *Acinetobacter* were the predominant taxa detected in the sequences obtained from milk samples, and bacterial culture showed growth of *Staphylococcus* and *Corynebacterium* in 50% (7/14) of stripped milk samples and growth of *Staphylococcus* in 7% (1/14) of cisternal milk samples.

Conclusions: Our study suggests that microbiome data generated from milk samples obtained from clinically healthy bovine udders may be heavily biased by contaminants that enter the sample during the sample collection and processing workflows. A major reason for this finding likely stems from the fact that bovine milk contains very low bacterial biomass, and thus each contamination event (including the DNA extraction process itself) introduces bacteria and/or DNA fragments that easily outnumber the native bacterial cells. This finding has important implications for our ability to draw robust conclusions from milk microbiome data, especially if the data have not been subjected to rigorous decontamination procedures. We strongly urge researchers to include numerous negative controls into their sampling and sample processing workflows; and to utilize several complementary methods for identifying potential contaminants within the resulting sequence data. These measures will improve the accuracy, reliability, reproducibility, and interpretability of milk microbiome data and research.

Financial Support: This work was supported by the Multistate Research Project NE1748 as USDA Hatch Project MIN-62-126, and Organic Agriculture Research and Extension Initiative (OREI), and National Institute of Food and Agriculture (Grant Number: 2018-51300-28563).

Notes:

195 - Enriching without culture: Target-enriched metagenomics allows for strain-level characterization of *M. haemolytica*

Enrique Doster[1], Cory Wolfe[1], William B. Crosby[2], Michael L. Clawson[3], Amelia R. Woolums[2], Lee J. Pinnell[1], Paul S. Morley[1]

[1]Veterinary Education, Research, and Outreach Program, Texas A&M University, [2]Department of Pathobiology and Population Medicine, College of Veterinary Medicine, Mississippi State University, [3]USDA, Agriculture Research Service US Meat Animal Research Center. enriquedoster@tamu.edu

Session: Metagenomics, 2024-01-22, 5:15 - 5:30

Objective: Bovine Respiratory Disease (BRD) represents a significant economic burden in cattle farming, accounting for substantial losses in productivity and health management costs. As *Mannheimia haemolytica* is a key pathogen associated with BRD, understanding its role and diversity in respiratory infections is crucial. This study aims to develop a comprehensive target-enriched metagenomic workflow that would enable detailed characterization of strain-level diversity of *M. haemolytica* within bovine respiratory microbial communities, thereby facilitating a deeper understanding of its impact on BRD.

Methods: We first developed a custom Agilent SureSelect bait system for targeted metagenomic sequencing of *Mannheimia haemolytica*. Furthermore, we refined the bioinformatic classification process for metagenomic reads, which traditionally has been a challenge at the strain level due to limited metadata for each genome. By employing the Kraken2 classifier and modifying its taxonomic labeling based on the sequence similarity of all publicly available *M. haemolytica* genomes (n=2106), we created a unique database of "phylogenetic sequence cluster variants (PSVs)". This innovative approach aims to enhance the resolution of strain-level classification by removing the reliance on accurate metadata and instead adopting a data driven approach that better leverages the growing number of genome sequences. In addition, we developed the VARIANT++ pipeline, which incorporates QC trimming, host DNA removal, and taxonomic classification to identify PSVs for a given bacterial species. To evaluate our workflow, we analyzed 39 nasal swab samples from feedlot cattle, categorized into five groups based on *M. haemolytica* relative abundance (>30%, 10-30%, 1-10%, 0.1-1%, and 0%) determined through 16S rRNA amplicon sequencing.

Results: Our results indicate a substantial improvement in the classification of *Mannheimia haemolytica* at the strain level. The custom bait design successfully enriched *M. haemolytica* sequences in our samples, with an average of 90% (range 50-93%) of non-host reads being classified to at least the species level. Notably, on average approximately 10% of these reads could be classified at a more refined strain or "PSV" level, a marked increase compared to less than 0.25% achievable with standard taxonomic labeling. This enhanced classification allowed the identification of over 300 unique PSVs per sample, including samples considered to have a low abundance of *M. haemolytica* with 16S sequencing. Interestingly, preliminary results show that the overall composition of *M. haemolytica* PSVs was similar across different sample groups, the presence of certain low-abundance PSVs varied, suggesting potential strain-specific associations.

Conclusions: This study represents a novel metagenomic approach that enables strain-level characterization of *Mannheimia haemolytica* and provides a new perspective for investigating the complex dynamics within bovine respiratory microbial communities. The identification of a diverse community of PSVs highlights the fluid genetic landscape of *Mannheimia haemolytica* and underscores the need for continued exploration into their biological significance and potential implications for BRD epidemiology.

Financial Support: Funded by Texas A&M's VERO Program and employs datasets from projects funded with support from USDA NIFA, West Texas A&M's Agricultural Sciences Dept., Mississippi State's Veterinary Medicine Dept., and industry partner Phileo by Lesaffre.

Notes:

196 - Environmental, group, and individual sampling for characterizing the ecology of *Mannheimia haemolytica* in cattle

Stephen Tamm[1], William Crosby[2], Lee Pinnell[1], Enrique Doster[1], Benjamin Newcomer[1], Jenna Funk[1], Sarah Capik[1], Cory Wolfe[1], John Richeson[3], Sheryl Gow[4], Robert Valeris-Chacin[1], Amelia Woolums[2], Paul S. Morley[1]

[1]VERO Program, Texas A&M University, [2]College of Veterinary Medicine, Mississippi State University, [3]West Texas A&M University, [4]Public Health Agency of Canada, University of Saskatchewan. sctamm@tamu.edu

Session: Metagenomics, 2024-01-22, 5:30 - 5:45

Objective: *Mannheimia haemolytica* (Mh) is one of the most important pathogens associated with bovine respiratory disease (BRD) in feedlot cattle. Our research team is working to improve understanding of the ecology of Mh in cattle populations, with the goal of improving the ability to control and prevent BRD. Traditional methods for detection and characterization of Mh strains in individuals and populations involve intensive sampling of individual cattle, and genomic strain characterization has only followed from culture of individual isolates. The purpose of this study was to investigate use of culture-free molecular tools in combination with novel sampling methods for characterization of sequence variation and molecular ecology of Mh recovered from groups of cattle.

Methods: We used ropes hung above feed bunks, swabs of water bowls, and pools of respiratory swabs to investigate group sampling, comparing results to nasal swabs of individual cattle. Animals enrolled in this study included groups of different types of feedlot cattle, backgrounding cattle, and young calves in hutches. Target-enriched shotgun sequencing (TE) of extracted DNA was used to detect and characterize Mh phylogenetic sequence cluster variants (PSV) and antimicrobial resistance genes (ARG). The composition of the microbiome was characterized using 16S rRNA sequencing of extracted DNA. Sequence data were analyzed using AMR++, VARIANT++, and QIIME2 pipelines, and statistical analyses were performed using phyloseq in R.

Results: Analysis of TE data identified a total of 559 unique Mh PSVs unevenly distributed in all samples such that the majority of PSVs detected belong to only 15 PSV classifications. PSV richness was significantly lower in water bowl samples than rope and nasal samples although Shannon's diversity was similar among all sample types. Ordination using non-metric multidimensional scaling (NMDS) indicated that the PSV community composition was highly similar across all sample types with water bowls having the greatest variation, followed by nasal and rope samples, respectively. NMDS also illustrated that PSV composition was similar across all study groups. Analysis of 16S rRNA data revealed significantly lower richness in the microbiome of water bowl samples than rope and nasal samples. NMDS plots indicated that microbial community composition of individual and composite nasal swab samples were nearly identical and that all sample types shared some degree of overlap. Highly similar community composition was observed in samples collected from groups of cattle in similar locations while the greatest dissimilarity was observed in the two most distinct populations groups.

Conclusions: Similarity of the Mh PSV composition and microbial community composition detected in nasal swabs as well as environmental samples suggests that respiratory microbial communities of cattle were significantly represented in environmental communities. Findings also suggest that pooling DNA or swab material from individual nasal samples provides a reliable population-averaged perspective for groups of animals allowing for resources to be redirected towards more groups. Additionally, TE shotgun sequencing provided intriguing insight into the suitability of employing environmental sampling in detection and characterization of Mh.

Financial Support: This research was supported by USDA: APHIS:VS: Center for Epidemiology and Animal Health.

Notes:

197 - Coronaviruses: Past, present and future threats to animals and humans

Linda J. Saif[1]

[1]Center for Food Animal Health Research, Animal Sciences Department, Ohio Agricultural Research and Development Center, College of Food, Agricultural and Environmental Sciences; Veterinary Preventive Medicine Department, College of Veterinary Medicine, The Ohio State University. saif.2@osu.edu

Session: ACVM - Featured Speakers, 2024-01-23, 8:30 - 9:15

The precipitous global spread of the betacoronavirus (CoV), Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) ignited a devastating and ongoing pandemic. It was preceded in the past 2 decades by the deadly SARS (2003) and MERS (2012) betaCoV zoonoses. Most mammalian CoVs, including the endemic human CoVs, likely originated from ancestral CoVs in bat reservoirs, with some infecting intermediate animal hosts (wildlife: SARS--civet cats; domesticated animals: MERS--camels) prior to spillover into humans or other animals. Notably betaCoVs from wild ungulates (cervids) experimentally infect cattle; historically cattle likely transmitted them to other species (humans, pigs, dogs, poultry). Like other RNA viruses, CoVs frequently mutate and recombine to generate new CoV species or variants that escape existing immunity and acquire new tissue or host tropisms that increase their transmissibility and broaden their host range and interspecies/zoonotic transmission. To highlight these concepts, swine CoVs provide historical precedents for understanding CoV emergence and evolution in a host species, with lessons applicable to SARS-CoV-2.

Multiple factors influence interspecies and zoonotic CoV transmission: the environment (e.g., habitat loss and encroachment, wet markets, with animal/human contact); the pathogen (unique features of RNA viruses, stability, transmissibility); and reservoir-host interactions (viral receptors, type/frequency of exposure, superspreaders, etc). Live animal markets were implicated in SARS-CoV and potentially SARS-CoV-2 outbreaks. Besides the high transmissibility of SARS-CoV-2 in humans and the emergence of variants, a major concern for viral persistence is its spillover (reverse zoonoses) and adaptation to animals. SARS-CoV-2 spilled over from humans to pets (cats, dogs, hamsters), farmed mink and various wildlife (white-tailed deer) with evidence for spillback and secondary transmission to humans. To date at least 34 species, many of them wildlife, are susceptible to SARS-CoV natural or experimental infection. Ominously, if SARS-CoV-2 becomes established in an animal reservoir(s), the virus can persist, mutate and continue to evolve in the new host species, extending its host range and its potential to reinfect humans or other species. Based on historical precedent and the continued presence of SARS-related CoVs in bats and new host reservoirs, novel CoVs will continue to emerge in animals and humans. New emergent CoVs that may pose future threats (WHO “Disease X”) include the human canine alphaCoV and porcine deltaCoV recently detected in children. A One Health approach encompassing global disease surveillance, international collaboration and trans-disciplinary research teams is critical to prevent and control future pandemic threats (reviewed in https://doi.org/10.1073/pnas.2202871119). My talk will address historical and comparative aspects of CoV infections/interspecies transmission and their past, present and future threats to animals and humans.

Notes:

198 - Pathogenesis of murine and human coronavirus infections

Stanley Perlman[1]

[1]Dept. of Microbiology and Immunology, University of Iowa. stanley-perlman@uiowa.edu

Session: ACVM - Featured Speakers, 2024-01-23, 9:15 - 10:00

Mouse hepatitis virus (MHV) is a group of sarbecoviruses with similarities to the severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2). Prior to the pandemic, it was considered a prototypic coronavirus and was used in many studies of the molecular biology and pathogenesis of these viruses. Mouse hepatitis virus is not a single virus, but rather includes several strains with the ability to infect many different organs, including the liver, gastrointestinal tract, the lungs and the brains. In some cases, single strains infect all of these organs while in other cases, a single strain will exhibit tropism for a single organ. In addition, some of the infections have immunopathological components. It is well known that enteric strains of MHV are highly contagious and are the bane of research mouse colonies. Remarkably, all of these viruses use the same host cell receptor to enter cells, showing that expression of the receptor is necessary but not sufficient for infection.

In this presentation, the clinical and pathophysiological manifestations of murine CoV infections with be discussed. One particular strain of MHV, the JHMV strain, causes acute and chronic demyelinating diseases with resemblance to the human disease multiple sclerosis. This strain shows a strict tropism for the brain and does not infect tissues outside of the brain except when mice are immunocompromised. Data showing the role of the adaptive immune response in causing demyelination after infection with JHMV will be presented. The relevance of these mouse studies to some of the manifestations observed in patients with COVID-19 will also be described.

Notes:

199 - The origin of the uterine microbiome in cattle

Federico Cunha[1], Kristi Jones[1], Modesto Elvir Hernandez[1], Rafael Bisinotto[1], Jimena Laporta[2], Kwangcheol Jeong[1], Ting Liu[1], Lilian Oliveira[3], Klibs Galvao[1]

[1]University of Florida, [2]University of Wisconsin, [3]University of Georgia. fcunha@ufl.edu

Session: "Omics" 2, 2024-01-23, 8:30 - 8:45

Objective: The objective of this project is to determine the origin of the uterine microbiome in cattle.

Methods: Holstein heifers were euthanized 4.6 ± 2.3 hours after birth (n=14) and at 62.9 ± 1.5 days of age (n=14) via non-penetrating captive bolt and exsanguination. The uterus, vagina, vulva, rectal feces, blood, and urine were aseptically sampled and cultured in varied media and atmospheric conditions. Swabs of the uterine serosa were taken as negative controls. Positive cultures were speciated by 16S rRNA gene sequencing. Quantitative real-time PCR was performed in all samples targeting a universal bacterial 16S rRNA gene sequence to identify and quantify bacterial DNA presence in the sampled tissues. The V4 hypervariable region of the 16S rRNA gene was amplified by PCR for metagenomic sequencing on the MiSeq platform. Data were log10 transformed prior to performing two-way mixed ANOVA to compare bacterial copy number differences between the groups.

Results: Bacterial growth did not occur in 27 of 28 cultured uterine samples. Only 1 Day-60 uterine sample and 1 Day-60 vaginal sample were culture-positive for bacterial growth of *Staphylococcus simulans* and *Corynebacterium glutamicum* respectively. All vulvar samples were culture positive from which 316 isolates were identified, predominantly composed of Firmicutes, Fusobacteria, Bacteroidetes, and Actinobacteria. Bacterial DNA was identified at significantly higher abundance in the vulva (4.35±0.51, $P<0.01$) and vagina (4.21±0.13, $P=0.04$) than in the uterus (3.91±0.33). There were no significant differences in copy number of 16S rRNA per mg of tissue for any site between Day-0 and Day-60 heifers. PCR products of V4 region for metagenomic sequencing of the uterus were minimal with few visible bands and no Illumina sequencing reads produced.

Conclusions: Together these results demonstrate scant presence of bacterial DNA in the bovine uterus and vagina immediately after birth with no microbial viability persisting up to 60 days of age. These results suggest a viable bovine uterine microbiome is not established until after 60 days of life.

Financial Support: This project was supported by the USDA-NIFA-AFRI (Accession No. 1026802).

Notes:

200 - Exploring the upper respiratory and fecal microbiomes in neonatal dairy calves

C. A. Sowers[1], M. M. Smithyman[2], M. Chung[3], R. Valeris-Chacin[3], M. A. Scott[4], G. C. Duff[2], J. T. Richeson[1], S. F. Capik[5]

[1]Department of Agricultural Sciences, West Texas A&M University, [2]Clayton Livestock Research Center, New Mexico State University, [3]Department of Veterinary Pathobiology, Veterinary Education, Research, and Outreach, Texas A&M University, [4]Department of Large Animal Clinical Sciences, Veterinary Education, Research, and Outreach, Texas A&M University, [5]Tumbleweed Veterinary Services, PLLC. casowers1@buffs.wtamu.edu

Session: "Omics" 2, 2024-01-23, 8:45 - 9:00

Objective: Early-life onset of bovine respiratory disease (BRD) can be detrimental to dairy calf health, growth performance, and possibly milk production if heifers are retained. Research is limited regarding how early BRD-associated bacteria can be detected in dairy neonates or their origin. Our objective was to evaluate the upper respiratory tract (URT) and fecal microbiomes of neonatal dairy calves during the first 24 hours of life and compare them to the dam's microbiomes at parturition.

Methods: Commercial Holstein dairy cows (n=26) were selected based on calving order. Dairy employees removed calves (17 heifer and 12 bull; 3 cows delivered twins) from the maternity pen immediately following parturition (0h) and placed them in a heated holding pen, where study calves were commingled. Following calving, cows were brought to a head gate for employees to check cows for a twin and for sampling. Cow sampling included left nasal swabs (LNS), right nasal swabs (RNS), vaginal swabs (VS), and fecal swabs (FS) using proctology swabs at 0h. Samples (LNS, RNS, and FS) were collected from calves at 0h, 6h, 12h, and 24h post-parturition. Calf samples were collected in the warming pen, however, 24h swab collection was completed in individual hutches. All swabs were flash frozen in an ethyl alcohol and dry ice solution. DNA was extracted via Power Soil Pro kits and prepared into equimolar DNA libraries for full-length 16S rRNA gene sequencing on a MinION Mk1C device (>1000 bp, min. 20K reads/sample). Sequenced reads were classified using Centrifuge, where microbiome analyses were conducted in R to estimate the changes in the microbiome according to animal type (cow versus calf), time of collection, and sample type (nasal, fecal, or vaginal) via PERMANOVA ($p<0.05$) and principal component analysis procedures.

Results: Cow upper respiratory tract microbiome differed at 0h from that of their offspring. Both LNS and RNS microbiomes of dairy cows following birth were more dispersed than in calves (P=0.002; P=0.0002, respectively). Using stratified PERMANOVA[RV1] , fecal microbiome by animal type was different (P=0.001); no differences (P=0.063) were found via dispersion testing. There was greater dispersion at 24h in LNS microbiomes as they became more dispersed compared to 0h or 6h ($P\leq$0.01). Microbiomes of the RNS were more dispersed at 24h than at birth (P=0.0009). At 12h, calf FS microbiome was more dispersed than at 6h (P=0.03). Calf age accounted for 10.6, 9.9, and 31% of the variance in the beta diversity of LNS, RNS, and FS microbiomes, respectively (P=0.001). No differences (P=0.925) were found between LNS and RNS microbiomes after adjusting for calf age.

Conclusions: At birth, the URT microbiome of cows significantly differed from calf URT microbiome. There was a clear transition of microbial communities associated with calf age in both the URT and fecal microbiomes as dispersion increased as time progressed.

Financial Support: This study was funded using internal Texas A&M University System funds.

Notes:

201 - Unraveling the effects of novel and wild type endophyte-infected tall fescue on Angus steers' metabolome

N. M. Filipov[1], I.M. Llada[1], J. M. Lourenco[1], G. Suen[2], D. P. Jones[3], Z. R. Jarrell[3], N. S. Hill[1]

[1]University of Georgia, [2]University of Wisconsin, [3]Emory University. filipov@uga.edu

Session: "Omics" 2, 2024-01-23, 9:00 - 9:15

Objective: Fescue toxicosis (FT) is the most serious pasture-related disease in the US. It is produced by an ergot alkaloid (EA)-producing fungus (*Epichloë coenophiala*), that symbiotically resides in fescue grass. Although decreased weight gain is a hallmark of FT and a major concern for the beef industry, causative mechanisms remain unclear. A novel endophyte (NT) with low or null capability of EA production has been developed. However, the potential metabolic impact of ingesting this foreign microorganism by ruminants remains unexplored.

Methods: Eighteen steers were randomly placed on nontoxic (NT), toxic (E+) and endophyte-free (E-) fescue pastures for 28 days. Body weight (BW) was measured pre, 7 and 14 days after pasture placement using a digital scale. Urine and ruminal fluid samples were collected at 6 different time points. To capture a diverse range of metabolites, an untargeted metabolomic approach (HRM) was performed on these biological samples, utilizing both C18 and HILIC columns. sPLS-DA was conducted using the most significant features (FDR=0.05) obtained for each column, while metabolic pathway analysis was carried out using the mummichog algorithm with a FDR cutoff of 0.2.

Results: Across the 28 days, steers on E+ gained about 60% less weight than the other two groups. sPLS-DA analysis showed a clear group separation in both urine and rumen samples for the metabolite features obtained from the HILIC column. Conversely, when we used features from the C18 column, in both matrices, an overlap was observed between the E+ and NT groups, while the E- group remained distinguishable. Pathway analysis revealed perturbations in amino acid and carbohydrate metabolism in both E+ and NT groups compared to the E- group. Importantly, the E+ group exhibited a significantly impacted lipid metabolism not observed in the other groups.

Conclusions: These data suggest that the presence of either toxic or novel endophytes in the fescue grass alters selected ruminal and urine metabolic pathways in grazing beef. The implications of this finding for the replacement of E+ fescue with NT fescue cultivars on animal's performance and health have to be evaluated fully. Additionally, we observed E+-specific disturbance in lipid metabolism that is possibly linked to the production of EAs. This metabolic disruption in the E+ group could be potentially contributing to the observed differences in weight gain compared to the other two groups. Future analysis will integrate these data with animal microbiome responses to fescue grazing, both in the presence and absence of endophyte infection.

Financial Support: USDA (NIFA), Grant Number 67015-31301.

Notes:

202 - Blood metabolomics and impacted cellular mechanisms during transition into lactation in dairy cows that develop metritis

Segundo Casaro[1], Jessica Prim[1], Tomas Gonzalez[1], Caio Figueiredo[2], Rafael Bisinotto[1], Ricardo Chebel[1], Jose Eduardo Santos[1], Corwin Nelson[1], Soojin Jeon[1], Rodrigo Bicalho[1], John Driver[3], Klibs Galvao[1]

[1]University of Florida, [2]Washington State University, [3]University of Missouri. segundocasaro@ufl.edu

Session: "Omics" 2, 2024-01-23, 9:15 - 9:30

Objective: The objective of this study was to identify metabolites associated with metritis and use them for identification of cellular mechanisms affected during transition into lactation.

Methods: Holstein cows (n = 104) had blood collected in the prepartum period (d-14 ± 6), at calving (d0), and at the day of metritis diagnosis (d7 ± 2). Cows with reddish or brownish, watery, and fetid discharge were diagnosed with metritis (n = 52). Cows with metritis were paired with herdmates without metritis (n = 52) based on days in milk. The metabolome of plasma samples was evaluated using untargeted gas chromatography time-of-flight mass spectrometry. Univariate analyses included t-tests and fold change analyses. Metabolites with false discovery rate (FDR) adjusted $P \leq 0.10$ on t-tests were used for partial least squares - discriminant analysis coupled with permutational analysis using 2,000 permutations. Metabolites with FDR-adjusted $P \leq 0.10$ on t-tests were also used for enriched pathway analyses and identification of cellular processes. Metabolic pathways with FDR adjusted $P \leq 0.05$ and impact ≥ 0.10 were affected. Compound network analysis was performed to visualize the changes in metabolites associated with the different metabolic pathways. Categories of cellular processes with a Bonferroni adjusted $P \leq 0.05$ were affected and further explored. Within affected categories, cellular processes with a Bonferroni adjusted $P \leq 0.05$ and a predicted Z-score were considered as affected.

Results: t-Tests showed that 89, 49, and 168 metabolites differed between cows that developed metritis and cows that did not develop metritis in the prepartum, at calving, and at the day of metritis diagnosis, respectively. During the prepartum period, cows that developed metritis had impacted metabolic pathways associated with amino acid metabolism. At calving, cows that developed metritis had impacted metabolic pathways associated with amino acid, energy, and lipid metabolism, and inflammatory response. The day of metritis diagnosis cows that developed metritis had impacted metabolic pathways associated with amino acid, energy, and pyrimidine metabolism. Cows that developed metritis had affected cellular processes associated with lower amino acid metabolism in the prepartum period, greater lipolysis, cell death, and oxidative stress at calving and at metritis diagnosis, and greater leukocyte activation at calving, but lower immune cell activation at metritis diagnosis.

Conclusions: Cows that developed metritis had plasma metabolomic changes associated with greater lipolysis, oxidative stress, and a dysregulated immune response, which may impair the cow's ability to prevent bacterial infection and predispose to metritis development.

Financial Support: This work was supported by U.S. Department of Agriculture Grant # 2019-67015-29836, Accession No: 1019435.

Notes:

203 - Antimicrobial resistance genes in clinical *Salmonella* isolates from Texas panhandle cattle

Max Chung[1], Ethan Dudley[1], Hatem Kittana[1, 2], Robert Valeris-Chacin[1]

[1] VERO, Department of Veterinary Pathobiology, College of Veterinary Medicine and Biomedical Sciences, [2]Texas A&M Veterinary Medical Diagnostic Laboratory. chunmn26@tamu.edu

Session: "Omics" 2, 2024-01-23, 9:30 - 9:45

Objective: Salmonellosis is one of the main causes of gastroenteritis in people in the United States. Salmonellosis is foodborne disease and cattle play a small role as a reservoir. However, the beef industry has a growing interest in deepening our understanding of *Salmonella* ecology in cattle during pre-harvest. Therefore, the objective of this study is to associate AMR genes found in *Salmonella* isolates from beef and dairy cattle with phenotypic AMR to aid in establishing industry specific approaches for mitigating AMR in *Salmonella*.

Methods: One hundred *Salmonella* isolated clinical cases archived at the Texas A&M Veterinary Medical Diagnostic Lab (TVMDL) were used for this study. Minimum inhibitory Concentration was performed on each isolate for 16 antimicrobials using Thermo Fisher's Sensititre Microdilution kit at the TVMDL. DNA was extracted using Qiagen's DNeasy Ultraclean Microbial kit and sequenced with Nanopore's MinION Mk1C device using the native barcoding kit (version 14). Reads were basecalled with Guppy's SUP basecaller and LongQC was employed to assess the quality of the reads. Genome assembly was conducted in a hybridized manner with Trycycler using both FLYE and Canu. Assemblies were polished with PEPPER, Homopolish, Medaka and JASPER, before evaluation by Quast and BUSCO with serotype specific references established by SISTR to assess assembly statistics and completeness. Downstream analyses were performed in a trilateral approach for format purposes. The pan genome was investigated with a Prokka-Roary-Scoary pipeline. Phylogeny was performed using Snippy-MEGA11, and a bacterial genome wide association study was conducted with ABRicate-Pyseer-Scoary using fisher's exact test and penalized regression.

Results: Antimicrobial resistance genes prevalence differed by serovar, host type (beef or dairy cattle) and location. The most prevalent serovars corresponded to recent literature. Most *Salmonella* isolates were resistant to tetracycline (81%) and ampicillin (81%). Six percent of the isolates were resistant to gentamicin and 13 percent were resistant to trimethoprim-sulfamethoxazole.

Conclusions: Understanding which serovars and which AMR genes are present in both beef and dairy cattle will aid in tailoring pre-harvest interventions and provide insight into the *Salmonella* landscape in the Texas Panhandle.

Financial Support: Texas A&M University

Notes:

204 - Comparison of the genetic content and arrangement between *Salmonella enterica* serovars Senftenberg and Infantis

David J. Bradshaw II[1, 2], Christopher L. Anderson[1], Bradley L. Bearson[3], Shawn M.D. Bearson[1]

[1]USDA-ARS, Food Safety and Enteric Pathogens Research Unit, [2]Oak Ridge Institute for Science and Education, [3]USDA-ARS, Agroecosystems Management Research Unit. david.bradshaw@usda.gov

Session: "Omics" 2, 2024-01-23, 9:45 - 10:00

Objective: The prevalence of poultry-associated *Salmonella enterica* subsp. *enterica* serovar Infantis (Infantis) has steadily increased since 2017. A concern associated with Infantis is the concurrent rise in the percentage of Infantis isolates carrying a megaplasmid known as pESI (plasmid for Emergent *Salmonella* Infantis). The genetic content of pESI varies but can encode multiple virulence, metal tolerance, and antimicrobial resistance genes (ARGs) which may lead to increased virulence or multidrug resistance in human infections associated with contaminated food. pESI has recently been identified in other *Salmonella* serovars including Agona, Muenchen, Schwarzengrund, and Senftenberg. Due to its concurrent increase in prevalence in poultry, this study focused on Senftenberg to determine its pESI carriage rate and the similarity of the genetic content and arrangement of Senftenberg pESI in comparison with Infantis pESI.

Methods: Assemblies of 2,029 Senftenberg genomes from the NCBI Isolates Browser were assessed for pESI carriage by requiring the presence of the pESI-associated *repA* gene encoding the RepB family plasmid replication initiator along with at least two out of five “core” pESI genes. Because publicly available complete Senftenberg pESI genomes are not available, six Senftenberg isolates predicted to contain (n=3) and lack (n=3) pESI underwent Oxford Nanopore MinIon long read sequencing. Trycycler assembled the reads while polypolish/polca polished the assemblies with publicly available short reads. Complete genomes of the three Senftenberg pESI were compared to the complete genomes of four Infantis pESI using MAUVE alignments in Geneious.

Results: Thirty-one Senftenberg isolates from 2019-2023 in the United States were predicted to have pESI, of which 29 isolates were from turkey-associated sources. In 2022, the carriage rate of pESI in turkey-associated Senftenberg isolates was 43%. Long read assembly of the six Senftenberg isolates confirmed the presence of the pESI megaplasmid in the predicted three (+)pESI isolates with sizes ranging from 300-321 kb. The three isolates containing pESI had 4-6 classes of ARGs (aminoglycoside, beta-lactam, phenicol, sulfonamide, tetracycline, and/or antifolate) encoded on the megaplasmid. Comparisons between the three Senftenberg pESI did not identify genetic rearrangements but did reveal regions of insertion/deletions. Of note was a ~18kb region present in two of the Senftenberg pESI isolates that conferred resistance to multiple ARG classes. This region was also present in three of the four complete Infantis pESI references analyzed and had variable insertions/deletions in comparison to the Senftenberg pESI megaplasmids.

Conclusions: Infantis acquisition of pESI may have contributed to the serovar’s enhanced prevalence and outbreak status in poultry over the last decade. With the detection of pESI in Senftenberg, this study provides a preliminary look into the carriage status of pESI in Senftenberg and comparisons of complete Senftenberg pESI genomes to one another and to Infantis pESI genomes. While pESI from the analyzed Senftenberg and Infantis isolates were generally similar in terms of genetic content, regions of insertions and deletions both within the Senftenberg serovar and between serovars were observed that led to a loss or gain of ARG classes.

Financial Support: We would like to thank the Agricultural Research Service Participation Program (Oak Ridge Institute for Science and Education) for this research opportunity.

Notes:

205 - Cutaneous myiasis by *Calliphoridae dipterans* in dogs from Chad, Africa

Jeremiah Cox[1], Rafael AN Ramos[2, 3], Hassan Hakimi[4], Sidouin Metinou[5], Wachellet Danzabe[5], Macon Overcast[1], Rebecca Garabed[1], Philip Tchindebet Ouakou[6], Bongo Nare Richard Ngandolo[7], Fernando Torres-Velez[8], Lucienne Tritten[9], Meriam N Saleh[3], Guilherme G Verocai[3]

[1]Ohio State University, [2]Universidade Federal do Agreste de Pernambuco, [3]Texas A&M University, [4]Texas A&M, [5]The Carter Center, [6]PNEVG T'Chad, [7]Laboratoire de Recherches vétérinaires et Zootechniques de Farcha, [8]High-Consequence Pathogens and Pathology, CDC, [9]University of Zurich. cox.1276@buckeyemail.osu.edu

Session: One Health / Public Health 2, 2024-01-22, 3:15 - 3:30

Objective: To provide geospatial, epidemiological, and molecular data on myiases in dogs from Chad.

Methods: From September to October 2022, a total of 1,561 dogs from 56 tropical and sub-tropical villages along the Chari River were inspected for cutaneous myiasis. Dogs in the study are also enrolled in a tethering intervention under the supervision of the Chadian Guinea Worm Eradication Program, with some villages participating in a study of flubendazole and others used as the control arm. The dog's age, gender, village, and coordinates were recorded. Maggots that were found were removed with tweezers and stored in 70% ethanol for later laboratory analysis. Individual maggots were morphologically assessed via microscopy. Subsequently, DNA of representative maggots was extracted, amplified, and sequenced for molecular confirmation of identification and phylogenetic analysis. A prevalence map was created using ArcGIS Pro, with proportional symbols used to display infested dogs normalized by the total number of dogs in each village. A data frame for analysis was created using the information gathered in the field. Using backwards selection and controlling for village as a random effect, multivariable mixed-effects logistic regression models were then used to determine which variables and interactions played a significant role in a dog's risk of cutaneous myiasis. Overall model fit was determined by comparing Akaike information criterion and Bayesian information criterion, and the final model's interclass correlation coefficient (ICC) was used to assess if there was clustering at the village level.

Results: 65 dogs from 21 villages were found infested by maggots causing myiasis. A total of 151 maggots were morphologically and molecularly identified as *Cordylobia anthropophaga* or *Chrysomya bezziana*. Most of infested dogs were from the southern region, and were found to be exclusively infested with one maggot species, but some villages were found to have both maggot species present. Molecular characterization of maggots corroborated initial morphological identification. The mixed-effects model showed that enrollment in the flubendazole arm, a more northern latitude, and an interaction of study arm and latitude was the best fitting model and returned ICC indicating that there was no case clustering at the village level.

Conclusions: The overall prevalence was less than five percent, with 21 of 56 villages having at least one case. While some dogs had multiple maggots present, none were found to be co-infested with both *C. anthropophaga* and *C. bezziana*, even though some villages had cases of both species. The statistical analysis suggested some protective effect of flubendazole against myiasis, which was further amplified in more northern villages. More investigation is needed to assess causation.

Notes:

206 - Concurrent therapeutic and behavior interventions reduce emerging Dracunculus medinensis worms in dogs in Chad

Amy C. Dupper[1], Rebecca Garabed[1], Christopher A. Cleveland[2], Michael J. Yabsley[2], Fernando Torres-Velez[3], Karmen Unterwegner[3], Mario Romero[3], Hubert Zirimwabagabo[4], Metinou Sidouin[4], Philip Tchindebet Oaukou[5], Mbang Mahammat Ada[5], Adam J. Weiss[3]

[1]The Ohio State University, [2]University of Georgia, [3]The Carter Center, Atlanta, Georgia, [4]The Carter Center, N'Djamena, Chad, [5]Programme National d'Eradication du Ver de Guinee, Ministry of Health, N'Djamena, Chad. dupper.3@buckeyemail.osu.edu

Session: Parasitology 2, 2024-01-23, 8:45 - 9:00

Objective: Identify the individual and concurrent effects of flubendazole (FBZ) and proactive tethering in reducing emerging *Dracunculus medinensis* worms in dogs based on the best-fitting model fit to existing data.

Methods: This is a secondary data analysis using Carter Center data and data collected from a previously published study examining the effectiveness of flubendazole (FBZ) in reducing *D. medinensis* infections in dogs. The previous study enrolled 435 dogs in 23 villages in Chad, half of which were randomized to receive three injections of FBZ six months apart in May 2019, November 2019, and June 2020. No significant difference in treated and control dogs were found at the individual level. The current study sought to examine the effect of FBZ at the village level in the presence of existing eradication interventions. Our data set included monthly counts of emerging worms in dogs from January 2019 through September 2021 from 56 villages, of which 11 (19.6%) were involved in the 2019 clinical trial. Eight models were fit using a generalized linear mixed model (GLMM) with a random effect for village and represented the different biologically plausible scenarios in which FBZ is believed to act on *D. medinensis* within the host based on expert elicitation. Each model's AIC and BIC values were compared to the AIC and BIC values from a base model where no intervention was present. Models with a difference in AIC and BIC greater than 10 from the base model were selected for inclusion in a final model. We then fit four separate GLMM models looking at the effect of proactive tethering on the number of emerging worms over time. Again, models with DAIC and DBIC > 10 were selected for inclusion in a final model. The models from the individual interventions were combined to assess the effects of concurrent interventions, and the model with the lowest AIC and BIC values was selected as our final model. The predicted number of *D. medinensis* infections per month was estimated from fixed effects in the final model over the study period, and confidence intervals were calculated from 1000 iterations of bootstrap samples. All analyses were performed using R version 4.3.1.

Results: The predicted counterfactual number of *D. medinensis* infections for the average village over the study period with no interventions present was 17.5 (95% CI, 13.0, 23.4). Both flubendazole alone and proactive tethering alone decreased the average estimated number of infections per village to 14.4 (95% CI, 9.6, 21.1) and 12.9 (95% CI, 9.5, 17.2), respectively. The predicted number of infections further decreased in the presence of both interventions to 11.9 (95% CI, 8.4, 17.9).

Conclusions: Though not directly addressing causality, our secondary data analysis supports the hypothesis that flubendazole treatment has a prolonged effect on emerging *D. medinenesis* worms at the village level. Flubendazole may be an important tool when used concurrently with existing eradication interventions.

Financial Support: This work was supported by The Carter Center. The Global Campaign to Eradicate Dracunculiasis receives financial support from a large coalition of organizations and agencies. Please refer to: ww.cartercenter.org/donate/corporate-government-foundation-partners/index.html.

Notes:

207 - Characterization of ABC transporter genes in Toxocara canis using RNA-Seq.

Matthew T. Brewer[1], Jeba R. Jesudoss Chelladurai[2], Theresa A. Quintana[2]

[1]Iowa State University, [2]Kansas State University. taq@ksu.edu

Session: Parasitology 2, 2024-01-23, 9:00 - 9:15

Objective: *Toxocara canis* (canine roundworm) is a ubiquitous canine nematode that presents risks to human health. Eradication is complicated by the ability of *T. canis* third-stage larvae to undergo developmental arrest in the host somatic tissue. Macrocyclic lactone anthelmintics do not kill somatic larvae, despite their adequate distribution throughout the host's body.

Our study aimed to investigate the differential expression of mRNA transcripts in *T. canis* third-stage larvae treated with ivermectin (IVM) or moxidectin (MOX) compared to untreated controls to identify up- or downregulated genes, with particular interest in ABC transporter genes. Our working hypothesis is that ivermectin (IVM) and/or moxidectin (MOX) induce transcriptional changes among ABC transporter genes in third-stage infective larvae. Several gaps exist in our understanding of their role in anthelmintic tolerance. Our central hypothesis is that ATP-binding cassette (ABC) transporters, such as P-glycoproteins (Pgps), play a role in drug tolerance via the efflux of anthelmintic compounds.

Methods: In this study, we isolated eggs from the uterus of adult *T. canis* female worms and incubated the eggs at 25°C to allow development to third-stage larvae. Using a chemical hatch protocol, we isolated larvae (n = 500 each) and subjected them to three conditions: incubation with IVM, MOX, or no treatment (controls), in biological triplicates. Adult male and female worms were also used as controls. We isolated RNA from each sample with Trizol and quantified it with Qubit. We then prepared libraries using an Illumina Stranded RNA prep kit and sequenced it on a NextSeq sequencer. We analyzed the data using the HISAT2-featurecounts and RNA STAR pipelines. Using DESeq2, we quantified the expression of ABC transporter genes and identified differentially expressed transcripts.

Results: Compared to higher eukaryotes, parasitic nematodes have a diverse repertoire of ABC transporter genes. We have identified 62 ABC transporters in the genome of *T. canis*. In the preliminary analysis, IVM-treated L_3s exhibited the upregulation of ≥100 genes and the downregulation of ≥49 genes compared to the control group. Similarly, MOX-treated L_3s showed the upregulation of ≥237 genes and the downregulation of ≥82 genes compared to the controls. Further investigation identified that some ABC transporters were differentially expressed.

Conclusions: Our study highlights the importance of further characterizing these ABC transporters and their role in drug disposition to identify new targets for further investigation. Future research will focus on investigating several ABC genes that were found to be upregulated in treated larvae to determine their functional transport capabilities of macrocyclic lactones. This may aid the development of nematode-specific ABC transport inhibitors that can enhance antiparasitic efficacy against somatic larval reservoirs, potentially mitigating human toxocariasis.

Financial Support: This study was supported by a pilot grant awarded to JJC from the Center on Emerging Zoonotic Infectious Diseases, an NIH Center of Biomedical Research Excellence at Kansas State University.

Notes:

208 - Fibrinogen-specific *Haemonchus contortus* cysteine proteases

Athira Karunakaran[1], Mariam Bakshi[1], Raffi Aroian[2], Erich Schwarz[3], Jane Homan[4], Gary Ostroff[2], Wenbin Tuo[1]

[1]Animal Parasitic Diseases Laboratory, USDA/ARS, [2]University of Massachusetts Chan Medical School, [3]Cornell University, [4]ioGenetics LLC. athira.karunakaran@usda.gov

Session: Parasitology 2, 2024-01-23, 9:15 - 9:30

Objective: *Haemonchus contortus* is one of the most pathogenic blood feeding gastrointestinal nematode of small ruminants. The current rise of anthelmintic resistance demands viable alternatives for prevention. Our objective is to produce functional parasitic cysteine proteases that may play a role in regulating the blood feeding process of *H. contortus*. The blood feeding regulators can be potential vaccine candidates to prevent haemonchosis.

Methods: Five cysteine proteases of *H. contortus*, namely cathepsin-B like protein 1 and 2 (HcCBP1, HcCBP2) and cysteine proteases 1a, b, and c (HcCP1a, HcCP1b and HcCP1c), were selected for this study. These proteins are collectively referred to as *H. contortus* cysteine proteases (HcCPs). The genes encoding these proteins were cloned into pET29b and pcDNA3.1 vectors for *E. coli* and HEK293 cell expression, respectively. The purified proteins were analyzed for proteolytic functions on host blood proteins as substrates, including hemoglobin (Hb), bovine serum albumin (BSA), IgG, and fibrinogens (Fg) from sheep, cattle, and swine. The time and pH (4-8) dependent degradation of substrates by HcCPs were tested at 37°C. The digestion was terminated by adding LDS-PAGE sample buffer and heating prior to analysis by SDS-PAGE. Proteins were visualized by Coomassie blue staining. Additionally, to investigate structural requirement for degradation, HcCPs and Fgs were heat-inactivated at 80 ºC for 8 min. Western blotting was also performed to confirm degradation.

Results: None of the bacterial-expressed recombinant HcCPs (rHcCPs) was active in degrading the substrates under any assay conditions. Fgs from all three species were degraded by the mammalian cell expressed rHcCPs (mrHcCPs) using acetate buffer (pH 5.5), cathepsin assay kit buffer, PBS (pH 7.2), and Tris buffer (pH 8). Hb, BSA and IgG were not susceptible to mrHcCPs. The degradation of Fg by mrHcCPs appeared to be initiated by cleavage of mrHcCPs themselves as early as 3 h, followed by significant degradation of Fg 6 h in incubation. Complete degradation of Fg was observed by 12-15 h of incubation. Heat-inactivation of either Fg or mrHcCPs resulted in complete loss of the ability of mrHcCPs to degrade Fgs. Cleavage of mrHcCPs failed to occur when co-incubated with non-susceptible substrates. mrHcCP activity was reduced or abrogated in the presence of a cysteine protease inhibitor, E64.

Conclusions: Results from this study indicate that mrHcCPs are active cysteine proteases/cathepsins even at neutral pH. Unlike HcCPs reported, HcCPs of this study degrade Fgs of three mammalian species, but not Hb, BSA and IgG, suggesting HcCPs play a role in regulating blood clotting. Further, we show that degradation of Fg requires native structures of both Fg and mrHcCPs, implicating that mrHcCPs-Fg interaction requires intact structures. Fg, also known as Factor I in the clotting cascade, mediates blood clotting. The degradation of Fg by parasite-derived cysteine proteases can prevent clot formation and facilitate blood feeding by the parasite. Thus, these active mrHcCPs are potential vaccine candidates against *H. contortus infection.*

Financial Support: USDA Research Project #8042 32000 105 00D and NICHD R01 HD099072.

Notes:

209 - Unique glycolytic enzymes as targets for novel anti-*Cryptosporidium* drugs in bovine calves

Shahbaz Khan[1], Rashid Bajwa[1], Mukthar Mia[1], Abdur Rehman Azam[1], Zaheer Abbas[1], Jozef Stec[2], William H. Witola[1]

[1]University of Illinois Urbana-Champaign, [2]Marshall B. Ketchum University. whwit35@illinois.edu

Session: Parasitology 2, 2024-01-23, 9:30 - 9:45

Objective: *Cryptosporidium parvum* is a highly prevalent protozoan parasite that causes a serious diarrheal syndrome in calves, lambs and goat kids in the United States and world-wide. There are neither effective drugs nor vaccine available against *C. parvum* infection. In our preliminary work, we found that inhibitors for *C. parvum*'s unique bacterial-type lactate dehydrogenase (CpLDH) and plant-like pyruvate kinase (CpPyK) enzymes of the glycolytic pathway can stop the growth of this parasite and prevent disease development in infected mice models. In this project, we aim to derivatize optimized CpLDH and CpPyK inhibitor combinations that are efficacious and safe in treating *C. parvum* infection in bovine calves.

Methods: We investigated the safety and anti-cryptosporidial synergistic efficacy of CpPyK and CpLDH inhibitors. We used the fixed-ratio ray design to derive concentration ratios for CpPyK and CpLDH combinations and tested those combinations for cytotoxicity and anticryptosporidial efficacy in mice and bovine calves. Further, we synthesized new analogs of NSC638080 (CpPyK inhibitor) that are currently being tested in search for improved anti-cryptosporidial efficacy.

Results: We identified combinations of CpPyK and CpLDH inhibitors with strong synergistic effects against survival of *C. parvum in vitro* and *in vivo*. In infected mice and bovine calves, compound combinations of NSC303244 + NSC158011, NSC252172 + NSC158011, and NSC638080 + NSC158011 depicted enhanced efficacy against *C. parvum* reproduction, and ameliorated intestinal lesions of cryptosporidiosis at doses 4-fold lower than the total effective doses of individual compounds. Importantly, NSC303244 + NSC158011 combination was effective in clearing the infection completely without relapse. Studies are ongoing to test the efficacy of new chemical derivatives of those compounds as combinations for treating cryptosporidiosis in bovine calves as well as to perform pharmacokinetic studies.

Conclusions: Collectively, our studies have unveiled compound combinations that simultaneously block two essential catalytic steps for metabolic energy production in *C. parvum* to achieve improved efficacy against the parasite. These compounds and their combinations are, therefore, viable lead-compounds for the development of a new generation of efficacious drugs for treating cryptosporidiosis.

Notes:

210 - Prevalence of canine intestinal parasites at public dog parks in Central Appalachia

Matthew R. Kolp[1], M. Marcello[1], A. Holt[1], B. Hamilton[1], A. Lagos[1], K. Hill[1], K. Rossi[1]

[1]Richard A. Gillespie College of Veterinary Medicine, Lincoln Memorial University. matthew.kolp@lmunet.edu

Session: Parasitology 2, 2024-01-23, 9:45 - 10:00

Objective: Dog parks are valuable environments for dogs and owners to socialize and exercise, yet parks represent risky places for parasitic contamination. Neglecting to pick up dog feces is a public health concern as a source of infection and communal outbreaks, including zoonotic parasites. Recent studies have investigated prevalence of parasitic taxa (e.g., *Giardia*, *Ancylostoma*) in dog parks nationally, and evidence suggests contamination varies across regions. The Eastern US, especially Appalachia, is a unique landscape with urban centers, small towns, and public lands popular for recreation and tourism that are all connected by interstate highways. In this mountainous, mosaic landscape of humans, companion animals, livestock, and wildlife, there could be a higher risk for encountering parasites, which is true of ticks and tick-borne diseases in this region. Additionally, dog owners' attitudes about canine health and visiting dog parks likely varies across this region. Understanding risks of encountering parasites in dog parks warrants comprehensive data collection. We began a year-long experiment in Spring 2023 to characterize the prevalence of intestinal parasites at dog parks in Central Appalachia (KY, TN, VA), and test for differences among dog parks in rural and urban counties.

Methods: To date, fecal samples (n=206) have been collected from abandoned poop piles (i.e., dog feces not discarded) across seven dog parks from counties representing a range of USDA Rural-Urban Continuum Codes. Ongoing convenience sampling allowed us to collect samples multiple times at each park throughout 2023. Chi-square tests of independence were used to identify differences in the prevalence of parasites among parks. Two diagnostic methods were used to identify parasites. For each sample, centrifugal fecal flotation (CFF) was used to identify parasites microscopically and record their abundance. In a subset of samples (n=127), PCR was used to screen samples for the presence of *Ancylostoma* spp., *Toxicara canis*, and *Trichuris vulpis* DNA.

Results: Across all dog parks, 33.5% (69 of 206) of abandoned poop piles contained at least one parasite identified via CFF and microscopy, including *Ancylostoma* spp. (detected in 21.4% of samples), *Trichuris vulpis* (14.6%), and *Toxicara canis* (8.7%). In addition, 24 samples (11.6%) contained at least two of these parasitic taxa. Most positive samples (59 of 69) contained > 30 individual eggs per $1cm^3$ of feces. Total parasite prevalence differed between dog parks ($\chi^2 = 19.9$; $p = 0.0028$) and between parks located in counties designated as either rural or urban ($\chi^2 = 18.8$; $p < 0.0001$). Rural county dog parks had more positive samples (49 of 106) than parks in urban counties (20 of 100). In addition, the abundance of *A. caninum* was significantly greater in rural parks (30.2%) than urban parks (12%). Preliminary results of PCR suggest different sensitivities and specificities for detecting parasite DNA in CFF positive and negative samples.

Conclusions: Canine intestinal parasites were detected in abandoned poop piles at all dog parks sampled, suggesting fecal pollution as potential infection sources for park attending dogs and humans. Rural county dog parks may also host a community of dogs with a higher prevalence of intestinal parasites.

Notes:

211 - Systematic review of the impact of respiratory disease vaccination on antibody titer and health outcomes in cattle

Hudson McAllister[1, 2, 3], Bradly Ramirez[1, 2, 3], Molly Crews[1, 4], Alexis Thompson[1, 5], Sarah Capik[6], Matthew Scott[1, 2, 3]

[1]Texas A&M University, [2]Vero Program, [3]Large Animal Clinical Sciences, [4]Small Animal Clinical Sciences, [5]Texas A&M Veterinary Medical Diagnostic Lab, [6]Tumbleweed Veterinary Services, PLLC. hmcallister15@tamu.edu

Session: Vaccinology 3, 2024-01-23, 8:30 - 8:45

Objective: Bovine respiratory disease (BRD) is a multifactorial disease complex commonly affecting beef and dairy operations. Vaccination against major BRD-related pathogens is commonly performed for disease prevention, however uniform reporting of health and performance outcomes is infrequent. Our objective was to systematically evaluate the effect of titer response to vaccination for respiratory pathogens on the health and performance of beef and dairy cattle.

Methods: This study was conducted under Prisma 2020 guidelines for systematic reviews and utilized the following databases: CAB Abstracts, Ovid MEDLINE, Web of Science, AGRICOLA (EBSCO), and ProQuest Dissertations and Theses. Criteria for study inclusion were: research conducted in the USA or Canada, between 1982 and 2022, on beef or dairy cattle, a commercially available vaccine in the US or Canada labeled for a respiratory pathogen of interest, and must evaluate antibody titers, and performance, or morbidity. Respiratory pathogens of interest were *Mannheimia haemolytica*, *Pasteurella multocida*, *Histophilus somni*, bovine viral diarrhea virus, bovine herpes virus type 1, parainfluenza 3, bovine respiratory syncytial virus, and bovine coronavirus. Deduplication occurred in Zotero and full-text analysis including further deduplication was conducted in Covidence.

Results: Following deduplication, 3020 articles were uploaded into Covidence for title and abstract evaluation. Following title abstract a total of 466 articles were used for full-text analysis. Following full-text evaluation, 101 papers were included in the review; included articles were 74% beef cattle-based vs 26% dairy cattle-based. Approximately 52% of all studies did not have a negative control. Studies varied greatly in all categories making a meta-analysis not possible.

Conclusion: Vaccination for BRD is a heavily studied topic in animal health research, however, reporting of results remains nonuniform from individual studies. This review aimed to gain an understanding of how titer response to vaccination impacts the health and performance of both beef and dairy cattle. However, it is difficult to understand and make conclusions about data that are not comparable from study to study. Further work must be done to understand the impact vaccination makes on cattle health, performance, and antibody titers.

Financial Support: This project was internally supported by Texas A&M University Department of Large Animal Clinical Sciences. Any opinions, findings, conclusions, or recommendations supported by this publication are those of the author(s) and do not necessarily reflect the view of internal supporters.

Notes:

212 - Bovine ocular immune responses after vaccination with Carbigen®+Emulsigen®-D-adjuvanted *Moraxella bovis* cytotoxin

John Angelos[1], Boguslav Mandzyuk[1], Regina Agulto[1]

[1]University of California, Davis. jaangelos@ucdavis.edu

Session: Vaccinology 3, 2024-01-23, 8:45 - 9:00

Objective: The pathogenesis of *Moraxella bovis*, a Gram-negative bacterium implicated in pathogenesis of infectious bovine keratoconjunctivitis (IBK; 'pinkeye'), requires expression of pili used for attachment to the ocular surface and a cytotoxin that causes corneal epithelial cell injury leading to corneal ulceration. Commercially available and autogenous parenterally-administered *M. bovis* bacterins to prevent IBK have been reported to lack efficacy. Recent IBK vaccine research has focused on mucosal delivery of *M. bovis* antigens as a way to boost ocular immunity against *Moraxella* spp. antigens. Previous research showed that intranasal administration of a recombinant *M. bovis* cytotoxin subunit (MbxA) adjuvanted with a polyacrylic acid-containing adjuvant (Carbigen®) resulted in changes in ocular antigen-specific IgA. When evaluated in a randomized controlled field trial, intranasal MbxA-Carbigen® vaccinates experienced similar rates of IBK as in adjuvant controls, however, disease was less severe in vaccinates versus controls. This study was undertaken to determine if mucosal and systemic immune responses to MbxA could be enhanced with the addition of Emulsigen®-D, an oil-in-water emulsion containing the immune stimulant dimethyldioctadecyl ammonium bromide, to MbxA-Carbigen®.

Methods: Beef steers were randomly assigned to 3 groups (5 animals/group) that were administered either: 500 µg recombinant *M. bovi*s cytotoxin (MbxA) adjuvanted with 10% Carbigen®/15% Emulsigen®-D (Group 1); adjuvant alone (2ml water plus 10% Carbigen®/15% Emulsigen®-D; Group 2); or 500 µg MbxA adjuvanted with 10% Carbigen® (Group 3). Vaccines were administered intranasally (2cc) in one nostril on days 0 and 21. Tear and serum samples were collected on days 0 (prevaccination), 14, 28, 42, and 49. Immune response variables (MbxA-specific-tear IgA, -tear IgG, and -serum IgG) were quantitated by ELISA, and tear and serum *M. bovis* cytotoxin neutralizing antibody titers were measured. Nonparametric statistical methods were used to evaluate differences between groups with $P<0.05$ as the level of significance.

Results: Cattle in Group 1 had significantly higher MbxA-specific tear IgA compared to cattle in Groups 2 and 3. Group 1 animals had a significantly higher fold change from day 0 to 49 in tear cytotoxin neutralizing titer compared to Group 2 animals.

Conclusions: A combination of 10% Carbigen®/15% Emulsigen®-D was superior to 10% Carbigen at stimulating ocular IgA responses to recombinant *M. bovis* cytotoxin following intranasal administration. Further investigations are necessary to determine if this antigen-adjuvant combination is effective at preventing naturally occurring-IBK.

Financial Support: This study was funded by USDA Hatch Formula Funds.

Notes:

213 - Safety and immunogenicity of DNA vaccines directed towards avian influenza

Bubacarr JB Touray[1], Ahmed S Aly[1], Chungyi Hansen[1], Adel M Talaat[1]

[1]University of Wisconsin – Madison. btouray@wisc.edu

Session: Vaccinology 3, 2024-01-23, 9:00 - 9:15

Objective: Avian influenza poses a substantial threat to the global poultry industry. Vaccination strategies are crucial for mitigating its impact. This study explores the immunogenicity of mosaic hemagglutinin (mHA) DNA vaccines in day-old chickens, emphasizing the influence of adjuvants and diverse administration routes.

Methods: Day-old chickens were vaccinated with two doses of 100 µg of mHA DNA vaccine, either in naked form or adjuvanted with Quil-A and Chitosan (QAC) or Quil-A and DOTAP (QTAP). Various administration routes were employed, with a 2-week interval between doses. The experiment continued for 35 days, after which all chickens were euthanized for analysis. Chicken body weight was monitored throughout to assess vaccine safety. Hemagglutination inhibition (HI) titers were measured to evaluate immune responses. Serum immunoglobulin G (IgG) levels and immunoglobulin A (IgA) levels in tears were quantified to assess humoral immune responses.

Results: No significant changes in chicken body weight were observed throughout the study, indicating the safety of the vaccination regimens. Importantly, the single dose of QTAP-mHA vaccine administered subcutaneously (SQ) elicited significantly higher HI titers compared to all other vaccination groups. Furthermore, QTAP-mHA administered through either SQ/ON (oculo-nasal) or SQ/SQ routes induced higher levels of IgG in serum and IgA in tears, underscoring the versatility of the vaccine and administration methods in promoting both systemic and mucosal immune responses.

Conclusion: This study highlights the potential of mosaic hemagglutinin (mHA) DNA vaccines in day-old chickens, with adjuvants and diverse administration routes playing pivotal roles in enhancing immunogenicity. The absence of adverse effects on chicken body weight, coupled with superior HI titers and increased IgG and IgA levels, suggests the effectiveness of this vaccination strategy in providing protection against avian influenza. Further research should explore the vaccine's performance under field conditions and its potential impact on reducing disease transmission within poultry populations.

Financial Support: USDA/NIFA Award Number: 2023-67015-39884 and Animal Formula Fund, WIS04090.

Notes:

214 - Role of stem region of hemagglutinin and ectodomain of matrix protein 2 in protection against influenza viruses

Wen-Chien Wang[1], Ekramy E. Sayedahmed[1], Suryaprakash Sambhara[2], Suresh K. Mittal[1]

[1]Department of Comparative Pathobiology, College of Veterinary Medicine, Purdue University, [2]Influenza Division, Centers for Disease Control and Prevention. wang5382@purdue.edu

Session: Vaccinology 3, 2024-01-23, 9:15 - 9:30

Objective: Currently available seasonal influenza vaccines mainly provide strain-specific protection and thus are less effective against mismatched strains. Instead of stimulating the immune response against the hemagglutinin (HA) head domain (HA1), we targeted the relatively conserved stem region of HA (HA2) and ectodomain of matrix protein 2 (M2e) to explore their potential in providing cross-protective immunity against potential pandemic influenza viruses.

Methods: We developed both human and bovine adenoviral (Ad) vectored vaccines expressing H5N1 HA2 alone or with M2e. BALB/c mice were immunized with vaccine candidates, cellular and humoral immune responses were monitored, and the protection against homologous and heterosubtypic influenza viruses was investigated.

Results: The prime-boost immunization of mice with bovine and human Ad vectored vaccines showed a comparable increase in the number of interferon-gamma (IFN-γ) and interleukin 2 (IL-2) expressing cells in splenocytes, mediastinal lymph node cells, and lung mononuclear cells, indicating the development of HA2-specific cellular immunity. The HA-specific or M2e-specific serum antibodies were developed in immunized animals, signifying the induction of humoral immunity. Immunization and challenge study with an H5N1 reassortant influenza virus demonstrated a comparable decrease in lung viral titers. The challenge with heterosubtypic influenza viruses indicated partial protection in immunized groups.

Conclusions: The Ad vectors expression HA2 and M2e conferred partial protection against homologous and heterosubtypic influenza viruses, indicating the role of conserved domains in developing a universal influenza vaccine.

Financial Support: National Institute of Allergy and Infectious Diseases (NIAID) #AI059374.

Notes

215 - Cross-protective immunity in pigs vaccinated intranasal with mannose-chitosan nanoparticle-based influenza vaccine

Gourapura J Renukaradhya[1], Dina Bugybayeva[1], Ekachai Dumkliang[2], Veerupaxagouda Patil[1], Ganesh Yadagiri[1], Raksha Suresh[1], Sara Dolatyabi[1], Jennifer Schrock[1], Mithilesh K Singh[1], Juan F Hernandez Franco[3], Hadi Mohamad Yassine[4], Harm HogenEsch[3]

[1]The Ohio State University, [2]Silpakorn University, [3]Purdue University, [4]Biomedical Research Center, Qatar. gourapura.1@osu.edu

Session: Vaccinology 3, 2024-01-23, 9:30 - 9:45

Objective: To mitigate swine influenza A virus (SwIV) infections in swine, vaccination is the viable strategy. Lack of induction of mucosal and cellular immunity in the respiratory tract by commercial SwIV vaccines administered intramuscularly is responsible for limited cross protection. Our objective is to improve the mucosal and cellular immunity induced by whole inactivated SwIV delivered through mannose-chitosan nanoparticle (mChit-SwIV-NP) by including a STING (stimulator of interferon gene) adjuvant.

Methods: We developed mChit-SwIV-NP vaccine containing whole inactivated SwIV H1N2 and STING adjuvant ADU-S100, either encapsulated (mChit-SwIV+S100-**e**NP) or surface adsorbed (mChit-SwIV+S100-**s**NP). Influenza-free nursery pigs were vaccinated intranasally twice at 3-week interval and challenged with the heterologous pandemic 2009 H1N1 virus (78% HA gene identify). Nasal swabs collected at day post challenge (DPC) 2, 4 and 6, and peripheral blood mononuclear cells (PBMCs), bronchoalveolar lavage fluid (BAL) cells, and tracheobronchial lymph nodes mononuclear cells (TBLN MNCs) isolated at DPC 6 were used for analyses.

Results: The infectious challenge virus load was reduced by over 1 $\log_{10}$ in nasal passage of both mChit-SwIV+S100-**e**NP and mChit-SwIV+S100-**s**NP vaccinates, with the latter performing relatively better. Like the increased specific IgG and sIgA responses observed, we also detected high avidity in the antigen and antibody interaction, and virus neutralizing antibodies in the respiratory tract and serum of mChit-SwIV+S100-**s**NP vaccinates. In TBLN MNCs of mChit-SwIV+S100-**e**NP vaccinates observed an increased lymphocytes stimulation index, and in both PBMCs and TBLN MNCs detected enhanced frequency of activated $IFN\gamma^+$ and $IL\text{-}17A^+$ cytotoxic T lymphocytes and T-helper/memory cells, compared to mChit-SwIV+S100-**s**NP and commercial SwIV vaccine received groups.

Conclusions: Intranasal inoculated mChit-SwIV+S100-**s**NP vaccine elicited robust cross-reactive antibody responses while mChit-SwIV+S100-**e**NP vaccine induced robust mucosal and systemic cell-mediated immune responses in vaccinated pigs better than commercial SwIV vaccine group. Data suggest that mannose-chitosan nanoparticle vaccine delivery system has a promise in mitigating SwIV infection and virus transmission in swine herds.

Financial Support: Supported by USDA-NIFA AFRI grant # 2019-67015-29814 and # 2019-67015-29815.

Notes:

216 - Low-dose Epigraph vaccine provides broadly protective immunity against swine influenza A virus

A. Madapong[1], E.M. Petro-Turnquist[1], M.J. Pekarek[1], D. Steffen[2], E.A. Weaver[1]

[1]Nebraska Center for Virology, University of Nebraska-Lincoln, [2]School of Veterinary Medicine and Biomedical Sciences, University of Nebraska-Lincoln. amadapong2@unl.edu

Session: Vaccinology 3, 2024-01-23, 9:45 - 10:00

Objective: Swine influenza A virus (IAV-S) is a significant pathogen in swine and causes a considerable financial burden to the swine industry. Current vaccination strategies induce short-lived and strain-specific responses against IAV-S. The substantial genetic diversity of IAV-S necessitates consistent revaccination and updating of commercial vaccines. Computational platforms have recently emerged as promising tools to develop new-age vaccines against infectious diseases in livestock animals. Our previous studies have shown that the Epigraph platform, a computational algorithm that maximizes potential T-cell epitopes incorporated into a synthetic immunogen design, induces broadly protective, rapid, and durable immunity against IAV-S *in vivo*. Here, we expand on our previous findings to determine the minimum dose required to maintain protection against antigenically distinct IAV-S infection.

Methods: Groups of outbred swine (n=5/group) were intramuscularly prime/boost immunized with different doses of the Epigraph vaccine, including 1E11, 1E10, and 1E9 viral particles (vp), the commercial comparator vaccine (FluSure XP), or DPBS at 0- and 21-days post vaccination (DPV). Virus-specific antibody responses were determined by hemagglutination inhibition (HAI) and virus neutralization assays. Cell-mediated immune response was evaluated by ELISpot IFN-γ assay. At 35 DPV, pigs were intratracheally challenged with a Clade IV(A) H3 isolate (A/swine/Ohio/11SW87/2011). The protective efficacy was determined by the reduction of viral shedding in nasal swabs, reduction of lung lesions, and the presence of the virus in the lung tissues.

Results: Different doses of Epigraph vaccine vaccinations provided better immune responses than the FluSure XP and DPBS groups. Moreover, the Epigraph vaccine showed complete protection against the IAV-S challenge, evidenced by lower levels of viral shedding and less microscopic lung lesion scores ($p < 0.05$) without the virus in the lungs. This protection was in accordance with robustly protective circulating antibody and T-cell responses even at the lowest dose of Epigraph vaccine vaccination, which was equal to or better than the FluSure XP.

Conclusions: This study details the immune response and protective efficacy of a low-dose Epigraph vaccine compared to a commonly used commercial vaccine. Additional studies are underway to further analyze the protection with low-dose vaccination against a highly heterologous H3 Clade I H3 isolate (A/swine/Texas/4199-2/1998).

Financial Support: The U.S. Department of Agriculture

Notes:

217 - Exploring a mouse model for torque teno sus virus 1

M.T. Islam[1], Z. Aboezz[1], S. Ramamoorthy[1]

[1]Department of Microbiological Sciences, North Dakota State University. mdtariqul.islam.1@ndsu.edu

Session: Immunology 4, 2024-01-23, 8:30 - 8:45

Objective: While it is not clear if torque teno viruses (TTVs) are primary pathogens, experimental infection of torque teno sus virus 1 (TTSuV1) results in renal and hepatic lesions and exacerbation of clinical manifestations in co-infections with porcine circovirus type 2 (PCV2) and swine influenza virus (SIV). A major roadblock for TTV research is that it is not readily grown in laboratory cell culture systems. Therefore, its role in the host virome is poorly understood. The primary objectives of this study were to determine whether recombinant TTSuV1 derived from the infectious clone can infect replicate in mice and address the molecular pathogenesis of TTSuV1.

Methods: Using recombinant TTSuV1 rescued from a novel reverse genetics system, we infected 2-week-old *C57BL/6J* mice. Assessment of viral loads in whole blood and lungs tissues was done by a TTSuV1-specific by qPCR. The presence of TTSuV1 antigen in blood cells was evaluated by flow cytometry using a virus-specific polyclonal antibody and cell type-specific markers. TTSuV1 specific binding antibody responses and neutralizing responses were measured respectively by ELISA and modified virus neutralization assay method.

Results: TTSuV1-specific qPCR showed a viral load of 7.1±0.68 $\log_{10}$ genome copies in blood at DPI 15 (Day post infection) while at DPI 30 mean TTSuV1 load was 4±1.3-$\log_{10}$ genome copies. In lungs tissue, mean TTSuV1 load was significantly reduced by 2.5±0.5 $\log_{10}$ at DPI 30 compared to DPI 15. Flow cytometry analysis revealed that, peripheral blood mononuclear cells (PBMC's) are permissive to TTSuV1 infection and preferentially targets lymphocytes. Sero-conversion was detected after DPI 15 and moderate virus neutralizing responses of 70% fluorescent foci neutralization titer were detected at later time points (DPI 30).

Conclusions: Thus, the data generated is useful for future studies to understand the role of TTV's as primary or coinfecting agents. A mouse model could be critical to establish the parameters of how TTV affect health and disease.

Financial Support: NIH-NIAID competitive grant number 1R21AI137963.

Notes:

218 - Characterizing the spatial expression of the CHIR-B in the chicken intestine after coccidiosis vaccine challenge

Yvonne Drechsler[1], Theros Ng[1]

[1]College of Veterinary Medicine, Western University of Health Sciences. ydrechsler@westernu.edu

Session: Immunology 4, 2024-01-23, 8:45 - 9:00

Objective: The objective of this study is to investigate the role of the Clustered Homolog of Immunoglobulin-like Receptors-B (CHIR-B) in modulating immune responses in coccidiosis infection in chickens. Specifically, we aim to explore CHIR-B localization with Src-homology 2 domain (SH2)-containing SHP-1 and SHP-2, which are T-cell-modulating phosphatases that are associated with Ig-like receptors.

Methods: We will use the B19/B19 and BQ/BQ chickens to study the role of Ig-like receptors on coccidiosis. After hatching, homozygous B19 and BQ chicks, confirmed by LEI0258 PCR, were allocated into two treatments, control or infected, in cages. This resulted in a 2x2 factorial design with 2 chicken haplotypes and 2 infectious statuses (control and infected). The infected groups were housed separately from the control groups. On day 10 of age, chicks in the infected treatments were orally gavaged using a curved stainless-steel feeding needle with a 200 µL 10x dose of coccidia vaccine (COCCIVAC-D2, Merck, Kenilworth, NJ) in sterile water. The vaccine is a blend of live non-attenuated drug-sensitive strains of *Eimeria acervulina*, *E. brunetti*, *E. maxima*, *E. mivati*, *E. necatrix*, and *E. tenella* oocysts. Performance data, such as bird weight and feed intake, are collected weekly. On day 21, 4 birds per treatment (n=4) were euthanized for sample collection. Intestinal lesion scores throughout the intestine (jejunum, ileum, and cecum) were performed as described. Briefly, lesions were scored from 0 to 4, where a 0 score represents no gross lesions and 4 indicates severe lesions in the intestine. Tissues collected from the jejunum were frozen, or stored in neutral buffered formalin for spatial sequencing, using a combined ACD Biotechne and nanoString protocol for the localization of CHIR-B, SHP-1, and SHP-2, and the differential expression of genes in the region of interest.

Results: The study's findings will provide insight into the role of Ig-like receptors on the immune responses of chickens to coccidiosis. Collected samples indicated differences in severity in coccidiosis as evaluated by lesion scores in BQ vs B19. In the pending spatial sequencing analysis, we expect a dampening of immune response in the susceptible haplotype, B19/B19 compared to BQ/BQ, through the recruitment of CHIR-B signaled by SHP-1 and SHP-2 molecules.

Conclusions: This research aims to enhance our understanding of chicken immune responses to coccidiosis and necrotic enteritis infections, aiding the development of genetic biomarkers for selectively breeding disease-resistant chickens. The inhibitory receptor CHIR-B, expressed on various immune cells, has ITIMs in its cytoplasmic tail, which become phosphorylated upon ligand engagement. Upon activation, CHIR-B down-regulates immune responses. SHP-1 plays a crucial role in inhibitory signaling pathways in immunity. SHP-2 has dual roles in immunity, acting as a positive regulator for activatory receptors and potentially a negative regulator for inhibitory receptors. Investigating the spatial distribution of activated signaling molecules and their association with CHIR-B will shed light on coccidiosis resistance mechanisms. This study will uncover a previously unknown relationship between CHIR-B, SHP-1, and SHP-2, deepening our understanding of host-pathogen interactions. The proposed approach may have wider implications for enhancing immune responses in poultry farming.

Notes:

219 - TLR4 activation by endotoxin activates lipolysis in bovine adipocytes

Miguel Chirivi[1], G. Andres Contreras[1]

[1]Department of Large Animal Clinical Siciences, Michigan State University. chirivim@msu.edu

Session: Immunology 4, 2024-01-23, 9:00 - 9:15

Objective: Bovine periparturient diseases are often accompanied by endotoxemia. Recently, we demonstrated that endotoxin (LPS) triggers lipolysis and reduces insulin sensitivity in adipose tissue (AT) providing evidence for a link between metabolic and infectious diseases. However, the mechanisms by which LPS activates lipolysis are poorly understood. The goal of this study was to characterize the role of TLR4 in LPS-induced lipolysis in bovine adipocytes.

Methods: Adipocyte progenitors (AP) were obtained from subcutaneous AT from 6 non-lactating non-gestating multiparous Holstein dairy cows by non-enzymatic isolation. AP were expanded and induced to differentiate into adipocytes using a standard pro-adipogenic media for 7 d. Next, adipocytes were stimulated for 3 h and 7h with the lipolytic agent isoproterenol (**ISO**=1 µM, **Basal**=0 µM) or Lipopolysaccharide **(LPS;** O55:B5; 0.001, 0.01, 0.1, 1, and 10 µg/mL). To quantify TLR4 contribution to LPS-induced lipolysis, cells were transfected with siRNA targeting *TLR4* (si*TLR4*) or control non-coding (siNC). Lipolysis was determined by quantification of glycerol release and results are presented as units relative to Basal (±SEM). Cell viability was assessed using a Calcein-Ethidium assay. Statistical analyses were performed using a linear mixed model in JMP. LPS <1 ug/mL did not affect adipocyte viability.

Results: Compared to Basal, ISO increased glycerol release by 69.44±4.2% and 86.91±16% at 3 and 7 h respectively. LPS increased glycerol release in a quadratic response ($P<0.01$), reaching the effective lipolytic dose at 1 µg/mL with 39.10±4.2% more glycerol released at 3 h and 72.66±16% at 7h. Therefore, 1 µg/mL of LPS during 7 h was used for si*TLR4* experiments. *TLR4* transcription was reduced by 81.55±2.8% in si*TLR4* compared to siNC cells ($P<0.001$). si*TLR4* drastically reduced lipolysis induced by LPS (+3.5±4.5%) compared to siNC cells (+42.66±5.1%, $P<0.05$).

Conclusions: Collectively our results suggest that TLR4 signaling mediates lipolysis in bovine adipocytes. Future studies will evaluate the role of TLR4 during the development of insulin resistance by LPS in bovine adipocytes.

Financial Support: This research was supported by the USDA-National Institute of Food and Agriculture (Washington, DC; competitive grants 2021-67015-34563.

Notes:

220 - Effect of isoprostane class on bovine neutrophil microbicidal functions

Eric Owczarkak[1], Angel Abuelo[1]

[1]College of Veterinary Medicine, Michigan State University. owczar12@msu.edu

Session: Immunology 4, 2024-01-23, 9:15 - 9:30

Objective: Isoprostanes (isoP) are formed during conditions of oxidative stress through the oxidation of cell membrane polyunsaturated fatty acids (PUFAs). Depending on the PUFA being oxidized, different classes of isoP are formed. For example, the oxidation of n-6 PUFA results in the formation of F2-isoP whereas oxidation of n-3 PUFA produces F3-isoP. Although isoP have been extensively studied as a biomarker of oxidative stress, there is still a paucity of knowledge regarding the biological activity of these molecules. Neutrophils are innate immune cells that show decreased functionality during the transition period of dairy cattle, a time when oxidative stress is also prevalent. Thus, the objective of this study was to compare *in vitro* the effects of F2- and F3-isoP on neutrophil microbicidal functions.

Methods: Neutrophils were isolated from 6 healthy mid-lactation dairy cows and cultured for 8 h with different biologically-relevant concentrations (0, 10, 250, and 500 nM + vehicle control) of F2- and F3-isoP. Following incubation, the following neutrophil microbicidal functions were evaluated: (1) *E. coli* phagocytosis capacity via flow cytometry, (2) Oxidative burst via a luminescence assay, (3) myeloperoxidase release using a fluorometric assay, and (4) formation of neutrophil extracellular traps (NETs) via flow cytometry. Mixed models were built for the outcome variables phagocytosis, oxidative burst, myeloperoxidase, and extracellular trap formation. The fixed effects included the different treatments and animal was the random effect to account for individual variability. Tukey's honest significance test was used for post hoc pairwise comparisons.

Results: F3-isoP increased the phagocytic capabilities of neutrophils ($P<0.01$) compared to untreated controls, whereas phagocytosis was lower than controls at any of the F2-isoP concentrations tested ($P<0.01$). At any of the concentrations tested, MPO release was also increased in neutrophils treated with F3-isoP compared to controls ($P<0.032$) but there were no differences between control and F2-isoP-treated neutrophils ($P>0.62$).

Conclusions: Our *in vitro* results suggest that favoring the production of F3- over F2-isoP during periods of oxidative stress could ameliorate the extent of neutrophil dysfunction observed in transition cattle. This needs to be followed up with animal studies.

Financial Support: This work has been supported by competitive grant 2022-67015-36350 of the U.S. Department of Agriculture National Institute for Food and Agriculture.

Notes:

221 - Immune dysregulation at the cellular, cytokine, and transcriptomic level during African swine fever virus infection

D.W. Madden[1], B.L. Artiaga[1], C.D. McDowell[1], P.A. Assato[1], J.D. Trujillo[1], D.A. Meekins[1], D. Bold[1], K. Cool[1], T. Kwon[1], V. Balaraman[1], I. Morozov[1], N.N. Gaudreault[1], J.A. Richt[1]

[1]Department of Diagnostic Medicine/Pathobiology, College of Veterinary Medicine, Kansas State University. dwmadden@vet.k-state.edu

Session: Immunology 4, 2024-01-23, 9:30 - 9:45

Objective: Overwhelming pro-inflammatory responses are considered a primary pathogenic mechanism of African swine fever virus (ASFV) infection. To clarify the role of host immune responses in the pathogenesis of African swine fever (ASF), we sought to characterize innate and early adaptive immune responses to lethal ASFV infection and assess the temporal dynamics of major changes in immune parameters in relation to clinical progression of acute ASF.

Methods: Blood and plasma were serially collected from six pigs infected with a genotype II ASFV strain (Armenia 2007) prior to ASFV inoculation and at 1, 3, 5, and 7 days post-challenge (DPC). An array of pro- and anti-inflammatory cytokines were quantified in plasma using capture ELISA. Circulating immune cells were phenotyped using swine-specific flow cytometry antibody panels to evaluate changes in circulating lymphoid and myeloid cell populations. RNA-Seq was performed on whole blood RNA extracts using the Illumina NextSeq platform. Differentially expressed genes (DEGs) were identified for each timepoint compared to pre-challenge control using DESeq2, and gene ontology (GO) pathway enrichment was evaluated using the PANTHER software system.

Results: Following ASFV challenge, rapid increases in pro-inflammatory cytokines including type I interferons, IL-12p40, and TNF-α were observed at 5 and 7 DPC, coinciding with the onset of severe clinical disease and high viral DNA loads. Levels of the anti-inflammatory cytokine IL-10 were elevated at 5- and 7- days post-challenge (DPC), while concentrations of the immunoregulatory cytokine TGF-β1 decreased over time. Lymphopenia characterized by decreases in B cell, CD8+ T cell, and NK cell proportions was observed, while the level of CD4+ T cells remained stable and the percentage of CD4+CD8+ T cells increased over time. Significant fluctuations in circulating monocyte and macrophage levels were observed throughout infection, most notably an abrupt spike in CD203+ mature macrophages immediately prior to death. The number of DEGs found in whole blood increased over time, peaking at 7 DPC. Pathways associated with cytoplasmic translation and ribosome formation were downregulated at 1 DPC and 5 DPC, while processes involving innate immunity and host defenses against viral pathogens were strongly upregulated at 5 DPC and 7 DPC.

Conclusions: Lethal ASFV infection produces an immunological state characterized by large increases in circulating pro-inflammatory cytokines, inadequate anti-inflammatory cytokine responses, progressive lymphopenia, shifting monocyte/macrophage populations and phenotypes, and transcriptional activation of multiple host immune pathways. These results highlight the pro-inflammatory immune responses in acute ASF and can inform research into novel antiviral and vaccine development strategies for ASF.

Financial Support: This work was supported by National Bio and Agro-Defense Facility transition funds from the State of Kansas, and the AMP core of the National Institute of General Medical Sciences of the National Institutes of Health under award number P20GM130448.

Notes:

222 - Live-attenuated vaccine against contemporary pandemic genotype II African swine fever virus

Lihua Wang[1], Quang Lam Truong[2], Tuan Anh Nguyen[2], Hoa Thi Nguyen[2], Son Danh Tran[2], Anh Thi Vu[2], Anh Dao Le[2], Giap Van Nguyen[2], Phuong Thi Hoang[2], Yen Thi Nguyen[2], Luyen Thi Le[2], Thang Nguyen Van[2], Le My Thi Huynh[3], Thi Lan Huong Lai[2], Rachel Madera[1], Yuzhen Li[1], Lan Nguyen Thi[2], Jishu Shi[1]

[1]Center on Vaccine Evaluation and Alternatives for Antimicrobials, Department of Anatomy and Physiology, College of Veterinary Medicine, Kansas State University, [2]Key Laboratory of Veterinary Biotechnology, Faculty of Veterinary Medicine, Vietnam National University of Agriculture, [3]Department of Veterinary Microbiology and Infectious Diseases, Faculty of Veterinary Medicine, Vietnam National University of Agriculture. lihua@vet.k-state.edu

Session: Immunology 4, 2024-01-23, 9:45 - 10:00

Objective: African swine fever (ASF) is a lethal and highly contagious transboundary animal disease with the potential for rapid international spread. Currently, there is no safe and efficacious ASF vaccine commercially available worldwide. Here, we report the generation of a safe and efficacious live-attenuated vaccine against the genotype II African swine fever virus from a field isolate by cell passage.

Methods: Porcine alveolar macrophage (PAM) and an immortalized porcine alveolar macrophage cell line (3D4/21) were used to passage the African swine fever virus (ASFV) virulent VNUA-ASFV-05L1 strain (genotype II). The replication of viruses in different passages were titrated with hemadsorption (HAD) testing. The safety of the obtained live attenuated vaccine (LAV) candidate was evaluated in pigs. The efficacy of the LAV candidate was evaluated by challenging with virulent VNUA-ASFV-05L1. Quantitative RT-PCR was conducted to detect ASFV DNA in collected clinical samples of the experimental pigs. ELISPOT and ELISA tested the IFN-γ and IL-10 cellular responses in pigs. Statistical analysis was performed using GraphPad Prism 6.0 (GraphPad Software, CA, USA).

Results: We generated an ASF LAV candidate by serial passaging of an ASFV field isolate in PAMs (65 passages) and 3D4/21(55 passages). It provides 100% protection, even with the low dose of 10^2 HAD_{50}, for the vaccinated pigs against the challenge of contemporary pandemic ASFV field isolate. Pigs vaccinated with this LAV at a dose range of 10^2 to 10^5 HAD_{50} remained clinically healthy during both the 28-day observation period post-immunization and the 28-day observation period post-challenge. LAV was completely eliminated from blood by 28 days post inoculation (DPI), and from feces or oral fluids by 17 DPI when tested by ASFV real-time PCR. It remained a safe and attenuated phenotype after five passages in pigs. ASFV-specific IgG antibodies and significant cellular immunity, when tested by ELISA and ELISPO, respectively, were shown in vaccinated pigs before ASFV challenge.

Conclusions: In this study, we generated a safe and efficacious LAV vaccine VNUA-ASFV-LAVL2 from a field genotype II ASFV isolate by cell passage. This LAV can protect pigs 100% against contemporary pandemic ASFV challenge and can stably and efficiently replicate in commercially available 3D4/21 cell.

Financial Support: Vietnam Ministry of Science and Technology [2012R1A1A4A01015303]; NBAF Transition Fund, USDA NIFA, Hatch-Multistate project [1021491]; USDA ARS Non-Assistance Cooperative Agreements [58-8064-8-011, 58-8064-9-007, 58-3020-9-020, 59-0208-9-222]; USDA NIFA [2022-67015-36516], USDA NIFA Subaward [25-6226-0633-002[; National Pork Board [18-059]; Department of Homeland Security [70RSAT19CB0000027].

Notes:

223 - Effects of dietary zinc on the microbiota of the gestating cow and neonatal calf

Laurel Redding[1], Linda Baker[1], Mary-Jane Drake[1], Nagaraju Indugu[1], Dipti Pitta[1]

[1]University of Pennsylvania School of Veterinary Medicine. lredding@upenn.edu

Session: General Health & Physiology 3, 2024-01-23, 8:30 - 8:45

Objective: Zinc is included in the diet of dairy cows at varying levels in all stages of life as an essential trace element. While the effects of zinc have been studied in growing calves, little is known about the effect of zinc on the microbiota of the gestating cow or her neonatal calf. The early neonatal phase is a critical period, shaping the animal's intestinal microbial profile and future intestinal health. The aims of this study were thus to determine the effect of dietary zinc on the microbiota of the gestating cow and calf. A secondary aim was to determine whether zinc supplementation affected the likelihood of colonization with *Clostridioides difficile,* an opportunistic pathogen that thrives when the endogenous microbiota is disrupted.

Methods: Gestating cows were randomized to receive standard (40 ppm) or high (205 ppm) dietary zinc levels from drying off to calving. Fecal samples were collected from cows upon randomization and at calving and from neonatal calves. Fecal samples underwent 16s rRNA sequencing, and the effect of zinc supplementation on the diversity and composition of the cow and calf microbiome and on the presence of *C. difficile* were assessed.

Results: A significant effect of time but not treatment group was observed in the cow: alpha diversity of the microbiota of cows decreased from drying off to calving, and 14 and 7 genera were found at significantly higher relative abundances at calving and enrollment, respectively. No effect of the dam's treatment group was observed on the diversity or composition of the neonatal calf microbiota. *C. difficile* was found in only one cow (control group) and in two calves (one in control group, one in treatment group).

Conclusions: The impact of high levels of dietary zinc appeared to be minimal, with no observed changes in alpha or beta diversity, and few changes in the relative abundance of a small number of taxa. As noted previously, there appears to be a wide margin of safety in dietary zinc levels on the cow and calf and on their gut microbiota.

Notes:

224 - Characterizing mitochondrial function of dairy calf lymphocytes from birth to maturity

Katy Kesler[1], Angel Abuelo[1]

[1]Department of Large Animal Clinical Sciences, Michigan State University. keslerka@msu.edu

Session: General Health & Physiology 3, 2024-01-23, 8:45 - 9:00

Objective: Dairy calves are equipped with the necessary machinery to mount an effective immune response; however, they often fail to fully respond to immune stimuli until they reach maturity at 6 months of age. The inability of lymphocytes to mount a robust response severely limits the effective use of vaccination for disease prevention in dairy calves. Immune responses are bioenergetically demanding, and lymphocytes are dependent on mitochondrial outputs for their functionality. If mitochondrial function changes similarly to lymphocyte function over time, it would support immunometabolic targets for future immunomodulatory therapies aimed at improving calf health. The objective of this project was to determine the extent to which the mitochondrial function of dairy calf lymphocytes changes with age from birth to immunologic maturity.

Methods: Groups of dairy calves (n=4/group) were sampled pre-colostrum, 1 week (wk), 2 wk, 3 wk, 4 wk, 6 wk, 8 wk, 16 wk, 24 wk as well as mid-lactation adult cows (n=4) as controls. Whole blood was collected and B, CD4, CD8, and gamma-delta T lymphocytes were isolated using magnetic bead immunoprecipitation. Mitochondrial function was assessed with an extracellular flux analyzer. Non-mitochondrial oxygen consumption, basal respiration, maximal respiration, spare respiratory capacity, and proton leak were reported. Results were analyzed using a one-way ANOVA, and significant differences were calculated using a Tukey's honestly significant difference test and an alpha of 0.05. Tendencies were defined greater than 0.05 and less than 0.1.

Results: For CD4 Tcells, the pre-colostrum group had significantly higher maximal respiration than all other groups ($P<0.0001$). Additionally, the 3 wk group had higher non-mitochondrial oxygen consumption than the 8 wk ($P=0.03$), 16 wk ($P=0.02$), and 24 wk ($P=0.003$) groups and the pre-colostrum group was higher than the 16 wk group ($P=0.02$). Furthermore, the 3 wk group had more proton leak than the 8 wk ($P=0.046$), 16 wk ($P=0.04$), and 24 wk ($P=0.02$) groups. For CD8 T cells, there was a tendency for the pre-colostrum group to have higher maximal respiration than the 16wk ($P=0.052$) and 24 wk ($P=0.08$) groups. For gamma-delta T cells, the pre-colostrum group had significantly higher maximal respiration from all other groups ($P<0.0001$). Additionally, the pre-colostrum group had significantly higher spare respiratory capacity compared to the 1 wk ($P=0.008$), 2 wk ($P=0.01$), 4 wk ($P=0.03$), and 16 wk ($P=0.009$) groups. Overall, these results suggest that pre-colostrum Tcells appeared to have higher maximal respiration. However, this difference was absent by the first week of life and may be a transient effect accompanying the endocrine changes associated with the birthing process and should be explored further.

Conclusions: In conclusion, there is evidence that the T cells of pre-colostrum dairy calves appeared to have higher maximal respiration. Furthermore, starting from 1 week onwards, there were no metabolic differences observed in the mitochondria of dairy calf lymphocytes. Concluding that there is no evidence to support a metabolic change associated with age.

Financial Support: USDA-NIFA competitive grant #2023-67011-40483.

Notes:

225 - Probiotics in milk replacer affect the lung microbiome of neonatal dairy calves

Carol G. Chitko-McKown[1], Tara G. McDaneld[1], Janice E. Kritshevsky[2], Keith A. Bryan[3], Susan D. Eicher[4]

[1]USDA-ARS, USMARC, [2]Purdue University College of Veterinary Medicine, [3]Chr. Hansen, Inc., [4]USDA-ARS, LBRU. Carol.ChitkoMcKown@usda.gov

Session: General Health & Physiology 3, 2024-01-23, 9:00 - 9:15

Objective: Probiotics have been investigated for many health benefits, however, little research has been done to determine the effects of oral probiotics on the microbiome of the bovine respiratory tract. Previous data has indicated that probiotics administered to neonatal dairy calves in their milk replacer resulted in changes in the bacterial taxa in relative abundance with the addition of the probiotic compared to the control diet in both nasal and tonsil samples. Our hypothesis for this study was that this same probiotic treatment would result in changes in the lung microbiome as measured in lung lavage fluid.

Methods: A group of 20 dairy calves were split into two treatment groups: Control (N=10, milk replacer), and Probiotic (N=10, milk replacer + 0.5g/day Bovamine Dairy). On day 0 birth weight was obtained and the calves were provided colostrum as per the dairy SOP. On day 2, probiotics were added to the milk replacer of the treated group then included in their dry ration. Lung lavages were performed on day 52 on five calves from each treatment group. DNA was extracted from lavage fluid to evaluate the bacterial populations in the lung microbiome [MTRA1] [CMCRA2]. Hypervariable regions 1 through 3 along the 16S ribosomal RNA gene were amplified by PCR and sequenced by Illumina MiSeq to determine the bacterial taxa present.

Results: Preliminary data indicated that the bacterial genera identified in the lungs of probiotic-fed calves as compared to the control calves are significantly different ($P<0.05$). Additionally, when comparing diversity of taxa in the lung lavage samples to nasal and tonsil samples, taxa diversity of lung samples was significantly lower ($P<0.05$). As a result, numerous taxa [MTRA3] [CMCRA4] were identified to be significantly different in abundance in tonsil and nasal samplescompared to lung lavage samples ($P<0.05$).

Conclusions: Oral probiotics effect more than the gut microbiome.

Financial Support: USDA-ARS Project Number: 3040-32000-036-000D and Chr. Hansen, Inc., Milwaukee, USA.

Notes:

226 - Characterizing the developing fecal microbiome and resistome in foals and their dams

Maggie M. Murphy[1, 2], John L. Pipkin[1], Lee J. Pinnell[2], Cory Wolfe[2], Eryah Mora[3], Kelsey Shields[3], Jessica Looman[3], Gregg O. Veneklasen[3], Paul S. Morley[2]

[1]West Texas A&M University, [2]VERO Program, Texas A&M University, [3]Timber Creek Veterinary Hospital. mmurphy@wtamu.edu

Session: General Health & Physiology 3, 2024-01-23, 9:15 - 9:30

Objective: Changes in the gut microbiome during early-life can be associated with lasting effects on the structure and function of microbial communities, which in turn can affect the health and performance of animals. The purpose of this study was to characterize changes in the fecal microbiome and resistome of foals and their dams during early life.

Methods: Fecal samples were collected per rectum from 14 dam and foal pairs (n = 28) that were born and raised at Timber Creek Veterinary Hospital in Texas. Fecal samples were collected from dams at the estimated 300 days-of-gestation, and feces were collected from both foals and dams at 0, 2, 7, 14, 21, 28, 60, 90, and 120 days-of-age. Following DNA isolation, 16S rRNA and AMR target-enriched metagenomic sequencing was performed. Sequence data was analyzed using a combination of QIIME2, AMR++ (https://www.meglab.org/amrplusplus/), and phyloseq in R.

Results: Microbial richness significantly increased as microbial community structures changed over time across the first 4 sampling timepoints, and then stabilized. Interestingly, resistome richness followed an opposite pattern; decreasing significantly between day 28 and 60. The composition of the resistome changed significantly between day 28 and 60, but remained stable afterwards. The microbiomes and resistomes of the dams remained stable throughout the study.

Conclusions: The microbiome stabilized around 2 weeks of age, while the resistome stabilized later. Comparisons to foals with gut disease or treated with antimicrobial drugs may help elucidate potential impacts of dysbiosis.

Financial Support: Texas A&M University

Notes:

227 - Anti-IL-10 effects on broiler performance recovery following coccidiosis and necrotic enteritis challenge

K. Fries-Craft[1], S. Schmitz-Esser[1], E.A. Bobeck[1]

[1]Department of Animal Science, Iowa State University. kfcraft@iastate.edu

Session: General Health & Physiology 3, 2024-01-23, 9:30 - 9:45

Objective: *Eimeria spp*. in poultry induce host IL-10 production to gain a competitive advantage which also promotes conditions for necrotic enteritis due to secondary *Clostridium perfringens* (CP) challenge. Feeding anti-IL-10 may preserve broiler performance during these challenges. The study objective was to monitor broiler performance recovery over 3 weeks following *E. maxima* (EM) challenge ± CP across models ± d0 inoculation with *Salmonella* Typhimurium (ST).

Methods: Three replicate 42 d studies used Ross 308 broilers placed in 32 wire floor cages at hatch (20 birds/ cage) and assigned to diets ± 0.03% anti-IL-10. In replicates 1 and 2, birds were inoculated with $1*10^8$ colony forming units (CFU) of ST on d0 whereas birds in replicate 3 did not receive ST. In all replicates, half the birds were challenged with 15,000 sporulated EM M6 oocysts on d14 with half of the EM-challenged birds receiving $1*10^8$ CFU of CP on d18 and 19. On d24, birds were moved from cages to floor pens to monitor performance recovery following challenge over the course of a typical commercial grow-out period. Body weights (BW) and feed intake (FI) were measured weekly and prior to transition from cages to floor pens. Performance data encompassing weeks 4-6 within each replicate were analyzed using a mixed model with diet and challenge effects (SAS 9.4; $P \leq 0.05$).

Results: The challenge main effect reduced d28 BW in replicates 1 and 2 by 8.1-19.6% in birds challenged with EM±CP compared to their unchallenged counterparts ($P \leq 0.0006$). In replicate 1, the challenge main effect increased week 4 body weight gain (BWG) 20.8-22.4% in EM±CP-challenged vs. unchallenged broilers ($P < 0.0001$), whereas BWG was similar across all groups in replicate 2 as early as week 4. By d42, all performance measures were similar across challenged and unchallenged groups in replicate 1 and birds fed anti-IL-10 in replicate 2. Unchallenged birds fed control diets in replicate 2 weighed 6.3-11.3% more than their counterparts challenged with EM±CP on d42 ($P = 0.01$). In replicate 3, the challenge main effect contributed to 9.3-10.8% reduced d42 BW and 5.7-6.6% depressed FI in birds challenged with EM±CP vs. unchallenged birds ($P < 0.0001$). Birds challenged with EM-only displayed 13.2% reductions in week 6 BWG and FI compared to unchallenged birds, regardless of diet ($P \leq 0.01$).

Conclusions: Outcomes in this study suggest that anti-IL-10 has variable impacts on broiler performance recovery following coccidiosis or necrotic enteritis challenge. Performance recovery by the end of the grow-out period in replicates 1 and 2, but not 3, suggests that models implementing ST inoculation at hatch may facilitate post-challenge performance recovery. This provides unique insight into the potential protective role of ST on broiler performance in the aftermath of enteric disease challenges.

Financial Support: The authors thank USDA-NIFA for financial support of this work (grant 2021-67015-34533).

Notes:

228 - High energy diet does not affect body condition score in a subset of late lactation dairy cows

Thiago Cunha[1], Pedro Monteiro[1], JP Martins[1], Milo Wiltbank[1], Laura Hernandez[1]

[1]University of Wisconin-Madison. Thiago.cunha@wisc.edu

Session: General Health & Physiology 3, 2024-01-23, 9:45 - 10:00

Objective: To examine possible differences in energy partitioning and efficiency of use of energy for lactation in late lactation Holstein cows.

Methods: Beginning 75d prior to dry-off multiparous cows (BCS 3.16±0.03, 148±0.5 d post-AI) were blocked by lactation and BCS and randomly assigned to one of two diets, control (NEL=1.57 Mcal/kg; n=44) and high energy (NEL=1.82 Mcal/kg; n=45) fed cows until dry-off**.** BCS, milk production, and feed intake were evaluated. Circulating concentrations of glucose, non-esterfied fatty acids, and beta-hydroxybutyrate were measured. The goal of the experimental design was to increase body condition score (BCS) in the high energy group and maintain body condition score in the control group. This experiment focuses on the production and metabolic responses of the cows in the high energy diet group.

Results: At the end of the treatment period, 60% (27/45) of cows increased BCS) from 3.26 ± 0.1 to 3.92 ± 0.1 ($P < 0.001$) and 40% of cows (18/45) failed to gain BCS (from 3.15 ± 0.1 to 3.27 ± 0.1; $P = 0.32$). The latter group was considered as a non-responsive group (NR; primiparous = 8, multiparous = 10). Within the time period of the treatment diet, NR had both higher milk production ($P < 0.01$) and higher feed intake ($P < 0.01$), but no differences in net energy balance when compared to the responsive group ($P = 28$), nor interactions between time and parity (P = 0.18). No differences were detected in glucose ($P = 0.72$), non-esterified fatty acids ($P = 0.32$), or milk production ($P = 0.17$*)* between cows that did not respond to the high energy diet. However, non-responders tended to have increased feed intake for the first 90 DIM (*P = 0.02)* and had decreased circulating beta-hydroxybutyrate concentrations postpartum ($P = 0.01$).

Conclusions: Surprisingly, a subset cows in our study did not respond to the diet, despite the high energy content. While this was unexpected, the data collected from these animals will provide us information on the ability of dairy cows to adjust their milk production in order to adapt to diet. Our data will help us understand the metabolic factors that might affect energy partitioning in high producing lactating cows at late stages of the lactation period. Our findings suggest that certain dairy cows are able to make homeorhetic adjustments to milk production in order to compensate for diet.

Financial Support: "This project was supported by Agriculture and Food Research Initiative Competitive Grant no. 2020-67015031260 from the USDA National Institute of Food and Agriculture.

Notes:

229 - SARS-CoV-2 spillovers across the human-animal interface

Andrew S. Bowman[1]

[1]Dept. of Veterinary Preventive Medicine, The Ohio State University. bowman.214@osu.edu

Session: ACVM - Featured Speakers, 2024-01-23, 10:30 - 11:15

We made the first report of SARS-CoV-2 in WTD when 36% of the nasal swabs we collected during January - March 2021 from WTD in northeast Ohio were positive for SARS-CoV-2 by rRT-PCR. Phylogenetic analysis indicated that at least six human-to-deer transmissions occurred in that population and there was evidence of at least several weeks of deer-to-deer transmission.

Since then, SARS-CoV-2 has been detected at high rates in WTD across North America. Surprisingly, an Ontario WTD clade of SARS-CoV-2 that descends from the B.1 lineage was linked with deer-to-human spillback based on sequence similarity and an epidemiological link of deer contract prior to the human infection.

After our first detection of SARS-CoV-2 in WTD, the Ohio Animal SARS-CoV-2 Surveillance Consortium, whose members include: The Ohio State University Department of Veterinary Preventive Medicine, Ohio Department of Natural Resources (ODNR) Division of Wildlife, Cleveland Metroparks, Columbus and Franklin County Metro Parks and USDA Wildlife Services, has been collecting more than 2,000 samples from WTD annually. During November 2021-March 2022, we expanded our Ohio deer sampling effort to cover 94% of Ohio's counties by sampling hunter harvested and culled WTD. SARS-CoV-2 was detected in 12.2% (246/2012) of the nasal swabs, spanning Ohio's major metropolitan and rural areas. Phylogenetic analyses revealed that SARS-CoV-2 was independently introduced from humans into deer more than 30 times, seeding separate outbreaks across the state. Deer-to-deer transmission persisted for 2-8 months and disseminated hundreds of kilometers across multiple counties. SARS-CoV-2 evolved nearly three times faster in deer compared to humans, making divergent evolutionary pathways possible if the virus persists in deer long-term.

However, the timing of WTD sampling efforts presents a challenge for elucidating mechanisms of spillover and maintenance. Because deer shed viable virus for only ~7 days post experimental infection, we have a narrow window to detect an active infection. In the 34 (out of 83) Ohio counties where we did not identify active infections from deer nasal swabs, deer in 59% of those counties did have seropositive blood samples indicating previous infection. Compounded with the fact that very little is known about antibody persistence in WTD following infection, it is difficult to ascertain the transmission dynamics in WTD populations only from surveillance that focuses on the short window of hunting season.

To date, we have had success in detecting hotspots of infection. Sequencing reveals concerningly frequent human to deer transmission and onward transmission in the deer population, but it remains unclear how human to deer transmission occurs, if deer populations can maintain SARS-CoV-2, and if so, by what mechanisms.

Notes:

230 - Utilizing Aptamer-based proteomics to investigate feline infectious peritonitis pathogenesis and discover biomarkers.

Glenn A. Dean[1]

[1]Department of Microbiology, Immunology, and Pathology, College of Veterinary Medicine and Biomedical Sciences, Colorado State University. Gregg.Dean@colostate.edu

Session: ACVM - Featured Speakers, 2024-01-23, 11:15 - 12:00

Feline infectious peritonitis (FIP) is a highly variable systemic manifestation of Type I and Type II Feline Coronaviruses (FCoV). While infection with FCoV is common in cats, most infections are asymptomatic or cause only mild gastrointestinal signs due to a limited primary infection of the intestinal epithelium. This minimally pathogenic biotype is referred to as Feline Enteric Coronavirus (FECV). Mutation of the virus in individual hosts is thought to result in broader systemic distribution of virus that might manifest as the FIP biotype. Anti-coronavirus antibodies and the presence of virus in feces, blood or tissues can occur in the absence of clinical FIP making antemortem diagnosis of FIP difficult. To elucidate the systemic pathogenesis of FIP and attempt to identify potential diagnostic biomarkers, we measured >1300 serum proteins using an aptamer-based proteomics assay. Study groups include cats with active FECV infection, immunohistologically diagnosed FIP (dry and wet forms) and chronic FIV infection. Clinically normal cats that were negative for FIV and FCoV were also included. Pathway enrichment and associated analyses showed differentiable proteins were related to immune system processes, including the innate immune response, cytokine signaling and antigen presentation, as well as apoptosis and vascular integrity. Three candidate biomarker proteins were identified that successfully categorized all FIP versus non-FIP cats. The biomarkers identified by the aptamer assay were validated orthogonally by ELISA using the original sample set. These serum proteins represent potential antemortem diagnostic targets that can be quickly and inexpensively measured.

Notes:

231 - Quantifying trade-offs between therapeutic efficacy and resistance dissemination for enrofloxacin dose regimens in cattle

Liton Chandra Deb[1], Archana Timsina[1], ABM Shamim Ul Hasan[1], Suzanne Lenhart[2], Derek Foster[1], Cristina Lanzas[1]

[1]Department of Population Health and Pathobiology, North Carolina State University, [2]Department of Mathematics, University of Tennessee. lchandr@ncsu.edu

Session: Antimicrobial Use & Resistance 3, 2024-01-23, 10:30 - 10:45

Objective: Antimicrobials used in food-producing animals increase the selection pressure on bacteria to become resistant, which can be potentially transferred to humans. This study presents a deterministic mathematical model of within-host *P. multocida* infection, focusing on determining the treatment regimens (duration and dosage) of enrofloxacin. We demonstrate the effectiveness of our approach using a case study of a common antimicrobial treatment of respiratory infection due to *Pasteurella multocida* by enrofloxacin and discuss the potential implications for improving antimicrobial stewardship and preserving gut microbiota health. The main goal of this project is to study the trade-offs between the treatment costs and the level of the resistance of commensal bacteria (*E. coli*) and *P. multocida* bacteria.

Methods: We developed a within-host ordinary differential equation model to track the dynamics of antimicrobial drug concentration and bacterial populations in the site of infection (lung) and the gut. The model was parameterized to represent enrofloxacin treatment for bovine respiratory diseases caused by *Pastereulla multocida* in cattle. Three approved enrofloxacin dosing regimens were compared for their effects on bovine respiratory disease (BRD) treatment and resistance dissemination in the gut: 12.5 mg/kg and 7.5 mg/kg as a single dose, and 5 mg/kg as multiple doses. Additionally, we explored some non-approved dose regimes.

Results: The simulated results of three approved dosing scenarios reveal intriguing trends. Our results indicated that high-dose scenarios increased treatment costs and bacterial resistance levels in the gut and lungs compared to multiple low-dose scenarios. A proposed scenario (7.5 mg/kg, two doses 24hrs apart) showed promising results with lower economic costs and reduced bacterial resistance for treating BRD in cattle. This particular treatment exhibited not only demonstrated remarkable cost-effectiveness but also played a significant role in reducing bacterial resistance within both the gut and lungs. Moreover, it successfully cured the infection within a mere 48-hour time-frame following the administration of the treatment.

Conclusions: In conclusion, our study findings highlighted the significance of optimizing approved treatment dosing scenarios for managing BRD and emphasizes the importance of utilizing appropriate antimicrobial therapies for the shortest duration to mitigate the spread of antimicrobial resistance (AMR).

Financial Support: NIH R35GM134934

Notes:

232 - Impact of florfenicol dosing regimen on the phenotypic and genotypic resistance of enteric bacteria in steers

Jennifer Halleran[1], Hannah Sylvester[1], Megan Jacob[1], Benjamin Callahan[1], Ron Baynes[1], Derek Foster[1]

[1]North Carolina State University. jlhaller@ncsu.edu

Session: Antimicrobial Use & Resistance 3, 2024-01-23, 10:45 - 11:00

Objective: To assist with combating the antimicrobial resistance (AMR) crisis, the World Health Organization has recommended that food animal veterinarians use "lower tier" antimicrobials, such as florfenicol, to treat disease. Currently, there is limited knowledge about the development of resistance within the gastrointestinal tract when florfenicol is administered. The objective was to compare phenotypic and genotypic assessments of resistance in enteric bacteria following both FDA-approved florfenicol dosing regimens. The overall hypothesis was an increased prevalence of phenotypic resistance and antimicrobial resistance genes (ARGs) in the steers administered the repeated, lower dose of florfenicol and that the prevalence would be similar between detection methods.

Methods: Twelve steers were administered either two intramuscular (20 mg/kg q48hr; n=6) or a subcutaneous dose (40 mg/kg, n=6) of florfenicol. Fecal samples were collected for 38 days after administration. *E. coli* and *Enterococcus* were isolated and evaluated for susceptibility using broth microdilution. Fecal samples were submitted for metagenomic sequencing analysis. Raw reads underwent quality control, removal of bovine DNA and then were aligned to the Resistance Gene Identifier (RGI) within the Comprehensive Antimicrobial Resistance Database (CARD). The ARGs were normalized to account for differences in ARG length, the sample bacterial load, and length of the 16S rRNA sequence. To determine if there was a statistically significant difference in the MIC value or normalized AMR abundance, predetermined individual Wilcoxon ranked sum tests were conducted with Bonferroni correction ($p < 0.0125$).

Results: Fecal *E. coli* and *Enterococcus* were isolated and enumerated from all samples. Phenotypically, the general trend of an increasing MIC value after florfenicol administration with a return to the baseline MIC was observed for *E. coli* against cefazolin and ampicillin. The largest number of ARGs detected was at 72 hours (n=273) post dose. Aminoglycosides were the most abundant resistance determinant. In general, there was an increase in mean normalized AMR abundance seen 72 hours following florfenicol administration, with a return to baseline at the end of the study period for both dosing groups. There was no statistical difference when comparing mean normalized AMR abundance between dosing groups at any of the predetermined time points. Multiple multidrug resistance patterns were detected, with 10 containing phenicol resistance, and this was not different by treatment.

Conclusions: MIC values interpreted as resistant were observed in both dosing groups but returned to baseline at the end of the study period (38 days). Genotypically, numerous ARGs were detected, but the mean normalized AMR abundance did not significantly differ between dosing groups. Phenicol resistance appears to be associated with co-resistance to multiple antibiotic classes, but the significance in the dissemination to the food supply and human health is unknown. Resistance assessment between detection methods was different, indicating the utility of either may depend upon the goal of the experiment. The use of florfenicol as a "lower tier" antimicrobial may not reduce the overall development and potential transmission of ARGs relative to other "higher tier" drug classes.

Financial Support: FARAD

Notes:

233 - Evaluation of efficacy and antimicrobial resistance of two dry-cow intramammary antimicrobials in dairy goats

Michelle P Buckley[1], Roselle C Busch[2], Orhan Sahin[1], Patrick J Gorden[1]

[1]Iowa State University, [2]University of California at Davis. mpbuck@iastate.edu

Session: Antimicrobial Use & Resistance 3, 2024-01-23, 11:00 - 11:15

Objective: The goals of this study were to evaluate the efficacy of intramammary antimicrobial treatment for curing subclinical mastitis caused by non-aureus S*taphylococci* (NAS) and evaluation of development of antimicrobial resistance as characterized by antimicrobial susceptibility testing (AST) during the dry period.

Methods: Does enrolled in this study were residents of one of three farms from Iowa, Wisconsin, or California. All eligible does had aseptic milk cultures collected one week prior to scheduled dry-off. Pathogens were isolated and identified using standard laboratory techniques and confirmed using Matrix Assisted Laser Desorption Ionization-Time of Flight (MALDI-TOF). Halves that cultured positive for NAS were randomly assigned to one of three treatment groups: cloxacillin benzathine (CLOX), cephapirin benzathine (CEPH), or no intramammary treatment (NT). If the contralateral half also cultured positive, it was assigned the same treatment group as the positive half. Contralateral halves that cultured negative were enrolled as negative controls (NC) and no therapy was administered. Each enrolled doe had post-kidding cultures collected within 7 days of parturition. New infection risk and cured infection risk were used to evaluate efficacy of each treatment. Non-cured infections were defined as halves with the same organism identified on dry-off (DO) and post-fresh (PF) samples. New infections were defined as halves with no infection at DO and a pathogen present at PF or halves with a different pathogen present in DO and PF samples. Antimicrobial susceptibility testing was performed on isolates from halves identified as non-cured as well as new infections using broth microdilution plates for veterinary mastitis pathogens.

Results: Basic descriptive data analysis was completed to evaluate efficacy using commercial statistical software. Preliminary data indicates that 71.6% of halves sampled had subclinical infection with NAS at DO. Across all farms 116 of 133 (87%) of CEPH halves experienced a cure while 99 of 114 (87%) of CLOX treated halves cured during the dry period and 83 of 107 (78%) of NT halves. A tendency was detected indicating higher cure rate for CLOX halves than NT halves (P=0.0513), while halves treated with CEPH were significantly more likely to experience a cure than those that did not receive treatment (P= 0.0361).

Isolates for California does have not yet been evaluated for antimicrobial susceptibility. All AST isolates from Iowa and Wisconsin demonstrated inhibition of growth to cephalothin and oxacillin at ≤2 µg/mL. Breakpoints for CNS in goats are limited for these drugs and interpretation of AST data was only available for *Staphylococcus lugdenensis* treated with oxacillin, which is considered susceptible at ≤2 µg/mL. We suspect that this data can be extrapolated to other CNS strains as well. Other antibiotics evaluated do have established breakpoints. These drugs included ampicillin (76% susceptible), ceftiofur (97% susceptible), and erythromycin (82% susceptible).

Conclusions: This study demonstrates increased efficacy of two intramammary antibiotics for curing subclinical mastitis due to NAS in goats as compared with no treatment. AST indicated no changes in susceptibility to either drug between DO and PF sampling and inhibition of growth at low concentrations was noted for both drugs.

Financial Support: This work is supported by the USDA National Institute of Food and Agriculture, Agricultural and Food Research Initiative Competitive Program, Antimicrobial Resistance grant number: 2020-04197.

Notes:

234 - Antimicrobial resistance and molecular epidemiology of ESBL-producing-*Klebsiella* species in dairy cattle farms

Benti D. Gelalcha[1], Ruwaa Mohammed[1], Aga E. Gelgie [1], Oudessa K. Dego[1]

[1]The University of Tennnessee. bgelalch@vols.utk.edu

Session: Antimicrobial Use & Resistance 3, 2024-01-23, 11:15 - 11:30

Objective: *Klebsiella* species commonly inhabit the gut of dairy cattle. Frequent use of ceftiofur in dairy farms may impose selection pressure and result in the emergence of extended-spectrum beta-lactamase (ESBL)-producing strains. However, information on the status and spread mechanism of ESBL-*Klebsiella* spp. in dairy farms is largely unknown in U.S. dairy farms. This study aimed to determine the prevalence, antimicrobial resistance, and spread mechanisms of ESBL-*Klebsiella* spp. in dairy cattle farms.

Methods: Rectal fecal (n=508), manure (n=30), water (n=19), and feed (n=15) samples were collected from 14 dairy farms in East Tennessee. The samples were processed and plated directly on CHROMagar™ ESBL. Presumptive ESBL-*Klebsiella* spp. were confirmed using MADLI-TOF MS. The isolates were subjected to antimicrobial susceptibility testing (AST) and whole genome sequencing (WGS).

Results: From 572 samples, 57 (10%) were positive for ESBL-*Klebsiella* spp. The prevalence of fecal ESBL-*Klebsiella* spp. was 7.2 % (95% CI: 6.5 - 8.0). Most (96.5%, n=57) ESBL-*Klebsiella* spp. were resistant to ceftriaxone. About 19% of ESBL-K*lebsiella* spp. were multidrug resistant (MDR; resistance to ≥3 antimicrobial classes). Six families of beta-lactamase (*bla*) genes (*bla*$_{CTX-M}$, *bla*$_{SHV}$, *bla*$_{TEM}$, *bla*$_{OXY}$, *bla*$_{OXA,}$ and *bla*$_{SED)}$ were detected in ESBL-*Klebsiella* spp. genomes. Most (93%) isolates harbored two or more *bla* genes. The isolates were genotypically MDR, with 26 distinct types of antibiotic resistance genes (ARGs) and point mutation in *gyrA*, *gyrB*, and *par*C genes. The *Klebsiella* genomes also harbored 22 different plasmid replicon types. The IncFII and Col440I plasmids were most frequent and were associated with *bla*$_{CTXM-27}$ and *qnrB19* genes, respectively. Fifteen distinct sequence types (STs) and ten unknown STs of *K. pneumoniae* were detected. Clusters of ESBL-*Klebsiella* strains with identical STs, identical plasmids, and ARGs were detected in multiple farms indicating possible animal-to-animal transmission and inter-farm spread. The same ESBL variant was linked to identical plasmids in different *Klebsiella* STs, suggesting a possible horizontal spread of the ESBL genes.

Conclusions: The high burden of ESBL genes per *Klebsiella* spp. genome coupled with the dual spread mechanism could rapidly increase the prevalence of ESBL-*Klebsiella* and pose a risk to the health of humans, animals, and the environment.

Financial Support: The study was funded by The University of Tennessee, Knoxville.

Notes:

235 - The effect of metritis treatment with ceftiofur on fecal microbiome and resistome of dairy cows

Gerardo R Diaz[1], Tui Ray[1], Paulo R Menta[2], Vinicius S Machado[2], Noelle R Noyes[1]

[1]Department of Veterinary Population Medicine, College of Veterinary Medicine, University of Minnesota., [2]Department of Veterinary Sciences, Davis College of Agricultural Sciences and Natural Resources, Texas Tech University. diazo005@umn.edu

Session: Antimicrobial Use & Resistance 3, 2024-01-23, 11:30 - 11:45

Objective: Ceftiofur is the treatment par excellence for metritis in US dairy farms. Although it has been associated with several positive outcomes, some caveats, such as antimicrobial resistance (AMR), have been pointed out as negative consequence. Thus, we aimed to evaluate the effect of ceftiofur treatment for metritis on the AMR development in dairy cattle.

Methods: We selected 30 Holstein cows with metritis from a randomized controlled study that assessed the effectiveness of ceftiofur as metritis treatment. Fifteen fecal samples from treated cows (6.6 mg/kg of ceftiofur crystalline-free acid IM) and 15 from control cows were collected at day 0, 5 and 14 post-treatments. The fecal microbiome was investigated using 16S rRNA gene sequencing (V3-V4 region, 2x300bp PE). The bioinformatic analysis was conducted using cutadapt, DADA2, decontam and phyloseq in R software. The fecal resistome was interrogated using a bait enrichment approach with a panel of 31,250 unique 120-mers AMR gene probes, coupled with shotgun metagenomic sequencing. This enabled a 2-3x order-of-magnitude increase of on-target sequencing for antimicrobial resistance genes (ARGs) and virulence factors. The bioinformatic analysis was performed by AMR++ v2 and MEGaRES database. Regression models were used for hypothesis testing, considering timepoint and ceftiofur treatment (and interactions) as fixed effects, diversity metrics and differential abundance of a given feature as outcomes, and cow ID as random effect.

Results: A significant decrease in richness (R) and Shannon's diversity (ShD) of AMR genes in the treated group (R=359, CI95% 307-411; ShD=5.01, CI95% 4.85-5.18) compared to the control group (R=470, CI95% 418-522; ShD=5.33, CI95% 5.17-5.49) ($P = 0.0034$ for R; $P = 0.008$ for ShD). The beta diversity of AMR genes was significantly partitioned to treatment only at day 5 (PERMANOVA test, $R2 = 9.2\%$, $P = 0.009$). We observed that treatment across time-points significantly increased 4.71 LogFC the relative abundance of blaCMY AMR gene (Class-C-betalactamases mechanism) in treated cows compared to controls across time-points (Zero-inflated gaussian mixture model, p-adjusted < 0.001). Fecal Microbiome composition did not present diversity differences on treated vs control cows and presented minor changes on relative abundances at the genus level.

Conclusions: Our results suggest that IM ceftiofur treatment for cow metritis had an effect on fecal AMR gene diversity significant only at day 5 post-treatment and that overall, the blaCMY gene was significantly more abundant in treated cows. Further research is needed to understand the dynamics of Class-C-betalactamases resistance on the bacterial community of feces, which in our study seemed to be undisturbed.

Financial Support: USDA-NIFA-AFRI program (grant number: 2019-67015-29865).

Notes:

236 - Resistance-Enhanced variant of *Campylobacter's* CmeABC efflux pump utilizes distinct residues for drug binding and resistance

Nicholas Lizer[1], Zuowei Wu[1], Qijing Zhang[1]

[1]Iowa State University. nlizer@iastate.edu

Session: Antimicrobial Use & Resistance 3, 2024-01-23, 11:45 - 12:00

Objective: *Campylobacter* is a major cause of foodborne illnesses worldwide and has also been noted for it's startling increase in antibiotic resistance, which has led the U.S. Centers for Disease Control and Prevention to designate the organism a series antibiotic resistance threat. A major mechanism utilized by *Campylobacter* species to resist antibiotics is a resistance-nodulation-cell division (RND) type multidrug efflux pump, named CmeABC. Recently, a resistance enhanced variant (RE-CmeABC) of this efflux pump has been discovered in some *Campylobacter* strains, which greatly enhances their ability to resist higher concentrations of antibiotics, when compared to the typical CmeABC variant. How RE-CmeABC confers the enhanced resistance is unclear, but we hypothesize that amino acid changes in the drug-binding pocket of RE-CmeB are responsible for this phenotype.

Methods: To test this hypothesis and understand the molecular basis of RE-CmeABC mediated multidrug resistance, we generated site-specific mutants of RE-CmeB with several amino acid substitutions in its drug-binding pocket and evaluated their antibiotic resistance phenotypes in comparison with the strain carrying a non-mutated RE-cmeB.

Results: The results indicated that while the I136A and L662E mutations produced little discernable changes in the mutant's antibiotic resistance profile, the F625A, L610A, L607E, and M570A mutations showed significant shifts in resistance to ciprofloxacin, erythromycin, and tetracycline. Mutations that converted non-polar residues to other nonpolar residues produced little change in resistance, while mutations in amino acids from non-polar to charged polar residues significantly impacted the antibiotic resistance function, most likely by disrupting the hydrophobic interactions required for drug binding. Removal of large residues, such as phenylalanine, also lead to an increase in susceptibility to antibiotics.

Conclusions: These findings define the role of several key amino acids in antibiotic efflux and provide new insights into the molecular basis of the enhanced function of RE-CmeABC in mediating antibiotic resistance.

Financial Support: This work was supported by NIH Grant R01AI140669.

Notes:

237 - Molecular evaluation of multi-drug resistant *Enterococcus faecium* isolated from patients and surfaces of a veterinary medical center

Elizabeth A Hamilton[1], Margaret Krueger[1], Shelbey Todd[1]

[1]Michigan State University, College of Veterinary Medicine. hameliz@msu.edu

Session: Biosecurity & Infection Control 3, 2024-01-23, 10:30 - 10:45

Objective: Nosocomial infections have significant impacts on the survivability of both human and veterinary patients. Each day, one in 31 patients in human hospitals across the United States has a hospital acquired infection (HAI). While there is currently no monitoring system for HAIs within veterinary hospitals, the impact is believed to be similar. *Enterococcus faecium* is frequently found in the environment of veterinary medical centers (VMC). While *E. faecium* can cause infections, it is more often found as a contaminate due to lapses in infection control and can develop or acquire antimicrobial resistance and create biofilms that may transfer resistance genes. The objectives of this study were to compare clonal relatedness and the phenotypic and genotypic antimicrobial resistance of *E. faecium* isolated from clinical and environmental samples from a VMC.

Methods: *E. faecium* identified from VMC environmental samples (n=32) and clinical canine and feline samples (n=30) collected in 2020 were used in this study. *E.* faecium isolates were identified using MALDI-tof and antimicrobial susceptibility results were obtained by broth microdilution using the Sensitre companion panels for gram positive organisms. MIC data were interpreted using Clinical Laboratory Standards Institute breakpoints. Data on clinical signs of infection and surgery/procedures performed were abstracted from patient medical records from whom clinical samples were collected. Whole genome sequencing (WGS) was performed on DNA extracted from these isolates. Multi-locus sequence typing was also performed on the raw reads; and the sequence types were entered into pubmlst.org to determine each isolates' clonal cluster (CC). Resistance genes were determined using ABRicate.

Results: WGS revealed that 6 of the 62 isolates were misclassified and were truly *E. faecalis* (n=1) and *E. lactis* (n=5). Of 56 *E. faecium* isolates, 8 sequence types (ST) and one CC were identified. The most common (46.8%) ST was ST80:CC17, which included 21 clinical samples and eight environmental samples. Within this cluster, four samples were collected from fine needle aspirate clinical samples (N=3) and the stainless-steel table where fine needle aspirate samples were collected (n=1) over a 12-day period. All three patients showed no clinical signs of infection at the time the sample was drawn. Of the 56 isolates, one from a clinical sample was resistant to all antibiotics tested. While all isolates were resistant to trimethoprim/sulfamethoxazole, only 69.6% carried the trimethoprim/sulfamethoxazole gene, dfrG (33.3% environmental, 66.7% clinical). Additionally, 2 clinical samples (3.6%) were resistant to chloramphenicol, only one carried the chloramphenicol gene, catA8.

Conclusions: ST80 was the most prevalence ST found among both clinical and environmental samples. While none was identified in this study, vancomycin-resistant *E. faecium* has been reported in association with ST80, which is also included in the hospital-acquired clade (A1/A2) recently defined. Based on the ST, sample type, and timing of sample collection, environmental contamination of clinical samples was documented in the VMC during this study. Infection control protocols were reinforced for FNA procedures. More work needs to be done to explore the application of these findings on infection control throughout the VMC.

Financial Support: This research was supported by the Michigan State University, College of Veterinary Medicine Endowed Research Fund.

Notes:

238 - Assessing risk factors for contamination of needleless connector ports in veterinary intensive care units

Sabrina Dauer[1], Elizabeth Hamilton[2], Nyssa Levy[2], Leslie Landis[2]

[1]College of Agriculture and Natural Resources, Michigan State University, [2]College of Veterinary Medicine, Michigan State University. dauersab@msu.edu

Session: Biosecurity & Infection Control 3, 2024-01-23, 10:45 - 11:00

Objective: Antimicrobial resistant bacteria are frequently isolated from surfaces and medical equipment in both human and veterinary medical facilities. Breaches in infection control protocols can create opportunities to introduce these resistant bacteria to patients, causing hospital-acquired infections. Use of intravenous (IV) catheters increases the risk for bacteria to be introduced into the bloodstream through the broken skin barrier causing catheter-related bloodstream infections (CRBSI). While the prevalence of CRBSIs are not tracked in veterinary medicine, approximately 80,000 CRBSIs occur in human intensive care units (ICUs) each year. The primary objective of this study was to determine risk factors associated with bacterial growth on needleless connector (NC) ports used on IV catheters for patients admitted to a veterinary hospital. The secondary objective was to determine susceptibility profiles for isolates of *Staphylococcus aureus, Staphylococcus pseudintermedius* and *Enterococcus faecium* found on NC ports.

Methods: An observational prospective study was conducted at the Michigan State University Veterinary Medical Center (MSU VMC). Over the course of three months, NC ports were collected from IV catheters when removed from cats or dogs admitted for at least one day at the ICU. These ports were then swabbed with transport media and submitted for bacterial identification and susceptibility testing. Medical record abstraction for all patients admitted into the ICU was performed to collect risk factor data. Chi-square, Fisher's exact tests, and univariable odds ratios were applied to determine the association between bacterial growth and risk factors.

Results: A total of 117 samples were collected from 23 cats and 73 dogs (n=96). Of the 117 samples collected, 32.5% (38/117) had bacterial growth, representing 34 patients (34/96). Fifty percent (17/34) of patients with growth on their NC ports were admitted due to GI symptoms, compared with 19%(12/62) of patients with no growth. Twelve percent (4/34) of patients with growth were admitted for injury/trauma, compared with 29% (18/62) of patients without growth. These differences produced a significant chi-square (p=.0466) and OR (4.427 [(1.494, 13.118]). Sixty-four bacteria were identified from the 38 positive samples. The majority were gram positive (71.9%, 46/64) of these, half were *Staphylococcus* spp. (23/46). *S. pseudintermedius* was identified on 23.7% (9/38) of the NC ports with growth, and five of these were methicillin-resistant *S. pseudintermedius* (MRSP). *E. faecium* was found on 7.9% (3/38) of the NC ports with growth; 100% were ampicillin resistant and 0% were vancomycin resistant. Of the 18 Gram negative bacteria (28.1%), the majority (44.4%) were of the *Enterobacteriaceae* family. *Klebsiella* spp. and *Pseudomonas aeruginosa* were both identified once on the same NC port and *Pantoea* spp. was identified on two NC ports.

Conclusions: We have identified a strong association between the reason for admission and contamination of IV catheter NC ports. Additional disinfection may be prudent for patients with IV catheters experiencing GI symptoms. Potential hospital-acquired pathogens were also identified, suggesting increased risk of CRBSIs from infection control breaches. Regardless of reason for admission, current protocols for handling IV catheters should be reviewed.

Financial Support: College of Veterinary Medicine, Michigan State University

Notes:

239 - The effect of an electrostatic precipitator on mitigating aerosol transmission of influenza A virus in pigs

M Torremorell[1], L Wang[1], J Moran[2], M Yang[1], B Olson[2], C Hogan[2]

[1]Dept. of Veterinary Population Medicine, University of Minnesota, [2]Dept. of Mechanical Engineering, University of Minnesota. wang9036@umn.edu

Session: Biosecurity & Infection Control 3, 2024-01-23, 11:00 - 11:15

Objective: Airborne viruses pose a threat to both human and animal health. The rapid spread of airborne viruses makes them difficult to contain and protect against in animal premises, including swine barns. Although air filtration is used to reduce incidence of airborne infections into swine farms, there are still shortcomings, such as high costs and pressure drop due to resistance to the airflow.

Electrostatic precipitators (ESP) are filterless air cleaning devices that utilize charged electrodes to ionize airborne particles and remove them. ESPs tend to have high collection efficiency across a wide range of particle sizes and have potential for virus inactivation. ESPs also have lower pressure drop and energy consumption compared to air filtration systems. We have developed a novel ESP for application as a biosecurity technology to remove infectious pathogens from aerosols. In this study, we assessed the effect of an ESP on mitigating the airborne transmission of influenza A virus (IAV) using experimentally infected pigs.

Methods: Four H1N1 IAV inoculated pigs were placed in two air-connected isolators (2 pigs per isolator) upstream of the ESP, and two uninoculated pigs were placed downstream in one isolator to serve as sentinels. The airflow moved at 30 cfm unidirectionally from the inoculated pigs to the sentinel pigs. The ESP was installed and operated in between the isolators containing the inoculated and sentinel pigs. A positive control test with the same settings but with the ESP turned off was also performed. Sentinel pigs and inoculated pigs were sampled daily by collecting a nasal swab from 0 to 6 DPI (days post inoculation). Air samples were collected daily with two Andersen cascade impactors (ACI). The ACIs sampled the air simultaneously from upstream and downstream isolators, respectively. Environmental wipes were collected daily from surfaces inside each isolator. Samples were tested for IAV by RT-qPCR to detect and quantify viral RNA. PCR positive air samples were titrated in MDCK cells using the $TCID_{50}$ method to quantify viral viability.

Results: During the positive control test (ESP OFF), inoculated pigs started shedding IAV at 1 DPI, and sentinel pigs started shedding virus at 2 DPI indicating that sentinel pigs became infected through IAV contaminated air. Viral RNA was detected from multiple stages of ACIs both upstream and downstream in particle size ranges from 0.22 μm to > 8 μm.

During the ESP test (ESP ON), sentinel pigs in two distinct replicates tested positive at 5 DPI and 7 DPI, respectively, when the ESP operated at 12 kV, and at 6 DPI when the ESP operated at 14 kV. Viral RNA was mainly detected from upstream isolators (air and surfaces). No viable viruses were isolated from the air.

Conclusions: Under the conditions of this study, the ESP efficiently removed IAV from the aerosols generated by infected pigs and delayed the onset of infection in the sentinel pigs. In this study, the ESP showed promising potential to reduce the spread of airborne viruses in swine.

Financial Support: This project was supported by the Agriculture and Food Research Initiative Competitive Grant no. 2021-68014-33655 from the USDA's National Institute of Food and Agriculture.

Notes:

240 - Biocide tolerance of *Salmonella* isolates recovered from cattle

Samantha Locke[1], Poonam Vinayamohan[1], Dubraska Diaz-Campos[2], Greg Habing[1]

[1]Department of Veterinary Preventive Medicine, The Ohio State University College of Veterinary Medicine, [2]Department of Veterinary Clinical Sciences, The Ohio State University College of Veterinary Medicine. locke.91@osu.edu

Session: Biosecurity & Infection Control 3, 2024-01-23, 11:15 - 11:30

Objective: Strict biosecurity and consistent cleaning and disinfection are the primary control methods to reduce *Salmonella* transmission on-farm. However, cleaning and disinfection protocols have had varied effectiveness against *Salmonella* in farm environments, and residual contamination is often reported. Biocide choice is critical when designing effective cleaning and disinfection protocols, yet little is known regarding *Salmonella* susceptibility to commercial products. Our objective was to determine the minimum bactericidal concentration (MBC), defined as ≥3 log reduction, of 6 biocides commonly used in livestock production or veterinary medicine. We hypothesized that some biocide label guidelines would not be effective at significantly reducing *Salmonella*.

Methods: To assess this, a 48-well microtiter assay was developed that included two-fold serial dilutions of Clorox® Germicidal Bleach, chlorine dioxide, chlorhexidine gluconate, KennelSol™, Rescue™, and VirkonS®. The lowest concentration tested was chosen based on the label directions for use and increased to 12 to 14 times the initial concentration, depending on the biocide. A neutralizer was used to inactivate biocides and simulate 10 minutes of contact time between bacteria and biocide in accordance with some biocide label instructions. A second assay was run concurrently, using sterile water as a sham for neutralizer. Six isolates each from *Salmonella* serovars Dublin, Newport, and Typhimurium recovered from ill cattle were tested in duplicate. Bacteria were enumerated to determine the log reductions after biocide exposure.

Results: Dilutions specified on KennelSol™ and VirkonS® labels resulted in a complete kill (no bacterial growth) for all isolates after 10 minutes of contact and subsequent neutralization. For all isolates, Clorox® was effective at either 625 or 1250 parts per million (ppm) within the label guideline for hard, nonporous surfaces (2400 ppm). However, the 200-ppm guidance for food contact surfaces failed to significantly reduce or eradicate any isolates tested, which is a public health concern for cattle-derived *Salmonella* strains that are transmitted through the food supply. Rescue™ required a 2- to 4-fold concentration above the label suggestion to achieve biocidal effects within 10 minutes when neutralized, but MBCs fell within suggested guidelines after 24 hours in the sterile water assay. Chlorine dioxide had limited efficacy in the contact time tested, with only 1 isolate susceptible at 3000 ppm. When exposed to sterile water MBCs were 3,000 ppm (n=15) or 1,500 ppm (n=2), far above suggested use guidelines. One Newport isolate exhibited tolerance to chlorine dioxide at all concentrations tested.

Conclusions: Results from this assay identified rapidly effective biocides and, as isolates were recovered from ill cattle, are particularly pertinent for guiding biocide choice on cattle farms experiencing salmonellosis outbreaks. Future work should define MBCs required to significantly reduce *Salmonella* biofilms, as well as account for potential variation in biocide effectiveness based on surface material.

Financial Support: This work was supported by a USDA Animal Health Formula Funds intramural grant in the College of Veterinary Medicine at The Ohio State University.

Notes:

241 - Detection of *Salmonella* Dublin from environmental samples by end point multiplex PCR.

Alejandra Arevalo-Mayorga[1], Jamie Ridolfo[1], Samantha Locke[1], Greg Habing[1]

[1]Dpto. Veterinay Preventive Medicine, The Ohio State University. arevalomayorga.1@osu.edu

Session: Biosecurity & Infection Control 3, 2024-01-23, 11:30 - 11:45

Objective: *Salmonella* Dublin (S. Dublin) causes severe disease in humans and production losses in the dairy industry due to acute, persistent, or subclinical infections. Detection by culture is laborious, time-consuming, and has remarkably low sensitivity in environmental samples, limiting our detection capacity and, subsequently, our understanding of its overall prevalence and epidemiology. Thus, using a culture-independent technique is a necessary complement to efficiently identify affected premises and provide insight into transmission pathways. The objective of this study was to develop and implement a multiplex end-point polymerase chain reaction (PCR) for the detection of *S.* Dublin in cattle environmental samples. We hypothesized that the use of PCR would increase the detection of *S.* Dublin when testing the pre-enrichment broth.

Methods: A total of 152 boot swabs were collected from pens and alleyways at one surplus calves' auction (n=50), eight veal farms (n=52), and ten dairy beef farms (n=50). At the auction, weekly sampling encompassed pens with animals present and the corresponding alleyways. In veal farms, only the alleyways were sampled, while in dairy beef farms, samples were obtained from both alleyways in pre-weaned calves and pens in post-weaned calves. Each farm underwent sampling once during the summer of 2023.

For the screening of *S.* Dublin, each sample underwent both the culture method and PCR. In the culture method, samples were enriched in liquid media (BPW, RV, and SB) and semisolid medium (MSRV). Subsequently, aliquots were streaked onto XLT4 agar, and presumptive colonies were serogrouped using Wellcolex™ Color Salmonella Rapid Latex Agglutination. Group D-positive isolates were then confirmed using monovalent antisera (Difco™ Salmonella O:9 Antisera). The DNA was isolated from the pre-enrichment broth of each sample utilizing a column-based commercial kit (Quick-DNA™ Fungal/Bacterial Miniprep Kit, Zymo). The endpoint PCR followed the protocol established by the One Herd Lab.

Results: Overall, 31.6% (48/152) of the cattle environment samples tested positive for *S.* Dublin via PCR, while 1.3% (2/152) showed positive results through culture. Specifically, 46% (23/50) of the auction samples tested positive for *S.* Dublin via PCR, with the highest number of positive samples originating from pen 6, housing animals over 100 pounds. None of the colonies were confirmed as *S.* Dublin. In the veal environments, *S.* Dublin was not detected by either PCR or culture.

When applying PCR to dairy beef samples, 50% (25/50) tested positive for *S.* Dublin, while only 4% (2/50) were positive by culture. One originated from the milk mixing room, and the other from the post-weaned environment. Each isolate came from a different farm.

Conclusions: The use of PCR increased the detection of *S.* Dublin from environmental samples when compared to culture. Detection via PCR provides insight into the environmental presence of *S.* Dublin across different cattle environments and has the potential to enhance our understanding of its transmission.

Financial Support: This study was funded by the USDA (Grant No. 2022-6801536628), under the Agriculture and Food Research Initiative (AFRI) of the National Institute of Food and Agriculture (NIFA).

Notes:

242 - Antimicrobial resistance and antimicrobial residues in the different stages of commercial poultry environment

P Gaonkar[1], C Higgins[1], R Golden[1], Y Adhikari[2], M Bailey[2], L Munoz[2], K Macklin[3], L Huber[1]

[1]Department of Pathobiology, Auburn University, [2]Department of Poultry Science, Auburn University, [3]Department of Poultry Science, Mississippi State University. ppg0001@auburn.edu

Session: Biosecurity & Infection Control 3, 2024-01-23, 11:45 - 12:00

Objective: Antimicrobial resistance (AMR) is a threat to the poultry industry, resulting in significant economic losses. This is due to reduced effectiveness of antimicrobials in treating infectious diseases in poultry, which results in increased mortality rates and higher expenses for alternative treatment. AMR transmission can take place between poultry, humans, and environment. However, the status of AMR in the environment is less studied. The objective of this study was twofold: first, to determine the AMR level in the environment of different stages of commercial poultry farms; and second, to quantify the antimicrobial residues present in the same environment.

Methods: Commercial poultry farms in different stages of production and practicing restricted antimicrobial use were included in this study. Farm practices data, especially on antimicrobial use (AMU) were collected as well as litter samples from inside the poultry house, and soil and fecal samples belonging to wild or domestic animals in the vicinity of the house. Broiler flocks were followed to the processing plant, and carcass rinses were collected from post-pick and post-chill stages. The frequency of 3 mobile genetic elements (MGE) and 14 antimicrobial resistance genes (ARGs) that confer AMR to 8 antimicrobial classes was assessed using qPCR. Data analysis was performed using RStudio. Liquid Chromatography-Mass Spectrometry (LC-MS) will be used to detect antimicrobial residues in the samples.

Results: Poultry farms enrolled in this study had a history of using antimicrobials for therapeutic purposes: penicillin and sulfonamide in pullet farms, tetracycline and aminoglycoside in breeder farms, and bacitracin in broiler farms. In broiler farms, AMR to majority of antimicrobial classes was higher compared with breeder and pullet farms. However, pullet and breeder farms showed higher frequency of sulfonamide ARGs, while beta-lactam ARGs were more commonly detected in breeder farms. Litter samples exhibited a higher level of AMR to majority of antimicrobial classes compared with soil and fecal samples, whereas soil samples showed lower level of AMR. Further, in fecal samples beta-lactam, bacitracin, and sulfonamide ARGs were most frequently observed. In the processing plant, the majority of ARGs were restricted to post-pick stage and aminoglycoside ARGs were found in post-chill stage. Overall, MGEs were most frequently found on broiler farms, litter samples, and post-chill stage compared with other farm types, sample types and post-pick stage, respectively. Apart from these findings, we will also present the detection of antimicrobial residues in farms' environment and their correlation with AMR.

Conclusions: Despite restricted AMU, there is potential for AMR spread across the food chain in commercial poultry. The historic use of antimicrobials may have resulted in the persistence of antimicrobial residue in the environment of farms with restricted AMU. Subsequent studies will explore the impact of antimicrobial residue on AMR and will be presented. Understanding the impact of historic AMU on AMR and accumulation of antimicrobial residues in the environment will provide potential pathways of AMR transmission across the food chain. Knowledge of the environmental aspects of AMR spread will help protect the health of poultry, humans, and the environment.

Notes:

243 - Swine air-liquid interface respiratory culture system for studying Influenza A subtypes H1N1 and H3N2 coinfections

A.F. Castillo-Espinoza[1], R.K. Nelli[1], L. Yen[1], G. Castillo[1], JC Mora-Díaz[1], L.G. Giménez-Lirola [1]

[1]Department of Veterinary Diagnostic and Production Animal Medicine. afc@iastate.edu

Session: Virology 5, 2024-01-23, 10:30 - 10:45

Objective: To describe the use of porcine-derived air-liquid Interface (ALI) primary respiratory epithelial cells (PRECs) as a model to characterize single vs. coinfection of Influenza A (IAV) subtypes H1N1 and H3N2, under strictly controlled *in vitro* conditions.

Methods: Tracheas collected from seven-days-old CD/CD pigs (n=3) were dissected, washed, and enzymatically digested to isolate PRECs, which were seeded into pre-coated transwells and cultured under ALI conditions for 4-5 weeks to allow them to differentiate. Differentiated ALI-PRECs were then inoculated with H1N1 (A/Swine/Minnesota/37866/1999), H3N2 (A/Swine/Texas/4199-2/1998), or both, along with mock uninfected controls. ALI-PRECs were exposed to viral/mock inoculum at a multiplicity of infection (MOI) of 0.1 and 1 for 6 h at 37°C, 5% CO2, after that the inoculum was removed, the wells were washed, and further incubated for 24, 48, 72, and 96 h post-inoculation (hpi). The outcome of the infection in ALI-PRECs was assessed microscopically and by immunocytochemistry (ICC), while the subnatants collected from the basolateral compartment were tested for IAV RNA detection by RT-qPCR.

Results: Microscopic evaluation showed active ciliary motility in both virus- and mock-inoculated ALI-PRECs up to 24 hpi. However, by 48 hpi, virus-inoculated ALI-PRECs exhibited significantly reduced ciliary motility, and characteristic cytopathic effects (CPE), including dead/lifting cells, became more pronounced by 72 hpi. The CPE was qualitatively assessed, and by 96 hpi, distinct virus-specific CPE was observed in all IAV-inoculated wells, including cytoplasmic stranding, vacuolation, cell rounding, clusters of rounded cells, cell shrinkage, and cell detachment. Mock-inoculated ALI-PRECs preserved ciliary beating and cell integrity; no morphological changes were observed in any biological replicate. ICC staining for IAV nucleoprotein further demonstrated that ALI-PRECs were permissive to initial virus entry and replication with detection at 24 hpi in fixed cells. Virus RNA by RT-qPCR was detected in the plate well subnatants of ALI-PRECs co-inoculated and single inoculated with H3N2 (MOI 1) as early as 24 hpi, and at 48 hpi in H1N1-inoculated ALI-PRECs. In summary, microscopic evaluation, ICC, and RT-qPCR data suggest that the ALI-PREC model is a suitable culture system for studying IAV single and coinfections in vitro.

Conclusions: Organotypic ALI-PREC is a suitable culture system for studying IAV single and coinfections of H1N1 and H3N2 subtypes *in vitro*, contributing to the advancement of research in a more ethical and innovative manner. Ongoing research will focus on characterizing gene expression profiles associated with the innate immune response during H1N1 and H3N2 coinfection and developing an ALI-PREC and macrophages/dendritic cells co-culture system to bridge innate and adaptive immunity against IAV.

Financial Support: Boehringer Ingelheim Animal Health.

Notes:

244 - Characterization of classical swine fever virus genotype 2 strains responsible for 2013-2018 outbreak in Colombia

Erin Robert[1], Kalhari Goonewardene[1], Lindsey Lamboo[1], Orlando Perez[1], Melissa Goolia[1], Charles Lewis[1], Cassidy Erdelyan[1], Oliver Lung[1], Katherine Handel[1], Estella Moffat[1], Carissa Embury-Hyatt[1], Nancy Naranjo Amaya[2], Alfonso Clavijo[1], Aruna Ambagala[1, 3, 4], Claudia Patricia Calderon Parra[2], Diana Cristina Gomez Rueda[2], Maria Antonia Rincon Monroy[2]

[1]Canadian Food Inspection Agency, National Centre for Foreign Animal Disease, [2]National Veterinary Laboratory, Instisuto Colombiano Agropecurio, [3]Department of Animal Science, University of Manitoba, [4]Department of Comparative Biology and Experimental Medicine, Faculty of Veterinary Medicine, University of Calgary. Erin.Robert@inspection.gc.ca

Session: Virology 5, 2024-01-23, 10:45 - 11:00

Objective: Classical swine fever (CSF) caused by CSF virus (CSFV) is a fatal hemorrhagic disease of domestic and wild pigs. CSF is reportable to the World Organization for Animal Health (WOAH) due to its significant losses to the pig industry. North America and a few countries in Latin America and Caribbean are free of the disease. Colombia is one of the largest pig producing countries in Latin America, and it suffered two major CSF outbreaks in the last decade - one in 2005 and the second in 2013. The purpose of this study was to characterize the virus responsible for the outbreak that began in June 2013.

Methods: A total of thirteen serum and ten tissue samples collected from northern Colombia between 2013-2017 were analyzed by PCR, virus isolation and whole genome sequencing. To determine the pathogenicity of the virus responsible, one of the CSFV isolates was inoculated into five-week-old weaned piglets.

Results: Phylogenetic analysis based on the full-length E2 sequence shows that the virus is closely related to the CSFV genotype 2.6 strains circulating in Southeast Asia. The pathotyping experiment suggests that the virus responsible for the outbreak is a moderately virulent strain. The 190 nucleotide stretch of the E2 hypervariable region of these isolates also shows high similarity to the CSFV isolates from Colombia in 2005 and 2006.

Conclusions: The emergence of genotype 2.6 in Colombia suggests a potential transboundary spread of CSFV from Asia to the Americas. The results also suggest that there is a common origin for both the 2005-2006 and 2013-2018 outbreaks. Presence of CSFV genotype 2.6 complicates the ongoing eradication efforts in the Americas, and emphasizes the need for continuous surveillance in the region.

Financial Support: This research was funded by the CFIA A-base projects N-000269 (Molecular Characterization of Reference Viral Strains at The NCFAD Mammalian Diseases Unit) and N-000305 (High consequence emerging viral diseases of swine in Caribbean region).

Notes:

245 - Naturally acquired equine parvovirus-hepatitis is associated with a wide range of hepatic lesions in horses

Joy E. Tomlinson[1], Mason C. Jager[1], Gerlinde R. Van de Walle[1]

[1]Cornell University College of Veterinary Medicine. jet371@gmail.com

Session: Virology 5, 2024-01-23, 11:00 - 11:15

Objective: Equine parvovirus-hepatitis (EqPV-H) is the causative agent of Theiler's disease, or severe acute hepatic necrosis, in horses. However, it is poorly understood whether EqPV-H is associated with other histologic findings in horses with clinical liver disease. The objective of this study was to examine the prevalence and severity of EqPV-H infections in diagnostic liver samples.

Methods: Archived formalin-fixed paraffin-embedded (FFPE) liver samples (n = 98) from Cornell University and University of California, Davis collected between 2007 and 2022 were assigned a histologic diagnosis, evaluated for 15 individual histologic features, scored for EqPV-H viral load by *in situ* hybridization (ISH), and tested for EqHV by RT-qPCR. The prevalence of EqPV-H positive cases among diagnoses were compared to the estimated population prevalence of 15/100 by the hypothesis test for two populations. Chi-square analysis was used to assess whether individual histologic features were associated with increased risk of liver being EqPV-H positive or >2+ using ISH. These features were then built into a decision tree using partition predictive modeling to develop an algorithm for recommending EqPV-H diagnostic testing on clinical samples.

Results: EqPV-H was detected in 48% (n=47) of samples. Horses with a diagnosis of cholangitis ($p = 0.0033$), lobular hepatitis ($p = 0.0003$), toxic hepatopathy (0.0036), and hepatic necrosis ($p < 0.0001$) were more likely to be infected with EqPV-H compared to a healthy horse. The most common histologic features of EqPV-H-positive samples included individual hepatocyte death (n=40, 85%), lobular infiltrates (n=38, 80%), portal infiltrates (n=35, 74%), and ductular reaction (n=33, 70%). Centrilobular necrosis ($p < 0.0001$), portal infiltrate ($p = 0.015$), and individual hepatocyte death ($p = 0.046$) were positively associated with high viral load (>2+ ISH score). Neutrophil infiltrates ($p = 0.0035$), bridging fibrosis ($p = 0.0089$), and portal edema ($p = 0.032$) were negatively associated with high viral load. Only four of 49 tested samples were positive for equine hepacivirus (EqHV), but the qRT-PCR assay was unreliable for FFPE tissues.

Conclusions: This study demonstrates that EqPV-H is common in a variety of liver pathologies and should be considered as a differential diagnosis in cases of hepatitis other than Theiler's disease.

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Notes:

246 - Identification of the ex vivo and in vitro equine arteritis virus receptors

Come J. Thieulent[1], Sanjay Sarkar[2], Mariano Carossino[1], Udeni B. R. Balasuriya[1]

[1]School of Veterinary Medicine, Louisiana State University,[2]Southern Research Institute, Infectious Disease Research. cthieulent@lsu.edu

Session: Virology 5, 2024-01-23, 11:15 - 11:30

Objective: Equine arteritis virus (EAV) is the causative agent of equine viral arteritis (EVA), a respiratory, systemic, and reproductive disease of equids with worldwide distribution. EAV causes significant economic losses to the horse industry due to abortion storms, neonatal mortality, respiratory disease, and the establishment of long-term persistent infection (LTPI) in the reproductive tract of infected stallions. Equine CXCL16 (EqCXCL16S) was recently identified as a cell entry receptor for EAV *in vitro*. However, its role in EAV infection *in vivo* and in the establishment of LTPI is still unclear. Additionally, EAV has a broad host-cell tropism and infects a variety of cells that do not express EqCXCL16S. The objective of this work was to identify the host cell protein(s) involved in EAV infection.

Methods: Peripheral blood mononuclear cells (PBMCs) collected from healthy horses and seven equine and non-equine cell lines were used in this study. Virus overlay protein-binding assay in combination with Far-Western blot and LC-MS/MS analysis were performed to identify EAV-binding protein(s). Virological and classic cell culture methods (e.g., cell transfection, immunofluorescence, plaque assays) were used to confirm the role of putative EAV attachment factors. Single-cell RNA sequencing (scRNA-seq) coupled to flow cytometry analysis is in progress to identify the receptor(s) involved in CD3+ T cell susceptibility in EAV infection.

Results: A 57 kDa protein expressed in two EAV-susceptible cell lines: equine endothelial cells (EECs) and equine dermal cells (E. Derm), was identified as a possible EAV-binding protein. This unidentified protein, present in the membrane fraction of the cells, was subsequently identified as vimentin. Screening of a wide range of cells from different mammalian species determined that only those expressing vimentin are susceptible to EAV infection. Pre-treatment of EECs with an anti-vimentin antibody induces a significant reduction in viral input. CD163 is a well-known scavenger receptor for porcine reproductive and respiratory syndrome (PRRSV), and its role in EAV infection is also investigated. scRNA-seq analysis of infected CD3+ T cell is in progress and the results will support or refute the role of the CXCL16/CXCR6 in EAV infection. The results are currently being confirmed by flow cytometry analysis.

Conclusions: Collectively, our data provide first strong evidence that EAV binds to the host cell protein, vimentin, which possibly serves as an attachment factor, suggesting that EAV interacts with multiple host cell proteins that determine its diverse cell tropism *in vitro.* The role of CD163 and the CXCL16/CXCR6 axis in EAV infection are still under investigation. Overall, this study will enable us to better characterize EAV receptors.

Financial Support: This work is supported by the NIH-USDA NIFA R01 Research Grant Program Dual Purpose with Dual Benefit: Research in Biomedicine and Agriculture Using Agriculturally Important Domestic Animal Species grant number 2019-67016-29102 (award number AWD-47990-1) from the USDA NIFA to UBRB.

Notes:

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

Huai Xu[1], Nagendraprabhu Ponnuraj[1], Keith Jarosinski[1]

[1]University of Illinois at Urbana-Champaign. huaixu2@illinois.edu

Session: Virology 5, 2024-01-23, 11:30 - 11:45

ObjectiveS: Transmission from host to host is an essential part of the herpesvirus life cycle. Using our chicken model for alphaherpesvirus transmission, we identified a conserved viral gene - namely glycoprotein C (gC) - to be essential for the interindividual spread of Marek's disease herpesvirus (MDV). The main objective of this project is to determine whether other avian alphaherpesviruses require gC for host-to-host transmission which can be important in developing the next generation of MD vaccines or therapies.

Methods: We used *Gallid alphaherpesvirus* 2 (GaHV2) or MDV, *Gallid alphaherpesvirus* 3 (GaHV3) (chicken), and *Meleagrid alphaherpesvirus* 1 (MeHV1) or turkey herpesvirus (HVT) in our host-to-host transmission models to test the ability of recombinant viruses to spread from bird-to-bird when they lacked gC expression. We have established that MDV expresses secreted forms of gC due to alternative mRNA splicing. These secreted gC proteins are important for the transmission of MDV in chickens, therefore, we examined alternative mRNA splicing of GaHV3 and HVT gC in infected cell cultures and chicken feather follicle epithelial (FFE) cells using western blot and RT-PCR assays.

Results: Previously, we determined MDV and GaHV3 are essential for virus transmission in chickens and HVT does not transmit in our chicken-to-chicken experimental model. Here, we tested the ability of HVT to transmit in a turkey-to-turkey experimental and natural infection model and found HVT did transmit efficiently. Importantly, gC was also required for transmission as a mutant HVT lacking gC was unable to spread in turkeys. Western blot analysis indicated that GaHV3 and HVT gC are both secreted into the media in cell cultures. RT-PCR analysis of mRNA splicing of GaHV3 and HVT showed that both viruses produce mRNA splice variants of gC in cell culture and in birds. After sequencing, we determined that HVT gC produces two splice variants termed gC104 and gC145 in the same way as MDV gC while 301B gC only produces the gC104 variant.

Conclusions: Our results confirmed the conserved requirement for gC proteins during natural infection (transmission) for members of the *Mardivirus* genus. We also determined that the MD vaccines 301B/1 (GaHV3) and HVT produce mRNA splice variants in both cell culture and in chickens confirming mRNA splicing of gC and expression of secreted gC is not unique to MDV. We identified a single nucleotide in GaHV3 that might does not allow this gC145 variant to be produced.

Notes:

248 - Laying hen susceptibility to infectious bronchitis virus: a comparative analysis of Massachusetts (Mass) and Delmarva (DMV) 1639 genotypes

Muhammad Farooq[1], Reham M. Abd-Elsalam[1,2], Natalya Ratcliff[1], Mohamed S.H. Hassan[1,3], Shahnas M. Najimudeen[1], Susan Cork[1], Sylvia Checkley[1], Yan Dong Niu[1], Mohamed Faizal Abdul-Careem[1]

[1]University of Calgary Faculty of Veterinary Medicine, Health Research Innovation Center, [2]Faculty of Veterinary Medicine, Cairo University, [3]Department of Poultry Diseases, Faculty of Veterinary Medicine, Assiut University. muhammad.farooq3@ucalgary.ca

Session: Virology 5, 2024-01-23, 11:45 - 12:00

Objective: The aim of this study was to compare the pathogenicity of two IBV strains belonging to Massachusetts (Mass) and Delmarva DMV/1639 genotypes in mature laying hens in their peak of lay.

Methods: Specific pathogen-free laying hens (n=12 per group) were housed in three separate negative-pressure rooms. The hens in their peak of lay (30 weeks) infected groups were inoculated with 1×10^6 embryo infectious dose $(EID)_{50}$ of the DMV/1639 IBV and Mass IBV via intratracheal and oculo-nasal route, and with phosphate-buffered saline (PBS) for the control group via the same route. Oropharyngeal (OP) swabs, cloacal (CL) swabs, and serum samples were collected for monitoring antibody titers and virus shedding at different time points. On 21 days post-infection, all birds were euthanized and samples from the trachea, lung, kidney, spleen, cecal tonsils (CT), ovary, and oviduct were collected in RNA Save® and formalin for tissue viral genome load, histopathology, and immunohistochemistry analysis.

Results: A significant drop in egg production with miss-shaped and soft shells were observed in the DMV/1639 IBV-infected hens only. The DMV/1639 IBV infected group showed prolonged and higher cloacal viral shedding compared to the Mass IBV infected group. At the end of the study (21 days post-infection), the viral genome loads in the respiratory, urogenital, and immune tissues were significantly higher in the DMV/1639 IBV infected group compared to the Mass IBV infected group. Gross lesions such as distorted ova leading to egg peritonitis were observed only in the DMV/1639 infected group. Moreover, microscopic lesion scores were significantly higher in the lung, kidney, cecal tonsils, and oviduct of the DMV/1639 IBV infected group compared to the Mass IBV infected group. Finally, the apoptosis index in the kidney, ovary, magnum, isthmus, and shell gland was significantly higher in the DMV/1639 IBV-infected group compared to the control and Mass infected groups.

Conclusions: Both DMV/1639 and Mass IBV isolates are capable of infecting mature laying hens at their peak of lay, causing severe clinical manifestations such as respiratory distress, and decreased egg production. The DMV/1639 strain of Infectious Bronchitis Virus (IBV) has a wider range of tissue tropism and a particular affinity for the kidney and reproductive tract of laying hens, leading to severe damage and a significant drop in egg production. The results highlight the importance of strain-specific differences in IBV pathogenicity and their potential impact on the poultry industry.

Financial Support: Research Driven Agriculture Research (RDAR), Canadian Poultry Research Council (CPRC), Natural Sciences and Engineering Research Council of Canada (NSERC), Egg Farmers of Alberta (EFA), and Ph.D. studies of Muhammad Farooq are funded by the Higher Education Commission (HEC) of Pakistan.

Notes:

249 - Evaluating the occurrence of giant liver fluke and its hosts in Elk Island National Park, Canada

O. Meyers[1], R. Maraj[2], S. Kutz[1], J. Gilleard[1], M. Pruvot[1]

[1]Faculty of Veterinary Medicine, University of Calgary, [2]Parks Canada, Elk Island National Park, Alberta, Canada. Olivia.meyers@ucalgary.ca

Session: Epidemiology 5, 2024-01-23, 10:30 - 10:45

Objective: Giant liver fluke (*Fascioloides magna*) is a digenetic trematode that parasitizes cervid definitive hosts and aquatic snail intermediate hosts. In Elk Island National Park, Canada, moose (*Alces alces*) suffer from verminous hepatitis and mortality associated with giant liver fluke infection that has led to an 88% decline of the park's moose population in 20 years. Fluke control methods include flukicide treatment of cervid hosts and ecological prophylactic control measures that reduce larval parasite burden. The objective of this study is to determine the spatial distribution of the giant liver fluke and its hosts in the park to help guide the targeted control of fluke infections, while minimizing environmental disturbance.

Methods: Elk Island National Park was sampled at 90 wetland habitats in June-August 2022 for ungulate feces, aquatic snails, and fluke metacercariae, the infective larval stage. The Flukefinder sedimentation method was performed to determine fecal egg counts. Snail hosts were identified using shell morphology and DNA barcoding. Metacercariae were morphologically identified by microscopy. The occurrence of cervid and snail hosts, fluke eggs, and fluke metacercariae in the park were modeled using single-season occupancy models.

Results: We found that 71% of elk feces were infected with *Fascioloides magna* eggs, with a mean fecal egg count of 16 eggs per gram and a standard deviation of 61. Infected elk feces were homogenously distributed across the park; however, highly infected feces clustered in the northernmost region. Two Lymnaeid snail hosts of the fluke, *Stagnicola elodes and Fossaria spp.,* commonly occurred in the park. Fluke metacercariae distribution was more heterogenous than snail and cervid hosts, suggesting additional ecological factors driving infectious risk across the park.

Conclusions: Overall, elk were confirmed as the primary definitive host and two Lymnaeid snail species were identified as the intermediate hosts. Snail and cervid hosts widely occurred across the park, but the distribution of metacercariae and eggs suggested heterogenous transmission risk, which may facilitate control of the parasite in the park.

Financial Support: The research was funded by Parks Canada, the Alberta Conservation Association Biodiversity Grant, and the University of Calgary's Faculty of Veterinary Medicine.

Notes:

250 - Space-time clustering and climatic risk factors for lumpy skin disease of cattle in Uttar Pradesh, India, 2021-2022

Isha Agrawal[1], Csaba Varga[1], Barkha Sharma[2]

[1]Department of Pathobiology, University of Illinois Urbana Champaign, [2]Department of Veterinary Epidemiology UP Pandit Deen Dayal Upadhyay Pashu Chikitsa Vigyan Vishwavidyalaya Evam Go Anusandhan Sansthan Mathura. ishaa3@illinois.edu

Session: Epidemiology 5, 2024-01-23, 10:45 - 11:00

Objective: Lumpy skin disease (LSD) is an economically significant emerging infectious disease of cattle causing high morbidity. Outbreaks of LSD have been reported in India since 2019, affecting over 15 states, including Uttar Pradesh. The main objective of this study is to assess the distribution and identify spatial, temporal, and space-time clusters of LSD. In addition, we evaluate the impact of climatic factors on the incidence of LSD.

Methods: Outbreak data for the study was obtained from state veterinary officers, who collected the reported number of LSD cases in each district during outbreaks in 2021 and 2022 in Uttar Pradesh. Mapping and spatial and temporal cluster analysis were conducted using ArcGIS Pro and SatScan. District-level incidence rate choropleth maps were created for each month. Global and local clustering of LSD cases to identify areas with high and low rates was conducted for each month using Moran's I statistics. To identify areas and periods with higher than expected cases, retrospective discrete Poisson models were constructed to identify temporal, spatial, and space-time clusters using a circular scanning window that included 50% of the population at risk and/or study period. Statistically significant (p-value ≤ 0.05) clusters were estimated after 999 Monte Carlo simulations. A negative binomial regression model was built to investigate the impact of temperature and humidity on LSD incidence. The number of LSD cases in each district was included as the outcome, and the number of cattle in each district was included as exposure to account for the background population. Predicted LSD probabilities for variables from the negative regression model were calculated and illustrated in a figure.

Results: In the 2021 outbreak, 5,784 LSD cases were reported across six districts, and in the 2022 outbreak, 112, 226 cases across 33 districts. No outbreaks were observed in 2022 in districts affected by LSD in 2021. In 2021, two significant high-rate spatial clusters were observed. In 2022 one temporal, sixteen spatial, and two space-time high-rate clusters were identified by the retrospective discrete Poisson scan statistics. The negative binomial regression model identified that the number of LSD cases significantly increased with the temperature rise (IRR=2.09, p-value<0.001) and humidity increase (IRR=1.37, p-value<0.001), suggesting a seasonality in LSD outbreaks.

Conclusions: Several spatial, temporal, and space-time LSD clusters were identified. The rise in temperature and humidity impacted the increase in LSD cases across districts in Uttar Pradesh. Our study provided information on LSD spatial epidemiology and identified climatic factors impacting LSD incidence that can aid animal health authorities in creating effective LSD prevention, surveillance, and control strategies in India.

Notes:

251 - Initial detection and spread of bluetongue virus serotype 6 (BTV-6) in wild and domestic ruminants in Colorado

Mollie K Burton[1], Christie E Mayo[1], Tyler J Sherman[2], Molly J Carpenter[1], Case R Rodgers[1], Mia K Torchetti[3], Karen A Fox[4]

[1]Department of Microbiology, Immunology, and Pathology, Colorado State University, [2]Diagnostic Medicine Center, Colorado State University, [3]National Veterinary Services Laboratories, Animal and Plant Health Inspection Service, U.S. Department of Agriculture, [4]Colorado Division of Parks and Wildlife. Mollie.Burton@colostate.edu

Session: Epidemiology 5, 2024-01-23, 11:00 - 11:15

Objective: Bluetongue virus (BTV) is an economically significant pathogen affecting both wild and domestic ruminants around the world. Incursions of novel serotypes into the US are believed to be brought in by the insect vector, the *Culicoides* biting midge, with most introductions occurring within the last two decades. Historically, serotypes 2, 10, 11, 13, and 17 were considered endemic in the US, with serotypes 3, 10, 11, 13, and 17 previously detected in Colorado. This work documents the initial detection and sequencing of an exotic serotype, BTV-6, in wild and domestic Colorado ruminants and the subsequent spread throughout domestic livestock populations as recognized through surveillance efforts.

Methods: Initial samples were recovered from a Dorset sheep in 2020 and a male mule deer in 2021. Following nucleic acid isolation and RT-qPCR testing at the CSU Veterinary Diagnostic Lab, the samples were sent to the National Veterinary Services Laboratories (NVSL) for sequencing and bioinformatic analysis. Further analysis and comparisons of the resulting sequences was performed using NCBI's BLAST tool. For surveillance efforts, serum and whole blood samples were collected from five sheep and five cattle sites along the Front Range of Colorado from August through December 2021. Serial samples were collected from the same animals at four sheep sites and one cattle site. Cross-sectional samples were collected from the remaining sites during the sampling period. Pan-BTV and serotype-specific RT-qPCR and cELISA were used to evaluate viral RNA presence and serology in these samples, respectively.

Results: The BTV-6 USA2006/01 isolate (GQ506537.1) aligned most closely with both recovered Seg2 sequences (91.96% and 91.94% for sheep and deer, respectively). In contrast, the remaining nine segments displayed greater homology (95.56% - 99.53%) with BTV-3, -10, -11, and -13. Three of the sheep sequences (Seg1, 7, and 8) were nearly identical (99.08% - 99.39%) with a California isolate of BTV-13, while one segment (Seg3) was most homologous (99.53%) with a Washington isolate of BTV-11. Segment sequences from our deer sample share these same patterns, though with lower percent identity (98.45% - 98.80%). Surveillance collections found site-level seroprevalence and viral RNA prevalence in sheep flocks ranged from 7-93% (mean=48%) and 0-60% (mean=19%), respectively, while cattle herds ranged from 22-93% (mean=56%) and 7-40% (mean=22%), respectively. Of samples for which serotypes have been identified, BTV-6 and BTV-11 compromise most positive samples with BTV-6 accounting for 50% of positive sheep samples and 31% of positive cattle samples.

Conclusions: This work highlights the introduction of a previously unreported serotype of BTV into Colorado. Sequencing of these isolates reveals evidence of multiple reassortment events with other serotypes such as BTV-13 and BTV-11 and surveillance efforts in domestic ruminants found subsequent spread of BTV-6 throughout these populations. These findings support the continued circulation of BTV in domestic livestock populations, exemplify BTV-6 as a newly established serotype, and will provide data for predictive modeling efforts. In light of novel introductions, USDA APHIS recently changed the nomenclature associated with BTV to "established, reported, or not reported", with the inclusion of serotype 6 as established partially due to this work.

Financial Support: Funding for this project was provided by the USDA-NIFA AFRI grant 2019-67015-28982 as part of the joint USDA-NSF-NIH-BBSRC-BSF Ecology and Evolution of Infectious Diseases program and the NIH Ruth L. Kirschestein National Research Service Award Training Program T32.

Notes:

252 - Comprehensive outcomes from a feedlot trial comparing ractopamine hydrochloride and lubabegron feed additives

Taylor B. McAtee[1], Brandon E. Depenbusch[2], Tim Murphy[3], Nick B. Betts[4], David G. Renter[1]

[1]Kansas State University College of Veterinary Medicine, [2]Irsik & Doll Feed Services, Inc., [3]High Plains Consulting, Inc., [4]Elanco Animal Health. taylormcatee@vet.k-state.edu

Session: Epidemiology 5, 2024-01-23, 11:15 - 11:30

Objective: Lubabegron (Experior; Elanco, Greenfield, IN, USA), is the first U.S. Food and Drug Administration approved for reducing gas emissions from feedlot animals or their waste; it does not have claims for feedlot growth or carcass performance. Our primary objective was to determine the effect of lubabegron on feedlot performance and carcass traits in finishing beef steers compared to ractopamine hydrochloride (Optaflexx; Elanco, Greenfield, IN, USA). Secondary objectives were to evaluate the effect of lubabegron on health outcomes, mobility scores, economic returns, and emissions estimates.

Methods: A commercial feedlot trial using crossbred beef steers (n = 2117; 823 ± 34 initial body weight [BW]) was completed as a randomized complete block design. Experimental treatments consisted of two feed additives 1) OPT targeted to deliver 300 mg/animal/d of ractopamine hydrochloride for 28 ± 7 d prior to harvest and 2) EXP targeted to deliver 36 mg/animal/d of lubabegron 56 ± 7 d prior to harvest and a 4-d washout period. Twenty total 70-142 hd pens, with 10 pens per treatment were used. Cattle were fed for an average of 167 days. A net returns value was calculated for each pen, where net return is the difference between total revenue and total variable expenses for that pen. The Uplook® version 1.0 (Elanco Animal Health) system was used to estimate emissions based on calculated carbon dioxide equivalents (CO2e) for each pen. Data were analyzed using general and generalized linear mixed models, with pen as the experimental unit, treatment as a fixed effect, and block as a random intercept. A statistical significance threshold of $\alpha = 0.05$ was determined a priori.

Results: There was no evidence for a difference between treatments for initial body weight ($P = 0.70$), health-related outcomes (P values ≥ 0.43), or mobility scores ($P = 0.09$). The cattle fed EXP were more efficient and had significantly increased final body weight, average daily gain, and feed efficiency, with decreased daily feed intake (P values ≤ 0.01) compared to OPT. Carcasses were 25 lbs heavier in EXP group ($P \leq 0.01$), and also differed between treatments for both USDA Yield (YG) and Quality Grade (QG) distributions (P values ≤ 0.01). Cattle fed EXP had more YG 1 and 2, and select and sub-select carcasses compared to OPT, which conversely had more YG 3, 4, 5, and prime and choice carcasses. With the increased beef production and efficiency compared to OPT, the estimated CO2 equivalent emissions associated with finishing from production were reduced 6.2% per unit of live weight for EXP ($P \leq 0.01$). Net returns ($/animal) were $57.08 more for EXP than OPT ($P \leq 0.01$) fed cattle.

Conclusions: In conclusion, when cattle were fed for the same total number of days, feeding EXP compared to OPT increased net returns, feedlot performance, and efficiency in live animals, but resulted in carcass yield and quality characteristics that may impact beef marketing programs.

Financial Support: This study was funded by the College of Veterinary Medicine's Center for Outcomes Research and Epidemiology.

Notes:

253 - A stochastic framework to assess optimal allocation of limited vaccine doses in FMD outbreaks using game theory.

K Moreno Torres[1], M Sanderson[1], C Rigney[2], M Schoenbaum[2], A Delgado[2], J Arzt[3], J Heier Stamm[4]

[1]Department of Diagnostic Medicine/Pathobiology, Kansas State University, [2]Center for Epidemiology and Animal Health, Animal and Plant Health Inspection Service, United States Department of Agriculture, [3]Foreign Animal Disease Research Unit, Plum Island Animal Disease Center, Agricultural Research Service, United States Department of Agriculture, [4]Department of Industrial and Manufacturing Systems Engineering, Kansas State University. sandersn@vet.k-state.edu

Session: Epidemiology 5, 2024-01-23, 11:30 - 11:45

Objective: Vaccination for foot-and-mouth disease (FMD) has been considered as decision-makers recognize that depopulation and carcass disposal may not be sufficient, welfare-supported, or cost-effective to control an outbreak. However, response required to control an FMD outbreak may outpace available vaccine doses. Thus, policymakers need strategies for vaccine allocation. This study aimed to develop a stochastic framework that strategically examines vaccination allocation in a series of simultaneous multi-player decision sets, using game theory and rules of allocation.

Methods: We modeled 11 stochastic FMD scenarios using InterSpread Plus. Stakeholders were denoted as decision-maker 1 (DM1 the index state), and DM2 (a group of 3 neighboring states) requesting all or a share of vaccine. Outbreak size (OS) and duration (OD) were the outcomes. Vaccine allocation strategies were determined by 4 rules. Rule 1 prioritized allocation to the index state, rule 2 prioritized allocation to neighboring states, rule 3 provided equal prioritization and rule 4 prioritized allocation based on the percentage of dairy cattle in each state. For each scenario, 300 iterations were completed using matched random seeds. The outcome rankings of each matched iteration were treated as the DMs' payoffs and were analyzed as static games with perfect information. Nash equilibria and Pareto optimal solutions were summarized across scenarios and iterations within each rule.

Results: Shared allocation resulted in a Pareto optimal solution more commonly, benefiting both DMs. Rule 3 had the highest level of agreement in decision-states (DS) between Nash and Pareto outcomes. For rules 1-3, Pareto optimal solutions resulted in lower OS and OD (90th percentile) than Nash equilibrium solutions. Under rule 4, OS and OD showed less differentiation between DS.

Conclusions: This stochastic framework incorporates epidemiological data and accounts for the payoffs resulting from multiple stakeholders' choices. This guides optimal decision-making for scarce resource allocation in contexts where individual payoffs depend upon others' choices.

Financial Support: Kansas State University; U.S. Department of Agriculture, Animal and Plant Health Inspection Services; U.S. Department of Agriculture, Agriculture and Research Services; USDA-Center for Epidemiology and Animal Health.

Notes:

254 - Factors influencing Ontario dairy veterinarians' management and care of down dairy cattle.

J.E. Brindle[1], D.L. Renaud[1], T. Duffield[1], D. Haley[1], C.B. Winder[1]

[1]University of Guelph. brindlej@uoguelph.ca

Session: Epidemiology 5, 2024-01-23, 11:45 - 12:00

Objective: This cross-sectional study aimed to assess what management practices veterinarians recommended for down dairy cows in Ontario, Canada, while identifying factors influencing producers' adoption of optimal care protocols.

Methods: An online survey targeting veterinarians in Ontario was conducted between February and May 2021. Veterinarians were contacted through the Ontario Association of Bovine Practitioners (OABP) email newsletters. The survey encompassed 75 questions, 38 pertained to down cow management. Descriptive statistics were computed using Python.

Results: A total of 48 veterinarians responded, which was a response rate of 26.8%. Gender distribution was even (50%), with the majority falling within the 30 to 39-year age bracket (41.3%). Respondents spent 77% of their working hours per month dedicated to dairy herds in their clinics. Veterinarians also reported completing a median number of 15 (range: 0-60) regular health visits per month and a median number of 40 (range: 5-230) any service visits (e.g., herd health, emergencies) per month. The most common definition among veterinarians for a down cow was "a cow that is unable to rise on its own." Considerable disparities were observed in recommendations for providing feed and water to down cows. Veterinarians suggested providing feed in reach (58.3%), loose on the floor (48.0%), unsecured (45.8%), secured (16.7%), or depends (6.3%). Water methods were similarly varied within reach (54.9%), unsecured (47.9%), secured (35.4%), by hand (4.2%), and depends (2.0%). The frequency of providing feed and water per day were also mixed with responses consisting of 2 times (6.9%), three times (31.0%), four times (10.3%), five or more times (3.5%), and available at all times (48.3%). Housing recommendations were also inconsistent, with most recommending individual pens (40.7%), followed by pasture (29.6%), special pens for three or fewer animals (26%), and special pens for four or more animals (3.7%). For spacing provided to a down cow, most suggested a minimum of 100 square feet (30.0%), 120-250 square feet (53.3%), 300 or more square feet (10%), or space to lung (6.7%). Additionally, there was substantial diversity in suggestions for lifting and moving. Veterinarians most commonly recommended moving a cow via sled (62.5%), followed by stone boats (56.3%), front-end loader buckets (45.8%), wheeled carts (20.8%), hip-lifters (2.1%), and depends (2.1%). When lifting a cow, the majority of veterinarians recommend using multiband slings (56.3%), hip clamps (43.8%), floatation tanks (25.0%), single belly slings (14.6%), ropes (4.2%), daisy lifters (2.1%), and hip clamps with straps (2.1%).

Conclusions: There was considerable variation in veterinarians' suggestions on how to care for down cattle when assisting producers in implementing evidence-based management strategies. The management of down cows may be improved through more standardized recommendations by veterinarians to producers.

Financial Support: This work is supported by funding from the Ontario Agri-Food Innovation Alliance, the Saputo Dairy Care Program, and Dairy Farmers of Ontario.

Notes:

255 - Economic considerations for extended feedlot heifer days-on-feed - sensitivity analyses from pooled clinical trials

L. Horton[1, 2], B. Depenbusch[3, 4], T. Schroeder[1, 5], D. Pendell[1, 5], M. Streeter[6], J. Hutcheson[6], D. Renter[1, 2]

[1]Kansas State University, [2]College of Veterinary Medicine, Department of Diagnostic Medicine and Pathobiology, Center for Outcomes Research and Epidemiology, [3]Innovative Livestock Services, Inc., [4]Current: Irsik and Doll Feed Services, Inc., [5]College of Agriculture, Department of Agricultural Economics, [6]Merck Animal Health. lhorton@vet.k-state.edu

Session: General Health & Physiology 4, 2024-01-23, 10:30 - 10:45

Objective: In recent years, feedlot cattle have been fed for longer days and to heavier endpoints than past decades. Economic considerations for various market factors are likely influenced by changes in animal performance and carcass characteristics when feeding longer. Our objective was to evaluate the effects of differing days-on-feed (DOF) on net returns (pen revenue - pen cost) when varying specific pricing components using sensitivity analyses.

Methods: To estimate pen-level net returns for each sensitivity analysis, partial budgets were constructed for pooled data from three clinical trials. A total of 10,583 crossbred beef heifers were enrolled across the trials [initial weight 315 kg (± 20.1 SD)] using a 2×3 factorial in a randomized complete block design. Two implant programs (a single delayed-release implant vs a re-implant program) were incorporated in the treatment structure, but are not discussed further. Pens were randomized to DOF treatment, which were: heifers fed to a feedlot standard baseline endpoint (BASE), heifers fed for +21 days beyond BASE, or heifers fed for +42 days beyond BASE. Variable pricing components evaluated in sensitivity analyses were fed-cattle prices (live and dressed), the Choice-Select spread (CS-spread) for grid-based marketing, and feed and yardage prices (FYP). Partial budgets were built at the pen-level for each variable pricing component, and sensitivity analyses functioned by evaluating a range of prices for a specific component, while holding all other prices constant. For each variable component, a Low, Mid-Low, Middle, Mid-High, and High price was selected from reported prices (2017 through 2022). Statistical analyses (with significance threshold $\alpha = 0.05$) were performed with linear mixed models.

Results: There were no significant 2-way interactions between treatment factors ($P \geq 0.14$), therefore, only main effects of DOF are reported. Lower net returns for +21 and (or) +42 compared to BASE occurred at every live fed cattle price ($P < 0.01$). Selling dressed, lower returns were estimated for +21 and +42 compared to BASE at Low, Mid-Low, and Middle fed cattle base prices ($P < 0.01$), while there were no significant DOF differences at the other dressed fed cattle base prices ($P \geq 0.24$). Net returns were lower for +42 than BASE at all CS-spreads ($P \leq 0.03$), while +21 and BASE were not significantly different. With the exception of the Low FYP ($P = 0.14$), longer DOF resulted in lower net returns at every FYP when selling on a live basis ($P < 0.01$). Selling dressed, there was no evidence of net return differences between DOF at Low or Mid-Low FYP ($P \geq 0.11$); conversely, +21 and (or) +42 DOF heifers had lower returns than BASE at Middle, Mid-High, and High FYP ($P < 0.01$).

Conclusions: Sensitivity analyses of pricing factors allowed evaluation of the impact that individual components have when feeding heifers for longer DOF. Overall, there was no evidence to support extending feedlot heifer DOF economically, and producers should consider a variety of market factors when making this decision.

Financial Support: This research was supported by Merck Animal Health, Innovative Livestock Services Inc., and the Kansas State University Center for Outcomes Research and Epidemiology.

Notes:

256 - Bayesian estimation of failed transfer of passive immunity IgG cut-offs for predicting morbidity in beef calves

Alexis C. Thompson[1], Matthew A. Scott[2], Renee D. Dewell[3], Brandon J. Dominguez[4]

[1]Texas A&M Veterinary Medical Diagnostic Laboratory, [2]Veterinary Education, Research, and Outreach Program, Texas A&M University, [3]Center for Food Security/Public Health, Iowa State University, [4]Texas A&M Veterinary Medical Diagnostic Laboratory. alexis.thompson@tvmdl.tamu.edu

Session: General Health & Physiology 4, 2024-01-23, 10:45 - 11:00

Objective: Calves with failed transfer of passive immunity (FTPI) are reported to have an increased risk of pre-weaning morbidity. The objective of this study was to determine the predictive value of FTPI classification on pre-weaning morbidity in beef calves.

Methods: Neonatal beef calves (2-7 days of age; n=1973) from seven commercial cow-calf herds had IgG concentrations measured by radial immunodiffusion. Generalized pre-weaning morbidity was recorded by animal caretakers within each herd. Calves were classified as having FTPI using 7 IgG cut-off values (500, 800, 1000, 1200, 1800, 2400 mg/dL). A Bayesian latent class model assuming conditional independence between the radial immunodiffusion and the detection of morbidity was used to obtain estimates of the test sensitivity, specificity, and positive and negative predictive value for the IgG cut-off values using informed priors.

Results: Estimated herd cumulative incidence of morbidity ranged from 4.6% to 37.9%. IgG had a median (95% credible interval) sensitivity that ranged from 0.27 (0.21, 0.34) for 500 mg/dL to 0.50 (0.43, 0.57) for 2400 mg/dL and a median (95% credible interval) specificity that ranged from 0.97 (0.94, 0.99) for 500 mg/dL to 0.64 (0.62, 0.67) for 2400 mg/dL. The positive predictive value for 500 mg/dL ranged from 0.27-0.87 to 0.05-0.31 for 2400 mg/dL. The negative predictive value for 500 mg/dL ranged from 0.61-0.96 to 0.80-0.97 for 2400 mg/dL.

Conclusions: Lower FTPI cut-off values were more predictive of morbidity than higher cut-offs. Less than half of calves with FTPI would have a morbidity event during the pre-weaning period. Most calves that did not have FTPI would remain healthy during the pre-weaning period. Testing for FTPI did not aid in the detection of calves that would remain healthy or overtly predict which calves would develop pre-weaning morbidity.

Notes:

257 - The effects of the equine mesenchymal stromal cell secretome on acute and chronic wound healing in vivo

Aarthi Rajesh[1], Rebecca M. Harman[1], Gerlinde R. Van de Walle[1]

[1]Baker Institute of Animal Health, Cornell University. ar889@cornell.edu

Session: General Health & Physiology 4, 2024-01-23, 11:00 - 11:15

Objective: The prevalence of chronic wounds continues to be a burden in equine medicine. Methicillin-resistant *Staphylococcus aureus* (MRSA) is commonly isolated from infected wounds. MRSA infections primarily delay healing by impairing local immune cell functions. This study aimed to investigate the potential of equine mesenchymal stromal cell (MSC) secreted bioactive factors, defined as the secretome, to improve innate immune responses *in vivo.*

Methods: The effects of equine bone marrow (BM)-derived MSCs on wound healing *in vivo* were assessed using a mouse model. Mice were subjected to two full-thickness punch biopsies and treated daily with either control medium or BM-MSC secretome, collected as conditioned medium (CM), until closure. Wounds were photographed daily, and tissues were harvested at days 2, 6, and 10 post wounding. Trichrome staining was carried out to compare the changes in the wound tissue histologically, followed by immunofluorescence analyses to assess the changes in immune cell infiltration. Experiments were then repeated with wounds inoculated with MRSA and analyses, as described above, are ongoing.

Results: Treatment with BM-MSC CM resulted in accelerated wound closure, with increased granulation tissue formation at days 2- and 6- post wounding, and advanced resolution of granulation tissue at day 10. Repaired tissue appeared more organised, with a higher density of collagen and increased hair follicles, when compared to the control group. Importantly, an increased infiltration of neutrophils and macrophages was observed at days 2- and 6- post-wounding, respectively. Morphometric analyses of MRSA-infected wounds treated with BM-MSC CM showed slightly faster wound closure as well when compared to controls.

Conclusions: BM-MSC secretome-treated wounds showed accelerated wound closure, characterized by enhanced granulation tissue formation and resolution, increased vasculature, regeneration of hair follicles, and increased neutrophil and macrophage infiltration, suggesting that the equine MSC secretome might have the potential to restore impaired immune cell functions in infected wounds.

Financial Support: This project was supported by an Agriculture and Food Research Initiative Competitive Grant no. 2022-67015-36351 from the USDA National Institute of Food and Agriculture.

Notes:

258 - Alternative anti-inflammatory drugs for pain management in neonatal calves with naturally occurring diarrhea

Keerra Holzapfel[1], Tyler Tubbs[1], Luciana M. Kluppel[1], Seth Chapman[1], Sarah Wagner[1], Fernanda Rosa[1]

[1]School of Veterinary Medicine, Texas Tech University. keholzap@ttu.edu

Session: General Health & Physiology 4, 2024-01-23, 11:15 - 11:30

Objective: Non-steroidal anti-inflammatory drugs (NSAIDs) are essential for management of pain in animals and humans affected by an array of acute and chronic disorders. Thus, the aim of this study was to evaluate different classes of NSAIDs that are extra label use for treating calves with naturally occurring diarrhea. We hypothesized that meloxicam and ketoprofen can be used for pain management in newborn calves with naturally occurring diarrhea without severely compromising the gastrointestinal tract.

Methods: A total of 81 newborn calves at 1-2 days of age were block by sex and breed and assigned into 3 different treatment groups (n = 27/treatment) at a commercial farm in Dalhart, TX. Preweaned calves were individually housed in calf hutches and assigned into: control (placebo treatment), meloxicam (oral administration, 1mg/kg), or ketoprofen (intramuscular administration, 3mg/kg). To be eligible for the trial, calves had a fecal score of two or higher (scale 1-5) and a respiratory score of 1 or 2 (scale 1-5). Blood samples for cytokine profile and complete blood count (CBC) were collected from each calf on day one (prior to treatments) and day five (4 days post-treatment) of the trial. Data were analyzed using GraphPad Prism v.9 using ANOVA-repeated measurements model. Significance was declared at $P < 0.05$.

Results: No treatment or interaction effects were observed for the absolute erythrocyte count, platelet count, lymphocyte count, and neutrophil count. However, neutrophils (K/uL) significantly decreased over time ($P < 0.001$) in the blood of all calves. Blood lymphocytes and platelets significantly increased over time for all groups ($P < 0.01$). The meloxicam treated calves had significant treatment and treatment × time interaction effects on the absolute monocyte count ($P < 0.001$ and $P = 0.01$, respectively). Blood cytokine profiling analysis showed no treatment or interaction effects for the pro-inflammatory cytokines TNFA, IL1a, and IFNG. However, a time effect ($P \leq 0.04$) was observed for these cytokines, which had an overall decrease in the blood of all calves. Similar blood concentration of interleukin 10 (IL-10), an anti-inflammatory cytokine, was observed in ketoprofen- and meloxicam-treated calves at 4-days post treatment ($P = 0.06$).

Conclusions: Overall, the similar blood levels of IL-10 between treated calves and the decreased concentration of neutrophils and blood pro-inflammatory cytokines in both ketoprofen- and meloxicam-treated calves suggest that these extra label use NSAIDs can potentially help newborn calves cope with underlying inflammatory conditions.

Financial Support: School of Veterinary Medicine, Texas Tech University

Notes:

259 - The significance of adipose tissue extracellular matrix microenvironment on adipogenesis

J. Fiallo Diez[1], C. Flesher[2], A. Tegeler[1], T. Michelotti[1], M. Hoque[3], B. Bhattarai[3], L. Flores[1], O. Benitez[1, 4], G. Christopher[3], C. Strieder-Barboza[1, 4]

[1]Department of Veterinary Sciences, Texas Tech University, [2]Department of Medicine, University of Pennsylvania, [3]Department of Mechanical Engineering, Texas Tech University, [4]School of Veterinary Medicine, Texas Tech University. jfiallod@ttu.edu

Session: General Health & Physiology 4, 2024-01-23, 11:30 - 11:45

Objective: The primary objective of this research was to examine the depot-specific adipogenic capacity (AC) in the subcutaneous (SAT) and visceral (VAT) adipose tissue of dairy cattle and define how the extracellular matrix (ECM) influences adipocyte function.

Methods: Flank subcutaneous adipose tissue (SAT) and omental visceral adipose tissue (VAT) samples from ten Holstein were obtained at a local abattoir. Through collagenase digestion, the stromal vascular fraction (SVF) and adipocytes were separated. To assess adipogenic capacity, adipocyte sizing and the frequency of adipocyte progenitors (AP; CD45-CD31-) in SVF were analyzed using flow cytometry, while adiponectin gene (*ADIPOQ*) expression during in vitro adipogenic differentiation was measured through qPCR. The viscoelastic properties of SAT and VAT were determined via rheology, and the native ECM was decellularized for further investigating depot-specific ECM-adipocyte crosstalk. In this 3D model, SAT and VAT APs were cultured and differentiated into adipocytes within depot-matched and mis-matched native ECM matrices for 14 days. *ADIPOQ* expression via qPCR and scanning electron imaging of adipocytes were analyzed as indicators of adipogenesis by the end of in vitro differentiation.

Results: SAT demonstrated increased adipogenic capacity in comparison to VAT, as indicated by a higher abundance of adipocyte progenitors (AP), greater *ADIPOQ* expression during the differentiation process, and a larger proportion of adipocytes with a larger size, indicative of augmented lipid accumulation. Additionally, the rheological analysis indicated that VAT was stiffer than SAT, thus suggesting a potential role for the ECM in mediating the observed differences in adipogenesis between SAT and VAT depots. Further investigation using the 3D culture model with native ECM confirmed the importance of the ECM microenvironment in regulating adipogenesis. Specifically, the ECM from SAT enhanced the adipogenesis of VAT adipocytes by promoting higher *ADIPOQ* expression. Conversely, the ECM from VAT hindered adipogenesis in SAT cells. These findings highlight the potential significance of the ECM microenvironment in modulating adipogenesis in different adipose tissue depots.

Conclusions: The research reveals that subcutaneous adipose tissue (SAT) in dairy cattle exhibits a higher adipogenic capacity compared to visceral adipose tissue (VAT). Moreover, the results imply that the differences in metabolic function between SAT and VAT are influenced in part by the unique extracellular matrix (ECM) microenvironment specific to each body depot. As a result, adipocyte progenitors and the ECM emerge as potential targets for regulating adipose tissue function in dairy cows.

Financial Support: For the sponsorship, this is mainly TTU funds, especially Davis College and Veterinary Sciences Dept.

Notes:

260 - Milk and tissue residue depletion of two dry-cow intramammary antimicrobials in dairy goats

Michelle P Buckley[1], Kristen P Hayman[1], Dwayne Schrunk[1], Laura Burns[1], Patrick J Gorden[1]

[1]Iowa State University. mpbuck@iastate.edu

Session: General Health & Physiology 4, 2024-01-23, 11:45 - 12:00

Objective: The goal of this study was to determine milk and tissue residue depletion times for two intramammary dry-cow antibiotics in dairy goats.

Methods: To evaluate milk residue depletion, 22 does had each half treated with 300 mg cephapirin benzathine after their final milking before dry-off, while an additional 21 does were treated with 500 mg cloxacillin benzathine in each half. Starting at the first milking after kidding, quadruplicate composite foremilk samples were collected from each doe for evaluation of antimicrobial residues. Does treated with cephapirin were screened using the Charm SLBL assay which is approved for detection of this drug in goat milk. Does treated with cloxacillin were evaluated using the Charm SL3 Beta-lactam assay, which was assessed for use in goat milk using samples spiked with known concentrations of drug by the ISU research group before initiation of this project. Does were sampled at each milking until they tested negative on the goat-side tests for two consecutive milkings via the goat-side assay.

To evaluate tissue residue depletion, forty-two lactating goats with two functional halves were enrolled and randomly assigned to treatment groups. Half of these does received intramammary infusions of 300 mg cephapirin benzathine into each teat while the remaining twenty-one does received 500 mg cloxacillin benzathine in each half of the udder at their final milking before dry-off. Four does from each treatment group were sacrificed at 21, 28, 35, 42, and 49 days post-treatment and 200 g of muscle, liver, and kidney tissue harvested from each animal from analysis of antimicrobial residues using LC-MS-MS. A second study was completed after tissue processing to more fully elucidate the pharmacokinetic properties of cloxacillin in tissues and six additional does were treated with 500 mg cloxacillin benzathine in each half immediately following their final milking. Three of these does were sacrificed on day 3 post-treatment while the remaining three does were sacrificed 7 days post-treatment.

Results: LC-MS-MS of milk samples, 18 of 22 cephapirin-treated does (81.8%) tested below the level of quantification (5 ppb) at the first milking. Three does had quantifiable residues at the first milking, with no residues detected in any animal after 60 hours. Cloxacillin-treated does had a less uniform response with 10 out of 21 does (48%) having no quantifiable residue at first milking, 3 does (14%) had no detectable residue by the second milking, and the remaining 8 does (38%) had residues for 36 hours or more with a mean of 84 hours required to clear detectable residues.

Cephapirin was not identified in any tissue at any point during the sampling period. Cloxacillin was not found in muscle tissue at any point during the sampling period. Peak concentrations of 0.0025 ppm and 0.0026 ppm for kidney and liver, respectively, were found at 3 days post-treatment.

Conclusions: The data obtained in this study indicates prolonged detection of cloxacillin in milk and tissue as compared with cephapirin. Our working hypothesis is that this is due to an exceptionally low solubility of the cloxacillin benzathine complex.

Financial Support: This work is supported by the USDA National Institute of Food and Agriculture, Agricultural and Food Research Initiative Competitive Program, Antimicrobial Resistance grant number: 2020-04197.

Notes:

P001 - Environmental antimicrobial resistance threats to food safety and security

Kiranmayee Bhimavarapu[1], Geoffrey Williams[2], Camila Rodrigues[2], Andre Silva[2], Melissa Boersma[3], Laura Huber[1]

[1]College of Veterinary Medicine, Auburn University, [2]College of Agriculture, Auburn University, [3]College of Sciences and Mathematics, Auburn University. kzb0148@auburn.edu

Session: Antimicrobial Use, **2023-01-22, 6:00 - 8:00 pm**

Objective: Antimicrobial use in food animals led to the emergence of Antimicrobial Resistance (AMR) in the environment and contamination of produce. Honey and pollen have shown to be reliable bioindicators of environmental AMR. However, the interconnectedness of antimicrobial use in animals, AMR environmental contamination, AMR transmission to produce, and impact on pollinator health is unknown. This study aims to infer the mechanisms of AMR spread from the environment to produce and its pollinator, and the risk of pollinator disease due to exposure to AMR following the use of composted or fresh poultry litter as a fertilizer.

Methods: In this controlled field experiment, two high tunnel greenhouses will be divided into 3 subsets. Yellow squash will be planted and fertilized with either fresh or composted poultry litter. *Apis mellifera* honey bee colonies will be introduced when squash plants have flowered. Soil, pollen, honey, honey bee, and produce samples will be collected every week for 8 weeks. The relative concentration of antimicrobial genes (ARGs), and mobile genetic elements (MGEs) will be compared using qPCR.

Results: Preliminary analysis revealed higher levels of ARGs and MGEs in raw manure when compared with the compost. Nutrient analysis of fertilizers showed higher levels of most of the minerals in composted litter except for nitrogen, which was higher in raw manure. Extraction of DNA from samples have been optimized. Tunnels had been set up to grow yellow squash crops. The differences in ARGs and MGEs in samples between groups receiving raw or composted poultry litter will be presented in the conference.

Conclusions: The use of antimicrobials in food-producing animals leads to the presence of antimicrobial residues and antimicrobial resistance (AMR) in their waste. When untreated production animal waste is used as fertilizer, residues and AMR accumulates in the environment, contaminating fresh produce, and potentially contributing to dysbiosis and higher predisposition to diseases in honey bees. This project will provide evidence to understand the ecologic interconnections between food production, farm environment, and pollinators relevant to AMR spread and its impact on food safety and the ecosystem's health.

Financial Support: This research is funded by the Animal Health & Disease Research Funds, Research and Graduate Studies, Auburn University.

Notes:

P002 - Effect of metaphylaxis on the nasopharyngeal microbiome, resistome, and *Mannheimia haemolytica* in stocker heifers

William B. Crosby[1], Brandi B. Karisch[2], Lari M. Hiott[3], Lee J. Pinnell[4], Enrique Doster[4], Cory Wolfe[4], Alexandra Pittman[2], Jonathan G. Frye[3], Charlene R. Jackson[3], John Dustin Loy[5], William B. Epperson[1], John Blanton, Jr.[6], Sarah F. Capik[7], Paul S. Morley[4], Amelia R. Woolums[1]

[1]Dept. Pathobiology and Population Medicine, Mississippi State University, [2]Dept.Animal and Dairy Sciences, Mississippi State University, [3]U.S. National Poultry Research Center, USDA-Agricultural Research Service, [4]VERO Program, Texas A&M University, [5]School of Veterinary Medicine and Biomedical Sciences, University of Nebraska, [6]South Dakota Agricultural Experiment Station, South Dakota State University, [7]Tumbleweed Veterinary Services. wbc95@msstate.edu

Session: Antimicrobial Use, **2023-01-22, 6:00 - 8:00 pm**

Objective: This study aimed to determine the effect of tulathromycin metaphylaxis and subsequent bovine respiratory disease (BRD) treatment on antimicrobial resistance (AMR) in *Mannheimia haemolytica* (*MH*) isolated from the nasopharynx, the respiratory microbiome and resistome, and the *MH* community in stocker cattle.

Methods: Crossbred beef heifers (n=331, mean weight=232, SD=17.8 kg) at high risk for BRD were randomly assigned to receive tulathromycin metaphylaxis (META, n=167) or not (NO META, n=164). Nasopharyngeal swabs (NPS) were collected for DNA extraction and *MH* isolation, antimicrobial susceptibility testing and whole genome sequencing at arrival and 3 weeks later (WK3). DNA extracted from NPS were pooled based on metaphylaxis administration and treatment for BRD, resulting in 4 treatment groups (TxGroups)—1) META-BRD, 2) META-NO BRD, 3) NO META-BRD, and 4) NO META-NO BRD. DNA pools underwent 16S rRNA amplicon sequencing, target-enriched antimicrobial resistance gene (ARG) and *MH* gene sequencing, and qPCR for *MH*. NPS were collected at arrival and 3 weeks later (WK3). Multidrug resistance (MDR) was defined as phenotypic resistance to 3 or more antimicrobial classes.

Results: META calves had higher odds of isolation of MDR *MH* at 3 weeks (OR (95% CI)=13.08 (5-30.9), $P<0.0001$) after arrival. There was no difference in risk of isolation of any *MH* (resistant or susceptible) between META and NO META groups at all timepoints. Animals in the NO META group had 3 times higher odds of being treated for BRD (WK3: OR (95% CI)=3.07 (1.70-5.52), $P=0.0002$; WK10: OR (95% CI)=2.76 (1.59-4.80), $P=0.0002$). ARGs found within MH were associated with integrative conjugative element (ICE) genes. There was no effect of tulathromycin on richness or diversity of the microbiome on WK3. However, Shannon's diversity index decreased at WK3 compared to arrival, and there was a difference in microbial community structure in all TxGroups at WK3 compared to arrival, due to an increase in *Mycoplasmataceae* at WK3 (50-80%) compared to arrival (25-50 %). At WK3 there was an increase in richness and diversity of ARGs compared to arrival in animals that received metaphylaxis, and an increase in diversity of ARGs in animals that received no antimicrobials. Aminoglycoside ARGs were the only class with differences in relative abundance among TxGroups at WK3.

Conclusions: Tulathromycin metaphylaxis increased risk of isolation of MDR *MH* and in this population, the increase in MDR *MH* appeared to be associated with ICE containing ARGs for multiple antimicrobial classes. This study highlights the complexity of studying AMR, because, though tulathromycin metaphylaxis increased the risk of isolation of MDR MH and affected the resistome, resistome changes were not associated with the antimicrobial class used, and time and BRD treatment contributed to larger differences in the microbiome and resitome than metaphylaxis.

Financial Support: Competitive grant #2019-67017-29111 from the USDA National Institute of Food and Agriculture and Mikell and Mary Cheek Hall Davis Endowment for Beef Cattle Health and Reproduction.

Notes:

P003 - Effects of bovine cathelicidins on bovine respiratory disease pathogens

Santiago Cornejo[1], Cassandra Barber[1], Merrilee Thoresen[1], Amelia Woolums[1]

[1]College of Veterinary Medicine, Mississippi State University. sc3025@msstate.edu

Session: Antimicrobial Use, **2023-01-22, 6:00 - 8:00 pm**

Objective: *Mannheimia haemolytica* (MH) and *Mycoplasma bovis* (MB) are bacterial pathogens associated with bovine respiratory disease (BRD) in beef cattle. Viruses such as bovine herpesvirus type 1 (BHV-1) or bovine respiratory syncytial virus (BRSV) predispose cattle to bacterial BRD. Antimicrobial metaphylaxis can limit BRD but does not consistently prevent disease due to MH or MB, and antimicrobials do not prevent viral infection. Moreover, antimicrobial resistance, which may impair metaphylaxis, is becoming prevalent. Antimicrobial peptides (AMPs) such as cathelicidins, with immune stimulating and nonspecific antimicrobial effects, could improve BRD control. Our objective was to test the hypothesis that the bovine cathelicidins BMAP-28 and Bac-5, and the synthetic BMAP-28 analog Syn-1, inhibit the growth of MH, MB, BHV-1, BRSV *in vitro*.

Methods: MH at 10^3 and 10^5 CFU/ml or MB at 10^5 CFU/ml were treated with a final concentration of 100 or 10 µg/ml of AMP and incubated for 0, 12, 24 h. Before treatment, and following 0, 12, 24 h of incubation, quantitative culture was performed. BHV-1 or BRSV at 10^3 and 10^4 IU/ml were treated with a final concentration of 100 or 10 µg/ml of AMP and incubated at 37ºC for 2 h. Treated virus and controls were then applied to bovine kidney cells (BK) in a $TCID_{50}$ assay, and plates were read after 5 (BHV-1) or 7-days (BRSV) of incubation. The effects of AMPs on BRD pathogens were tested in two trials, using a mixed procedure with trial as a random effect for normally distributed data, and by Kruskal-Wallis test with post hoc multiple comparisons for non-normally distributed data, with alpha = 0.05.

Results: BMAP-28 and Syn-1 at 100 µg/ml inhibited MH growth. BMAP-28 at 100 µg/ml decreased MB growth at 12 h. BMAP-28 at 100 µg/ml inhibited replication of BHV-1 and BRSV at 10^3 and 10^4 IU/ml, and Syn-1 and Bac-5 decreased replication of BRSV at 10^4 IU/mL.

Conclusions: Of the AMPs tested, BMAP-28 significantly inhibited MH growth and replication of BHV-1 and BRSV. These results provide support for further research to determine whether AMPs can be used to control BRD and delay the onset of antimicrobial resistance in BRD pathogens.

Financial Support: USDA formula funds; Mikell and Mary Cheek Hall Davis Endowment for Beef Cattle Health and Reproduction

Notes:

P004 - Exploration of descriptive machine learning approaches on antimicrobial resistance patterns in *Salmonella enterica*

Abdolreza Mosaddegh[1], Claudia Cobo Angel[1], Maya Craig[1], Kevin J. Cummings[1], Casey L. Cazer[1, 2]

[1]Department of Public and Ecosystem Health, Cornell University, [2]Department of Population Medicine and Diagnostic Sciences, Cornell University. cga54@cornell.edu

Session: Antimicrobial Use, **2023-01-22, 6:00 - 8:00 pm**

Objective: Salmonellosis is one of the most common foodborne diseases worldwide. We aimed to present the types of analysis that can be made using artificial intelligence (AI) to assess multidrug resistance (MDR) and determine if the antimicrobial use restrictions in livestock have impacted *Salmonella* MDR trends.

Methods: We used an integrated machine learning approach to analyze *Salmonella* antimicrobial resistance (AMR) and MDR patterns before and after the implementation of antimicrobial use (AMU) restrictions in livestock intended to reduce the burden of bacterial AMR. Patterns of antimicrobial resistance were investigated using a set of descriptive machine learning and analytical techniques including association rule mining, hierarchical clustering, and network analysis on a dataset of *Salmonella* isolated from cattle and tested for susceptibility to 11 antimicrobials from year to year.

Results: The analysis revealed a distinctive AMR pattern in the Dublin serotype. The results also indicated that each descriptive model provides insights on a specific aspect of resistance patterns and therefore, combining these approaches make it possible to gain a deeper understanding of antimicrobial resistance.

Conclusions: Most studies in the existing literature employed ML techniques focused on black-box models. Although most of such black-box models achieved high accuracy rates, especially in prediction and classification tasks, they don't provide deep understanding of AMR patterns. The results of various descriptive models in our study show the potential of white-box ML approaches to reveal different aspects of MDR.

Notes:

P005 - Small molecules as antibiotic-alternatives for the control of *Rhodococcus equi* infection in horses

B Lamichhane[1], M.M Islam[1], K. A. Shaaban[2], L. V. Ponomareva[2], J. S. Thorson[2], Y. A. Helmy[1]

[1]Department of Veterinary Sciences, University of Kentucky, [2]Center for Pharmaceutical Research and Innovation, and Department of Pharmaceutical Sciences, University of Kentucky. bla260@uky.edu

Session: Antimicrobial Use, **2023-01-22, 6:00 - 8:00 pm**

Objective: *Rhodococcus equi* is an intracellular pathogen responsible for pneumonia in young foals (between 3 weeks and 6 months of age). It can also infect immunodeficient adult horse and immunocompromised people. Foals get infection due to the inhalation of dust particles laden with *R. equi*. Currently, *R. equi* is treated with antibiotics such as macrolides and rifampin. However, the rapid development of antimicrobial resistance against these antibiotics has necessitated the development of new antibiotic alternative approaches for the control of *R. equi* in foals. Our study aimed to identify novel small molecules (SMs) to treat *R. equi* effectively in young foals.

Methods: In our study, we screened approximately 1900 different SMs from diverse libraries to evaluate their effects on the growth of *R. equi*. The bacteria were grown overnight. One hundred microliters of overnight grown bacteria (1×10^7; OD_{600}=0.05) was grown in the presence of 1 µL (at 10 µM final concentration) of the drug and incubated at 37 ºC for 24 hours in 96-well plate. The growth of the SM-treated *R. equi* culture was compared to that of the non-treated control, and the percentage of inhibition was calculated for each plate.

Results: Our results demonstrated that out of the 1900 SMs, 48 showed high growth inhibition (80-100%) for *R. equi* growth. Only 10 SMs inhibited the growth of *R. equi* up to 100%. These 10 selected SMs candidates were subjected to a dose-response assay to determine the minimal inhibitory concentration (MIC) and the minimal bactericidal concentration (MBC). The top six hits that showed efficacy at the lowest concentrations (≤2.5µM) will be used for further evaluation *in vitro*.

Conclusions: Our future studies will focus on evaluating the selected SMs (6 SMs) *in vitro* by testing their efficacy on other resistant *R. equi* serotypes, biofilm formation, and toxicity to equine bronchial epithelial cells. We will also investigate their effect on different virulence-associated genes of *R. equi*. Our study will facilitate the development of SMs as a novel antibiotic alternative for the control of *R. equi* in foals.

Financial Support: Start up fund for Helmy lab, Gluck Equine Research Center, University of Kentucky.

Notes:

P006 - Evaluating the efficacy of next-generation probiotic on *Rhodococcus equi* infection in vitro

Bibek Lamichhane[1], Yosra A. Helmy[1], Md Islam[1]

[1]University of Kentucky. mmis228@uky.edu

Session: Antimicrobial Use, **2023-01-22, 6:00 - 8:00 pm**

Objective: *Rhodococcus equi* is an intracellular pathogen that survive in the soil and causes pneumonia especially in young foals and immune-compromised horses. It is one of the emerging zoonotic pathogens, which can infect immunosuppressed humans. Currently, macrolides and rifampicin are being used to treat *R. equi* infection in foals. However, antimicrobial resistance (AMR) against these antibiotics has necessitated the development of antibiotic alternatives for the control of *R. equi.* In this study, we aimed to evaluate the efficiency of novel probiotic strains as an alternative antibiotic approach against *R. equi in vitro.*

Methods: In this study, we screened a total of 38 probiotics for their inhibitory effect against *R. equi* using an agar-well diffusion assay. *R. equi* (OD_{600}=0.05) was spread in the Mueller Hinton (MH) agar plate and 100 ul of each probiotic was placed into the wells, and the growth inhibition of the bacteria was evaluated after 6,12 and 24 hours. The probiotics with the highest zone of inhibition of *R. equi* were selected for further development *in vitro.* To better evaluate the inhibitory activity of the probiotics, we co-cultured the selected probiotics and *R. equi* in broth media. The log reduction of *R. equi* (log CFU/ml) grown together with the probiotics was calculated at 6, 12, 24, 48, 72, 96 and 120 hours of incubation. Furthermore, we also evaluated the effect of the selected probiotics cell free supernatants on the biofilm formation and preformed biofilm using a crystal violet. All the experiments were repeated at least twice and the data was analyzed using two-way ANOVA followed by Tukey test to determine statistical significance.

Results: We found that all the probiotics demonstrated large zone of inhibition of *R. equi* in agar-well diffusion assay, however we selected the top six probiotics that possessed highest zone of inhibition for further development. All the six selected probiotics significantly inhibited *R. equi* growth after 12 hours ($p<0.05$) and had complete clearance of the bacteria by 120 hours when co-cultured together. In the biofilm formation, the supernatants of five probiotics had more than 90% inhibition. However, only one of the probiotics had more than 90% inhibition and three of them had more than 80% inhibition of the preformed biofilm.

Conclusions: Our future studies will focus on evaluating the selected probiotics *in vitro* by testing their efficacy in adhesion, invasion and survival of *R. equi* in murine macrophages. We will also investigate the probiotic effect on different virulence-associated genes of *R. equi*. Our study will facilitate the establishment of probiotic therapy as a novel antibiotic alternative for the control of *R. equi* infection in foals.

Notes:

P007 - Antimicrobial resistance patterns and virulence determinants of *Salmonella* spp. in necropsied livestock and equines

A. Kabir[1], C. Glover[1], S. Locke[1], E. Erol[1], Y.A. Helmy[1]

[1]Department of Veterinary Science, University of Kentucky. ajran.kabir@uky.edu

Session: Antimicrobial Use, **2023-01-22, 6:00 - 8:00 pm**

Objective: *Salmonella* is a zoonotic bacterial pathogen causing foodborne illnesses worldwide. Poultry and poultry products are considered the main source and reservoir of infection; however, other animals play an important role in infection transmission to humans through contaminated food and water. The inappropriate or excessive use of antimicrobial drugs in both agriculture and human medicine has contributed to the development and spread of antimicrobial-resistant (AMR) strains of *Salmonella*. The goal of our research was to isolate *Salmonella* spp. from the necropsied livestock and equines and determine their phenotypic and genotypic patterns as well as their virulence determinants.

Methods: Samples were collected from the UK diagnostic laboratory. A total of 55 *Salmonella* isolates were obtained from necropsied bovine (n = 25), goat (n = 2), sheep (n = 2), and equine (n = 26) in Lexington, Kentucky. Samples were enriched in tetrathionate broth and then cultured on XLT4 plates. *Salmonella* serotypes were confirmed using polymerase chain reaction (PCR). The minimum inhibitory concentration (MIC) was determined for each of isolate using the broth microdilution method. The AMR and virulence genes were detected using PCR.

Results: Among these 55 isolates, we identified different *Salmonella* serotypes such as *S. Dublin, S.* Typhimurium, and *S. Thompson*. Virulence genes such as *invA*, *hilA, sopB, spi4D, spiA, spiC* were detected in more than 50% of livestock and equine isolates. Among livestock isolates, 100% were resistant to gentamicin and neomycin, 83.33% to clindamycin, and 50% to tetracycline. Among the equine isolates, 100% showed resistance to amikacin, cefazolin, and gentamicin, and 76.47% to ampicillin, clarithromycin, erythromycin, and rifampin. However, most of the isolates were susceptible to ceftazidime, chloramphenicol, doxycycline, imipenem, minocycline, tetracycline, and trimethoprim/sulfamethoxazole. A total of 76.47% of the isolates were multi-drug resistant in equines isolates, and 44.4% of isolates were MDR in livestock isolates.

Conclusions: AMR in *Salmonella* can limit the efficacy of antibiotics, resulting in more severe and sustained infections in humans and animals. This data is critical for determining the scope of the problem, identifying patterns, and implementing targeted interventions.

Financial Support: Startup fund for Helmy Lab, Gluck Equine Research Center, University of Kentucky

Notes:

P008 - Antimicrobial resistance surveillance pilot in healthy food animals in Pakistan

Sami Ullah Khan Bahadur[1], Muhammad Usman Zaheer[2, 3], Muhammad Farooq Tahir[3, 4], Muhammad Abubakar[5], Muhammad Athar Abbas[6], Muhammad Usman Talib[7], Javaria Alam[8], Hamid Irshad[9], Riasat Wasee Ullah[10]

[1]Colorado State University, [2]FAO Regional Office for Asia and the Pacific, Thailand, [3]Health Security Partners, Fleming Fund Country Grant, Pakistan, [4]Integral Global, Atlanta, [5]National Veterinary Laboratory, Pakistan, [6]National Reference Lab for Poultry Diseases, National Agricultural Research Centre, Pakistan, [7]DAI, Fleming Fund Country Grant Pakistan Islamabad, [8]FAO Pakistan, [9]Animal Health Program, National Agricultural Research Centre, Pakistan, [10]Ministry of National Food Security and Research, Pakistan. rdshrest@illinois.edu

Session: Antimicrobial Use, **2023-01-22, 6:00 - 8:00 pm**

Objective: Antimicrobial resistance (AMR) is considered an important global public health concern due to the emergence, transmission, and persistence of multidrug resistance (MDR) microbes across animals, humans, and environment sectors. Therefore, a one health approach at all levels (global, regional, and national) is required to contain AMR effectively. Pakistan developed its AMR National Action Plan (AMR NAP) in 2017 in line with One Health approach. Pakistan animal health sector developed a national surveillance guideline for AMR in healthy food animals through the support of Fleming Fund Country Grant Pakistan. The Animal husbandry Commissioner (AHC) office coordinated the implementation of a surveillance pilot in healthy food animals involving poultry and large ruminants at slaughterhouses. The intention of this pilot project was (i) to streamline guidelines for implementation of AMR surveillance in food producing animals in Pakistan; (ii) to estimate the prevalence of resistance amongst selected bacteria species in poultry and livestock (cattle and buffaloes) to the antibiotics that the WHO has specified as critical for use in humans.

Methods: The surveillance pilot system was simultaneously implemented in poultry and livestock. The National Reference Laboratory for Poultry Diseases (NRLPD) and the National Veterinary Laboratories (NVL) were designated as National Reference Laboratories (NRLs) for AMR in the animal health sector. The nine peripheral sentinel laboratories (PSL) across Pakistan were engaged to collect caecal/faecal contents from apparently healthy slaughtered poultry and cattle/buffaloes at slaughter shops and designated slaughterhouses, respectively. The samples were shipped to NRLs as per developed guidelines. The project focused on two commensal bacteria, i.e., *Escherichia coli (E. coli), and Enterococcus* spp. and one zoonotic foodborne bacterium, i.e., *Salmonella* spp. NRLs performed antimicrobial susceptibility testing (AST) of the isolates using disc diffusion method, shared the data on WHONET files to AMR Coordination Unit at Ministry of National Food Security and Research for analysis and policy decisions.

Results: "National Surveillance Strategy for AMR in Healthy Food Animals" was developed, followed by implementation through this AMR pilot project. All components of AMR surveillance were streamlined through capacity building of newly developed two NRLs and nine peripheral laboratories. NVL and NRLPD analyzed 3464 ruminants and 2317 poultry samples respectively. NVL isolated *E. coli* from 1237 samples, *Enterococcus* spp. from 560 samples, while NRLPD isolated *E. coli* from 1456 samples, and Salmonella spp. from 417 samples. The analysis indicated an association between AMR *E. coli*, *Salmonella* and *Enterococcus* isolates with season and geographical location. *E. coli* isolated from livestock showed higher resistance against Ampicillin, Cefotaxime, Ciprofloxacin, and Tetracycline while *Enterococcus* spp. from Livestock showed higher resistance against Ampicillin, Linzolid and Tetracycline. *E. coli* from poultry showed higher resistance against Ampicillin, Azithromycin, Chloramphenicol, Ciprofloxacin, Nalidixic acid, Tetracycline and Trimethoprim while *Salmonella* spp. showed higher resistance against Azithromycin, Chloramphenicol, Ciprofloxacin, Nalidixic acid, Tetracycline and Trimethoprim.

Conclusions: High resistance level of bacteria indicates that food animals may represent a source of their transmission to humans and highlights the need for establishment of AMR surveillance and control program at national level in Pakistan.

Financial Support: DAI, Fleming Fund Country Grant Pakistan Islamabad Health Security Partners, Fleming Fund Country Grant Pakistan, Washington US.

Notes:

P009 - Global trends in antimicrobial resistance on organic and conventional farms

Eldon Ager[1], Tamilie Carvalho[2], Erin Silva[3], Steven Ricke[4], Jessica Hite[5]

[1]Department of Integrative Biology, University of Wisconsin, [2]Department of Ecology and Evolutionary Biology, University of Michigan, [3]Center for Integrated Agricultural Systems, University of Wisconsin, [4]Department of Animal and Dairy Sciences, University of Wisconsin, [5]Department of Pathobiological Sciences, University of Wisconsin. eager2@wisc.edu

Session: Antimicrobial Use, **2023-01-22, 6:00 - 8:00 pm**

Objective: The important hypothesis that organic livestock management reduces the prevalence of antimicrobial resistance is either fiercely supported or bitterly contested. Yet, empirical evidence supporting this view remains fragmentary, in part because relationships between antimicrobial use and drug resistance vary dramatically across contexts, hosts, pathogens, and country-specific regulations. This study synthesizes global policies and definitions of 'organic' and asks if organic farming results in notable reductions in the prevalence of antimicrobial resistance when directly examined alongside conventional analogs.

Methods: A systematic literature review and metanalysis was conducted on studies from 5 continents including North America, South America, Asia, Oceania, and Europe. Specifically, we conducted literature searches for studies published between 2000-2022 using three electronic databases (PubMed, Web of Science, and PubAg). We developed search terms using the Boolean logic terms and reviewed all full English-language and Portuguese-language articles that directly compared patterns of antimicrobial resistance (AMR) from chicken, turkey, cattle, and pigs, and compared environmental samples from organic and conventional farms in a given geographic region. All data analyses were conducted in R version 4.2.0 and QGIS version 3.24.0-Tisler. To examine differences in the prevalence of AMR on organic and conventional farms, we used generalized linear models (GLMs) with binomial distributions and log link functions.

Results: Our results highlight substantial variations in country-specific policies on drug use and definitions of 'organic' that hinder broad-scale and generalizable patterns. Overall, conventional farms had slightly higher prevalence of antimicrobial resistance (28%) relative to organic counterparts (18%), although we found significant context-dependent variation in this pattern. Notably, environmental samples often exhibited high levels of resistance to medically important drugs, thus highlighting the role of horizontal gene transfer in antimicrobial resistance transfer across hosts.

Conclusions: Taken together, our results emphasize the challenges inherent in understanding the links between drug use and drug resistance and the critical need for global standards governing organic policies and greater investment in viable alternatives for managing disease in livestock.

Financial Support: This project was supported by the Global Health Institute at the University of Wisconsin-Madison.

Notes:

P010 - Investigating resistome SNP profiles of litter from pens of broilers exposed to different in-feed antimicrobials

Jared G. Young[1], Randall Singer[2], Noelle Noyes[1]

[1]Department of Veterinary Population Medicine, University of Minnesota, [2]Department of Veterinary and Biomedical Sciences, University of Minnesota. youn2635@umn.edu

Session: Antimicrobial Use, **2023-01-22, 6:00 - 8:00 pm**

Objective: Antimicrobial resistance genes (ARGs) are maintained within the genomes of diverse and numerous bacterial taxa, and the microbiome-wide profile of ARGs is termed the resistome. The composition and diversity of the poultry litter resistome has been associated with antibiotic exposures, host-level factors, and management practices. However, little is known about the factors that drive genotypic change both within individual ARGs, and collectively across all ARGs within a resistome. This knowledge gap must be addressed, as genotypic changes such as single nucleotide polymorphisms (SNPs) represent an important mode of ARG evolution within and across microbiomes. Our previous work demonstrated that the richness of SNPs in tetracycline resistance genes within broiler litter increased following administration of in-feed oxytetracycline, but this trend has not yet been replicated with other antibiotic classes and ARGs. Thus, the purpose of this study was to characterize the resistome SNP profiles of litter obtained from broiler pens exposed to different classes of antimicrobials via in-feed administration.

Methods: Pen-level litter samples were collected from broilers exposed to in-feed Narasin plus oxytetracycline (high and low concentration), bambermycin, virginiamycin, or bacitracin. Broilers were randomly assigned to pens across three flock cycles; samples were collected at 6 timepoints during each flock cycle; and all flocks were raised on the same litter. DNA extracted from litter samples then underwent shotgun metagenomic sequencing, and resistome analysis was performed using AMR++ v.3. Host-filtered fasta files were then aligned to MEGARes v.3 using NGLess, and SNP calling was performed using metaSNV v.2. SNP counts were concatenated into a single count matrix for downstream statistical analysis in R. Alpha and beta diversity analysis of SNP profiles (class-specific and resistome-wide) were conducted, and the diversity measurements were used as response variables in linear mixed-effect, generalized additive and PERMANOVA models.

Results: Across all samples (N=630), >4,000 unique ARG SNPs were identified. The resistome was largely dominated by tetracycline resistance genes, followed by ARGs for aminoglycoside and macrolide, lincosamide, and streptogramin (MLS) antibiotic classes. At the SNP level, SNPs in tetracycline ARGs composed only a small percentage of total SNPs, whereas SNPs originating from aminoglycoside and MLS ARGs were predominant. The abundance and richness of both tetracycline ARGs and SNPs increased for both oxytetracycline treatment groups, as compared to the control and other treatment groups, in flock cycles two and three following exposure to oxytetracycline, and these findings were significant. SNPs in both MLS and aminoglycoside ARGs were insignificantly variable for all treatment and control groups across all flock cycles, and GAM models demonstrated that flock cycle was a likely driver of observed changes in ARG SNP composition, independent of antimicrobial exposure.

Conclusions: These results may demonstrate an association between SNP accumulation in tetracycline ARGs and exposure to tetracycline, but this relationship was not observed for other ARG classes and relevant antimicrobial exposures. For these non-tetracycline ARG classes, SNP composition seems to be driven primarily by time, which in this study is a proxy for both flock cycle and age of the birds.

Financial Support: This work was supported, in part, by grants no. 2019-67017-29110 and 2015-68003-22972 from the USDA National Institute of Food and Agriculture.

Notes:

P011 - Organic acids mixture protects intestinal barrier function of broiler via adhesive proteins upregulation

D.M. Masiuk[1], V.S. Nedzvetsky[1], A.V. Kokarev[1], Roman Pogranichniy[1]

[1]Kansas State University. rmp1@vet.k-state.edu

Session: Antimicrobial Use, **2023-01-22, 6:00 - 8:00 pm**

Objective: Total antibiotic growth promotors application in the poultry industry stimulates the generation of antibiotic resistance strains. Antibiotic-free strategy is a prospective manner to limit microbial resistance growth. The expression of E-cadherin and fibronectin is a suitable marker of intestinal barrier health.

Methods: Present study was aimed to evaluate the efficacy organic acids mixture (OAM) on the intestinal barrier function with molecular markers application. Two broiler group (n=25) was exposed to 500 mg/l OAM with drinking water since 10th day of life after abrogated antibiotics treatment. Control group was fed with basal diet and standard antibiotics treatment. The innate immunity was assessed through the interferon-α production into duodenum. Intestine barrier function was evaluated via the E-cadherin and fibronectin contents by western blot.

Results: Obtained results have shown statistically significant upregulation ($P < 0.05$) all of aforementioned molecular markers in exposed to OAM broiler group in compare to control. The feeding with OAM induced the maximal upregulation of E-cadherin and fibronectin expression at 30 days of age. The maximum of interferon-α production was observed at 22 days. The morphometry of villi in duodenum has shown length increase (up to 7.4%) in exposed group in compare to control. Furthermore, there was observed the mortality reduction (4.2%) in the exposed broiler group compared with control in according farming groups which were exposed in poultry farm of Ukraine.

Conclusions: Observed results suggest minimum two mechanisms of presented antibiotic-free strategy First mechanism of protection may be mediated by the support of adherens junctions and extracellular matrix tightness. Second way could be linked with activation of IFN-related genes. The results of present study evidence that dietary supplementation of OAM enhances the innate immune response and intestinal barrier integrity of broilers without proliferation of enteric pathogens and mortality growth.

Notes:

P012 - In feed Bacitracin alters turkey microbiota functional and antibiotic resistance genes in a dose dependent manner

Torey Looft[1], Carmen Wickware[1, 2, 3], Tim Johnson[3], Julian Trachsel[1], Paul Oladele[3]

[1]USDA-Agricultural Research Service, [2]ORISE, [3]Purdue University. torey.looft@usda.gov

Session: Antimicrobial Use, **2023-01-22, 6:00 - 8:00 pm**

Objective: Bacitracin methylene disalicylate (BMD) is an in-feed antibiotic used to improve feed efficiency (subtherapeutic dose) or to treat disease (therapeutic dose) in poultry production. While antibiotic administration can improve animal health or production, it may increase antibiotic resistant bacteria. Thus, the impact of in-feed antibiotics is important to understand to gauge risk, but also identify pathways to target for maintaining animal health with non-antibiotic interventions.

Methods: We investigated the longitudinal effects of feeding subtherapeutic (50g/ton of feed) or therapeutic (200g/ton) BMD on intestinal metagenomes of commercial turkeys. Two-hundred and forty poults were randomly divided into three treatment groups (no antibiotic control, subtherapeutic BMD and therapeutic BMD). The therapeutic BMD group received 200g/ton BMD for 35 days, followed by 50g/ton until day 78. Ten turkeys from each treatment group were euthanized 7, 35 and 78 days after BMD administration began. The cecal content of the euthanized birds were collected for metagenomic analysis. DNA was sequenced on an Illumina HiSeq3000 and PacBio RSII SMRT sequencing technology for short and long reads, respectively. Sequence assemblies were generated with metaSPAdes and open reading frames were predicted with metagenemark. Annotations were created with CARD RGI for antibiotic resistant genes (ARG) and TIGRfam database for metabolic functional genes.

Results: Both doses of BMD reduced numbers of unique open-reading frames and there was a decrease in the number of genes involved in metabolism after early BMD exposure. The therapeutic dose of BMD increased antibiotic resistance genes, conjugation-related genes belonging to type IV secretion system, as well as transduction-related genes. The effect of BMD was transient in the subtherapeutic dose while the effect was observed until day 78 in the therapeutic dose. Estimated bacterial growth rate was reduced in the therapeutic dose on day 7, but restored by day 35, while tryptophan synthesis from chorismate increased in a dose dependent manner between days 7 - 35. Overall, the effect of subtherapeutic BMD on the turkey cecal microbiota was temporary while that of therapeutic dose was lasting. These metagenomic results are in general agreement with earlier metabolome analysis, indicating that metabolomic shifts are likely due to changes in the microbiota rather than a change in nutrient metabolism and absorption by the host.

Conclusions: These results show that although microbial metabolism was altered soon after BMD administration, there was recovery in the subtherapeutic dose (relative to non-medicated) with distinct metabolic functions in the therapeutic dose. The dose-dependent selection for antibiotic resistance genes is important to inform veterinary practices and risk evaluations of in-feed antibiotics used in turkey production. Identifying mechanisms of action for in-feed antibiotics may also lead to viable alternatives to antibiotics to support animal agriculture.

Notes:

P013 - Novel peptides derived from probiotics as antibiotic alternatives to control colibacillosis in poultry

Menuka Bhandari[1], Antonia Duran[2], Katie Galgozy[1], Dipak Kathyath[1], Yosra Helmy[1], James Fuchs[3], Gireesh Rajashekara[1], Dhanashree Lokesh[1], Mark Foster[2]

[1]Department of Animal Sciences, Ohio State University, [2]Department of Biochemistry, Ohio State University, [3]College of Pharmacy, Ohio State University. thenissery.1@osu.edu

Session: Antimicrobial Use, **2023-01-22, 6:00 - 8:00**

Objective: Avian Pathogenic Escherichia coli (APEC) is an extraintestinal pathogenic E. coli (ExPEC) that causes colibacillosis in poultry, leading to significant economic losses to the poultry industry worldwide. This study aimed to evaluate the efficacy of small peptides (P-1, P-2, and P-3) as potential inhibitors of APEC colonization in commercial broiler chickens. Additionally, the study aimed to determine the most effective therapeutic doses of the peptides and conduct chicken trials in conditions mimicking the natural infection to assess their anti-APEC activity. Furthermore, we sought to identify the potential targets and elucidate mechanisms of action of peptides (P-1 and P-2) in inhibiting APEC.

Methods: In a pilot study, peptides (P-1, P-2, and P-3) were administered orally at 50 mg/kg and 100 mg/kg doses to commercial broiler chickens (n=10/group). APEC colonization in the cecum and internal organs (lung, kidney, liver, and heart) was assessed. Dose optimization (50, 100, and 200 mg/liter; n=10/group) of P-1 and P-2 was performed by administering the peptides through drinking water. The birds were orally challenged with rifampicin-resistant APEC to determine the anti-APEC activity of the peptides. Bacterial cytological profiling, gene expression analysis, immunoblot, and in silico approaches were employed to identify potential targets of peptides. The ompC and mlaA genes from APEC O78 were cloned using gene-specific primers in E. coli. Membrane proteins were separated from cytoplasmic proteins and purified using affinity and ion exchange chromatography. Interaction studies with MlaA were performed using isothermal titration calorimetry.

Results: Peptides (P-1, P-2, and P-3) reduced APEC colonization in the cecum of chickens at both doses. P-2 demonstrated the most effective effect against APEC. In the dose optimization study, P-1 and P-2 at 50 mg/liter significantly reduced APEC load in the cecum. Peptides disrupted the APEC membrane and downregulated the expression of ompC, ompF, and mlaAgenes responsible for maintaining outer membrane lipid asymmetry in APEC. Peptides also reduced the level of OmpC and MlaA proteins. In silico binding predictions revealed higher binding affinity of peptides to OmpC. MlaA was purified using affinity chromatography, followed by cation exchange chromatography, and OmpC was partially purified using a Ni-NTA affinity column.

Conclusions: Peptides (P-1, P-2, and P-3) showed promising efficacy in reducing APEC colonization in chickens, with P-2 being the most effective. The dose optimization study identified 50 mg/liter as the optimum therapeutic dose for P-1 and P-2. Peptides were found to target the MlaA-OmpC/F system in APEC, disrupting membrane lipid asymmetry. Further studies using isothermal titration calorimetry and pulldown assays will validate and provide additional insights into the protein-ligand interactions. Our results highlight the potential of these peptides as effective alternatives to combat APEC infections in chickens. Large-scale chicken trials will further facilitate the translation of these peptides for commercial application to control colibacillosis in broilers.

Notes:

P014 - Investigating efficacy and mechanism of action of novel small molecule inhibitors of avian pathogenic E. coli

Anusree Thenissery[1], Dhanashree Lokesh[1], Yosra A Helmy[2], Dipak Kathayat[3], Gary Closs Jr[1], Katie Galgozy[4], James R Fuchs[4], Gireesh Rajashekara[1]

[1]Center for Food Animal Health, The Ohio State University, [2]Gluck Equine Research Center, University of Kentucky, [3]Dept. of Population Medicine and Diagnostic Sciences, Cornell University, [4]College of Pharmacy, The Ohio State University. thenissery.1@osu.edu

Session: Antimicrobial Use, **2023-01-22, 6:00 - 8:00**

Objective: Avian pathogenic E. coli (APEC) results in colibacillosis in avian species and possess a significant health threat to the poultry industry, leading to substantial economic losses. The primary objective of this study was to establish an appropriate disease model of infection, test novel therapeutic alternatives to antibiotics, and elucidate the mechanism of action of GI-7, a novel small molecule inhibitor of APEC. An APEC oral challenge model was developed to assess the effect of novel compounds. The novel anti-APEC leads, including growth inhibitor (GI-7) and quorum sensing inhibitors (QSI-5), were evaluated using the oral challenge model mimicking natural infection. Further, the mechanisms of action of the identified anti-APEC leads were investigated.

Methods: For APEC oral challenge model, chickens were infected with two different challenge doses (2.5 x 109and 2.5 x 108 CFU/chicken) at 2 and 7 days of age. Following euthanasia, pathological lesions in internal organs (liver, heart, lung, and kidney) and the APEC load in the cecum and internal organs were determined. The efficacy of small molecules GI-7 and QSI-5, both individually and in combination (GI-7 + QSI-5), in controlling APEC infections in chickens were evaluated using the oral challenge model. In vitro, a thermal proteome profiling (TPP) assay was conducted to gain insights into the mechanisms of action of these small molecules. Biotin-linked probes for GI-7 and QSI-5 were designed for target identification and to use in pull-down assays to identify specific target proteins. Furthermore, seven different analogs of GI-7 were synthesized and tested for activity against APEC. To investigate the interaction of GI-7 with LptDE, we cloned the lptD and lptE genes, co-expressed the proteins in E. coli, and purified the proteins using affinity chromatography.

Results: Chickens challenged with 109 CFU at two days of age demonstrated the progression of colibacillosis similar to natural infection. GI-7 and QSI-5 manifested superior efficacy against APEC at lower doses than the commonly used antibiotic sulfadimethoxine (SDM) when administered through drinking water. The TPP assay revealed potential targets, including the ABC transporter ATP-binding protein, carboxypeptidase/penicillin-binding protein 1A, and other cytoplasmic proteins involved in transcription and translation. Notably, one of the seven GI-7 analogues that contained a benzyloxy group instead of a methoxy group exhibited increased activity with a MIC of 50 μM, compared to the original GI-7 with a MIC of 100 μM. We have successfully obtained a partially purified LptDE using the Ni-NTA affinity column. Further, we plan to perform gel filtration chromatography to eliminate background proteins and conduct protein-ligand interaction studies using isothermal titration calorimetry.

Conclusions: Our findings highlight the GI-7 and QSI-5 as promising antibiotic-independent alternatives to control APEC infections in chickens. The combination of GI-7 and QSI-5 demonstrated synergistic anti-APEC effects through the bactericidal effect of GI-7 and the anti-virulent effect of QSI-5. The TPP assay provided valuable insights into these small molecules mechanisms of action and target identification. Additionally, the increased activity observed in the GI-7 analogue suggests a viable strategy to achieve higher efficacy with reduced cost.

Notes:

P015 - Impact of colistin loaded on alginate nanoparticles on pigs infected with resistant enterotoxigenic *E. coli* strain

Djamel Drider[1], Rabah Boukherroub[2], Laetitia Le Devendec[3], Yanath Belguesmia[1], Gwenaelle Mourand[1], Frédéric Paboeuf[3], Isabelle Kempf[3]

[1]Lille University France, [2]Lille University, CNRS, France, [3]ANSES France. yanath.belguesmia@univ-lille.fr

Session: Antimicrobial Use, **2023-01-22, 6:00 - 8:00**

Objective: Colistin is frequently used to control post-weaning diarrhea in pigs. Colistin resistance associated to DNA plasmidic genes is a public health issue. We evaluated, in experimental animal facilities, whether free colistin or colistin-loaded on alginate nanoparticles (colistin/Alg NPs) could select a colistin-resistant Enterotoxigenic (ETEC) Escherichia coli.

Methods: The Alg NPs were produced by a simple top-down approach through ball milling of sodium alginate polymer precursor, and colistin loading was achieved through physical adsorption. Colistin loading on Alg NPs was confirmed using various tools such Fourier transform infrared spectroscopy and dynamic light scattering measurements. Thirty-four piglets were orally inoculated or not with the mcr-1-positive, rifampicin-resistant 12-269M ETEC strain, and the inoculated pigs were either treated or not during five days with commercial colistin (100,000 IU/kg) or colistin/Alg NPs (40,415 IU/kg). Clinical signs were recorded. Fecal and post-mortem samples were analyzed by culture.

Results: The result clearly indicated that colistin/Alg NPs had a slightly better therapeutic effect. Both treatments led to a transitory decrease of the total E. coli fecal population with a majority of colistin-resistant E. coli isolates during treatment, but the dominant E. coli population was found susceptible at the end of the trial.

Conclusions: In conclusion, our study revealed that, under our experimental conditions, colistin/Alg NPs had a slightly better therapeutic effect than free colistin, on the diarrhea induced by the colistin resistant ETEC 12-269M. Further studies are needed to evaluate, in diverse experimental or field conditions, the therapeutic efficacy of colistin/Alg NPs for post-weaning diarrhea.

Notes:

P016 - Cellulose nanomaterials: A novel adjuvant and delivery system for aquaculture vaccine applications

Jacob Holbrook[1], Michael Mason[1], Kora Kukk1, Blake Turner[1], Deborah Bouchard[2], Sarah Turner[2]

[1]University of Maine Chemical and Biomedical Engineering, [2]University of Maine Cooperative Extension and Aquaculture Research Institute. michael.mason@maine.edu

Session: Aquaculture, **2023-01-22, 6:00 - 8:00**

Objective: Disease outbreaks are a major impediment to aquaculture production and are forecasted to continue as the industry grows and the climate warms. Vaccines are integral for disease management in aquaculture but they can be expensive, vary in effectiveness, and come with adjuvant-induced adverse effects causing fish welfare issues and negative economic impacts. The goal of this interdisciplinary project is to develop a new generation of vaccines for sustainable aquaculture. Our project uses novel nanomaterials produced from renewable wood fiber as depots/adjuvants in vaccine formulations to modulate the immune response of Atlantic salmon in a biocompatible, environmentally friendly, and cost-effective manner.

Methods: We are elucidating the role of CNM as a vaccine depot and mobile immunostimulant, the extent of CNM migration in vivo, and the efficacy of CNM bound antigen as an immunostimulant for protection against an Atlantic salmon pathogen. To accomplish this, our interdisciplinary research team: 1.) Prepared and conducted in vitro characterizations of CNM hydrogels and CNM/antigen (vaccine) formulations physically, chemically, and mechanically 2.) Conducted in vivo studies to determine biocompatibility, quantify the antibody kinetics in vaccinated fish serum using enzyme-linked immunosorbent assays, and examined gene expression regulation from head kidney and 3.) Will evaluate the efficacy of the CNM vaccine(s) in protecting against *Vibrio anguillarum* in Atlantic salmon by performing a pathogen challenge study.

Results: Initial results demonstrate TEMPO CNF hydrogels as a possible vaccine adjuvant for fish with delivery and biocompatibility being a primary obstacle. Alternative shear- thinning injectable hydrogels demonstrate promise with chemical toxicity being a hurdle. Performance of CNM formulations in stimulating antibody response and efficacy in preventing disease mortalities compared to a commercial vaccine and a negative vehicle control will be determined.

Conclusions: We anticipate the CNM vaccine formulations will perform as well or better than commercially available vaccines while being more cost-effective and sustainable for long-term aquaculture.

Finanical Support: U.S. Department of Agriculture, National Institute for Food and Agriculture AFRI Foundational Grant

Notes:

P017 - Effects of dietary glutamate supplementation on the respiratory burst of leukocytes in Hybrid Striped Bass

Karina Hissen[1], Wenliang He[1], Michael Criscitiello[1], Guoyao Wu[1]

[1]Texas A&M University. klhissen@tamu.edu

Session: Aquaculture, **2023-01-22, 6:00 - 8:00**

Objective: The freshwater teleost fish hybrid striped bass (HSB; Morone saxatilis ♂ x M. chrysops ♀) is constantly challenged by pathogens within their environments and rely on their immune defense mechanisms for survival. In particular, the intestinal mucosa is a significant barrier defending the fish against pathogens, yet this organ's immune function is understudied in fish. Amino acids play an essential role in the immune responses of fish. When carnivorous fish ingest protein, the small intestine absorbs dietary amino acids and small peptides. About 95% of dietary glutamate is utilized by the gut in its first-pass metabolism, and the responsible cell types may include mucosal immune cells to support local immune defense. We hypothesized that dietary glutamate may regulate intestinal immune defense through the respiratory burst (the consumption of oxygen for the production of superoxide anion and H2O2 as killers of pathogens) of intestinal mucosal leukocytes. For comparison, leukocytes from the head kidney were also studied.

Methods: Juvenile HSB with an initial weight of ~10 g were housed in a recirculating aquaculture system at the Kleberg Building of Texas A&M University. Fish were fed for 8 weeks purified diets containing either 3% (control) or 8% glutamate. The content of glutamate in the control diet is similar to that in commercial fishmeal-based diets. At the end of the 8-week feeding, fish in each tank were placed randomly into one of two tanks for single intraperitoneal administration of 0.1 ml of RPMI medium containing 0 or 100 μg of trinitrophenyl-lipopolysaccharide. Fish continued to consume their respective diets. On day 9, all fish in each diet group were euthanized for intestinal mucosae and the head kidney. Leukocytes were isolated from these tissues using Ficoll-Hypaque (specific gravity, 1.077). For the measurement of superoxide anion or H_2O_2 production, cells (~0.3 × 106) were incubated at 26ºC for 30 min in 0.2 ml of oxygenated (95% O_2/5% CO_2) Krebs bicarbonate buffer pH 7.4) with or without phorbol myristate acetate (PMA; 500 ng/ml) plus ionomycin (Iono; 7.5 ng/ml).

Results: The results display that exposure to LPS-TNP to HSB increases respiratory burst activity within the intestinal mucosal and head-kidney leukocytes from PMA plus Iono stimulation. The production of H_2O_2 was highest in HSB supplemented with 8% glutamate compared with the 3% glutamate diet, with the head kidney leukocytes producing more than the intestinal mucosa. Interestingly, superoxide anion production in the intestinal mucosa was higher in HSB supplemented with 3% glutamate while the head kidney had a greater production in HSB supplemented with 8% glutamate. This indicates that the rate of superoxide anion production increases within the gut mucosa of HSB when they are exposed to LPS-TNP compared with H_2O_2 to neutralize pathogens of respiratory burst.

Conclusions: We observed that dietary supplementation with glutamate may increase the production of superoxide anion and H_2O_2 by both the intestinal mucosal and head kidney leukocytes to neutralize pathogens. This suggests that dietary glutamate could modulate respiratory bursts activity and may play an important role in the intestinal mucosal immunity of HSB.

Financial Support: This research was supported by Agriculture and Food Research Initiative Competitive Grant no. 2021-67015-34534 from the USDA National Institute of Food and Agriculture.

Notes:

P018 - Investigate the impact of long-term exposure of *Edwardsiella ictaluri* to trans-cinnamaldehyde on resistance development

Sahar Rostami[1], Hossam Abdelhamed[1]

[1]Mississippi State University. sr2116@msstate.edu

Session: Aquaculture, **2023-01-22, 6:00 - 8:00**

Objective: Channel catfish (*Ictalurus punctatus*) has the highest economic value of any aquaculture sector in the United States, with an annual production value of $447 million in 2022. Disease control remains a major challenge for catfish farmers. Enteric septicemia of catfish (ESC), caused by *Edwardsiella ictaluri*, causes significant economic losses to industry. Antimicrobial (AM) feeds are the primary method to control ESC, but their use has raised concerns about the development of antimicrobial resistance. As a result, there is a growing interest in developing alternative methods for preventing and treating ESC. Trans-cinnamaldehyde (TC) is a natural compound found in cinnamon essential oil that has been shown to have antimicrobial activity against E. ictaluri. In this study, we aim to determine the resistance development of E. ictaluri after long-term exposure to a sub-minimal inhibitory concentration (sub-MIC) of TC.

Methods: We investigated the proteomic changes in *E. ictaluri* after continuous exposure to a sub-MIC of TC. To achieve this, *E. ictaluri* 93-146 was subcultured daily in the selected sub-MIC dose (0.016 µl/ml) of TC for 60 consecutive days. Then, we conducted susceptibility tests on isolates collected on day 30 and 60 using the disk diffusion method for TC, florfenicol, and sulfadimethoxine. Additionally, we analyzed the proteomic alterations in the day 30 and day 60 isolates compared to *E. ictaluri* 93-146. For all statistical analysis p-value < 0.05 was considered significant, besides proteins that met the criteria of a fold change over 1.5 were used for enrichment analysis.

Results: Using, Kruskal-Wallis and Dunn's post-hoc tests, our results showed that the inhibition zone against TC was notably smaller (p-value = 0.0120 and 0.002) in day 30 and day 60 isolates compared to the control group (diameter of inhibition zone is 6.78 cm and 6.48 cm vs. 7.27 cm). Additionally, the day 60 isolate showed reduced susceptibility to florfenicol compared to the wildtype (p-value = 0.0048). Based on enrichment analysis using STRING database, day 30 and day 60 isolates showed significant upregulation in the following KEGG pathways, pyrimidine and carbon metabolism and metal-ion binding GO function. In addition to that, we observed a significant downregulation of bacterial secretion system that involves proteins in the type III, and type VI secretion systems, when compared to *E. ictaluri* 93-146. Type III and VI secretion systems play crucial roles in delivering virulent factors to host cells. It is possible that disruption of these two-systems impact virulence of *E. ictaluri* 93-146.

Conclusions: In conclusion, subculture of *E. ictaluri* to sub-MIC of TC altered the metabolism and physiology of *E. ictaluri* in a way that might reduce its virulence. Further studies are required to confirm findings and investigate mechanism of action of TC, to interpret how this long-time exposure, reduced the sample susceptibility to florfenicol. Overall, TC is a promising natural compound for improving catfish health and performance without enhancing resistance.

Financial Support: The research was funded by the United States Department of Agriculture (USDA) National Institute of Food and Agriculture (NIFA) awarded number 2022-67015-36339 to Hossam Abdelhamed.

Notes:

P019 - Genomics analysis of multidrug-resistant *Plesiomonas shigelloides* strain MS-17-188

Sahar Rostami[1], Shahnewaj Bin Mannan[2], Hasan C. Tekedar[1], Mark L. Lawrence[1], Hossam Abdelhamed[1]

[1]Mississippi State University, [2]Moffitt Cancer Center. abdelhamed@cvm.msstate.edu

Session: Aquaculture, **2023-01-22, 6:00 - 8:00**

Objective: *Plesiomonas shigelloides* is a part of the normal intestinal flora of catfish. *P. shigelloides* strain MS17-188 was isolated from deceased catfish from East Mississippi and exhibited resistance to florfenicol, tetracycline, and sulfonamide. The whole genome sequencing of *P. shigelloides* strain MS17-188 contains three plasmids, named pPSMS-171881, pPSMS-171882, and pPSMS-171883 (GenBank accession no CP027853, CP027854, and CP027855, respectively). The objective of this study is to characterize the genetic structure of these three plasmids and conduct a comparative analysis to identify the potential source of the resistant elements. Moreover, we evaluated mobility and stability of plasmid-mediated antibiotic resistance.

Methods: The antimicrobial resistance genes and virulence genes were detected by the Comprehensive Antibiotic Resistance Database (CARD) and Virulence Gene Identifier. Additionally, plasmid mobility was evaluated by conjugation experiments on filter paper and stability was assessed using subculture and colony patching methods.

Results: pPSMS-171881 is 395,858 bp with an average G+C content of 49.07%. This plasmid harbors multidrug efflux complex (adef), two genes responsible for arsenic resistance, and one prophage-like region containing several potential virulence genes. pPSMS-171882 is 50,109 bp with an average G+C content of 43.94%. pPSMS-171882 also has a region of 7,085 bp encoding type IV secretion system (T4SS) proteins that mediate horizontal gene transfer. pPSMS-171883 is 18,970 bp with an average G+C content of 62.44%. pPSMS-171883 carries tetracycline resistance gene (*tetA*) and phenicol resistance gene (*floR*) flanked by two transposable elements (IS15 and ISVsa3) and mobilization protein, suggests that there is a conjugative mechanism by which this plasmid can be mobilized. The backbone (70%) of pPSMS11881 and a significant portion of pPSMS171882 were 96.95% and 69% identical to plasmid-1 and plasmid -2, respectively of *P. shigelloides* strain P5462, isolated from Gentoo Penguin. The backbone of pPSMS171883 was 99% identical to Chitinibacter sp. 2T18 plasmid isolated from freshwater mussels. Conjugation mating experiments indicated that pPSMS-171883 was capable of being transferred from *P. shigelloides* to *E. coli*. Results from stability experiment indicated that the pPSMS-171883 is lost over time in the absence of selective pressure.

Conclusions: This is the first study to report plasmid-mediated antimicrobial resistance in Plesiomonas isolated from cultured fish, which needs continued monitoring. We anticipate the data generated in this study will provide an understanding of the genetic mechanisms of antibiotic resistance of *P. shigelloides* in catfish.

Financial Support: This work is supported by the USDA National Institute of Food and Agriculture, Agricultural and Food Research Initiative Competitive Program, Antimicrobial Resistance number: 2021-68015-33502/project accession no. 2020-04194.

Notes:

P020 - Transcriptome profiling of lumpfish (*Cyclopterus lumpus*) head kidney to *Renibacterium salmoninarum* at early and chronic infection stages

Hajarooba Gnanagobal[1], Setu Chakraborty[1], Ignacio Vasquez[1], Joy Chukwu-Osazuwa[1], Trung Cao[1], Ahmed Hossain[1], My Dang[1], Katherine Valderrama[1], Surendra Kumar[2], Gabriela Bindea[3], Stephen Hill[2], Danny Boyce[2], Jennifer Hall[2], Javier Santander[1]

[1]Department of Ocean Sciences, Memorial University of Newfoundland, [2]Ocean Sciences Centre, Memorial University of Newfoundland, [3]INSERM, Laboratory of Integrative Cancer Immunology, France. hgnanagobal@mun.ca

Session: Aquaculture, **2023-01-22, 6:00 - 8:00**

Objective: *Renibacterium salmoninarum*, a Gram-positive pathogen, causes Bacterial Kidney Disease (BKD) in several fish species, including salmonids and non-salmonids like lumpfish (*Cyclopterus lumpus*), which is utilized as a living pest remover to biocontrol the sea lice (*Lepeophtheirus salmonis*) infestations in Atlantic salmon (*Salmo salar*) sea cages. Lumpfish susceptibility to *R. salmoninarum* has been reported recently. However, the transcriptome response of lumpfish to this immune-suppressive pathogen is unknown. Therefore, we aimed i. to profile the transcriptome response of lumpfish head kidney to early (28 days post-infection (dpi)) and chronic (98 dpi) *R. salmoninarum* infection, ii. to identify and compare the immune pathways differentially regulated in response to *R. salmoninarum* at 28 and 98 dpi, and iii. to examine lysozyme activity and antibody titers in lumpfish serum upon *R. salmoninarum* infection.

Methods: Fish were intraperitoneally (i.p.) injected with either a high dose of *R. salmoninarum* (1×10^9 cells dose-1) or PBS (control), and head kidney samples were collected at 28 and 98 dpi for RNA-sequencing. In addition, blood samples taken at 1, 14, 28, 42, 56, and 98 dpi were used for the lysozyme and indirect ELISA assays.

Results: Transcriptomic profiling identified 1971 and 139 differentially expressed genes (DEGs; cut-off: log2 fold-change ≥ |1| and false discovery rate $p\leq0.01$) in infected compared with control samples at 28 and 98 dpi, respectively. At 28 dpi, *R. salmoninarum*-induced genes (n=434, $p<0.001$) mainly involved in innate and adaptive immune response-related pathways, whereas *R. salmoninarum*-suppressed genes (n=1537, $p<0.001$) were largely connected to amino acid metabolism and cellular processes. Cell-mediated immunity-related genes showed dysregulation ($p<0.01$) at 98 dpi. Several immune-signalling pathways were dysregulated in response to *R. salmoninarum*, including apoptosis, alternative complement, JAK-STAT signalling, and MHC-I dependent pathways. The serum lysozyme levels of the infected fish at earlier sampling (1 dpi) were significantly higher ($p<0.05$) compared to the control fish at 1 dpi and the infected fish at 14, 28, 42, 56, and 98 dpi. Log antibody titers were not significantly different between control and *R. salmoninarum* infected fish in all the tested time points.

Conclusions: In summary, *R. salmoninarum* causes immune suppression at early infection, whereas lumpfish induce a cell-mediated immune response at chronic infection. This study provides a complete depiction of diverse immune mechanisms dysregulated by *R. salmoninarum* in lumpfish and opens new avenues to develop immune prophylactic tools to prevent BKD.

Notes:

P021 - Enhanced phagocytosis and complement-mediated killing of *Mannheimia haemolytica* with *neuA* gene deletion

Harish Menghwar[1], Fred M. Tatum[2], Robert E. Briggs[2], Eduardo Casas[2], Rohana P. Dassanayake [2]

[1]Oak Ridge Institute for Science and Education (ORISE), ARS Research Participation Program, [2]Ruminant Diseases and Immunology Research Unit, USDA Agricultural Research Service. Harish.Menghwar@usda.gov

Session: Bacteriology, **2023-01-22, 6:00 - 8:00**

Objective: *Mannheimia haemolytica* is the most significant bacterial pathogen associated with bovine respiratory disease complex. Although sialic acid is a known virulence factor in pathogenic members of the related Pasteurellaceae family, such as *Histophilus somni* and *Pasteurella multocida*, the significance of sialic acid in *M. haemolytica* virulence is currently unknown. Therefore, the role of sialic acid in the virulence of *M. haemolytica* serotype 1 was determined by constructing in-frame DneuA (CMP-N-acetylneuraminic acid (Neu5Ac/sialic) synthetase) gene deletion.

Methods: Temperature-sensitive plasmid pCT109GA189-Kan was used to introduce an in-frame deletion of *neuA* of *M. haemolytic*a serotype 1 strain D153. Lipopolysaccharides (LPS) were extracted from *M. haemolytica* wild type (WT) and neuA mutant by hot phenol-water extraction method. High-performance anion exchange chromatographic (HPAEC) analysis was used to determine the sialic acid content in the LPS preparations. Real-time qPCR and ELISA were used to detect proinflammatory cytokine (IL-1β, IL-6 and IL-8) transcripts and protein expression levels following cattle peripheral blood mononuclear cells (PBMCs) incubation with LPS preparations. Whole-blood and complement-mediated bacterial killing assays were performed with WT *M. haemolytica* and *neuA* mutant strains. Reactive oxygen species (ROS) generated by neutrophils and monocytes following incubation with WT and *neuA* mutant strains were determined by flow cytometry.

Results: Deletion of a major portion of *neuA* (amino acids 42-411) was confirmed by PCR, and lack of sialic acids in the extracted LPS of *M. haemolytica neuA* mutant, but not WT strain, was confirmed by HPAEC analyses. Both WT and neuA mutant strains exhibited similar growth rates in the growth curve assay. Real-time qPCR and ELISA analyses showed no differences in IL-1β, IL-6 and IL-8 expressions between the WT and neuA mutant strains when PBMCs were incubated with LPS. Interestingly, the neuA mutant was three to four logs more sensitive to a whole-blood bacterial killing assay than the WT parent. Similar results were also observed in plasma and serum bacterial killing assays. Flow cytometry analyses showed higher uptake of neuA mutant by phagocytes, compared to the WT strain, in the whole blood phagocytosis assay; however, no difference in ROS produced by neutrophils or monocytes was detected for neuA mutant or WT *M. haemolytica*.

Conclusions: These results indicate that sialylation of *M. haemolytica* LPS plays a vital role in reducing complement-mediated and phagocytic killing.

Financial Support: This research was supported by funding through internal USDA research dollars (USDA/Agricultural Research Service, National Animal Disease Center, 5030-32000-236-00D)

Notes:

P022 - Development and testing of *Mycobacterium avium* subsp. p*aratuberculosis* DIVA vaccines in ruminants

Mostafa Hanafy[1], Maria Alejandra Colombatti Olivieri[2, 3], John P. Bannantine[2], Denise K. Zinniel[1], Raul G. Barletta[1]

[1]School of Veterinary Medicine and Biomedical Sciences, University of Nebraska, [2]USDA-Agricultural Research Service, [3]Oak Ridge Institute for Science and Education (ORISE), ARS Participation Program. mhanafy2@unl.edu

Session: Bacteriology, **2023-01-22, 6:00 - 8:00**

Objective: Johne's Disease (JD) is caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP) and is a significant economic problem in the dairy industry worldwide. We successfully generated live-attenuated strains that can differentiate vaccinated from infected animals (DIVA) and demonstrated the promotion of apoptosis in the DMAP52 and DMAP56 marked and unmarked (unm) mutants. In this study, we infected bovine monocyte derived macrophages (bMDM) with MAP to determine bacterial survival, the cytokine response, and cell phenotyping and proliferation. We began a calf trial to determine pathogenicity and immune responses.

Methods: MAP strains were cultured in Middlebrook 7H9 media. For the in-vitro assays, peripheral blood mononuclear cells (PBMC) and bMDM were obtained from healthy control and JD infected cows, infected with MAP K-10, DMAP52-unm and DMAP52-unm-complemented. Activation markers CD25+ for LT (CD4, CD8 and gdTCR) and CD86+/MHCII for CD14 (monocytes) were measured for cell phenotyping and survival in bMDM by confocal microscopy at 24 and 72h post-infection. A non-stimulated/infected (NS) and a positive mitogen control (lipopolysaccharide, LPS) were included. For the calf trial, sixteen male Holstein calves were used in 3 experimental groups of 5 animals infected at 3 weeks of age with 3 doses of 2 x 10^{11} CFU/mL of MAP K-10, DMAP52-unm or DMAP52-unm-complemented, and an uninfected animal was used as a control. At pre-infection, 2 weeks, and at 1, 2, 3, 6, 9, 12 months post-infection, the immune response will be evaluated by antibody production, cytokines, and lymphocyte/monocyte populations.

Results: The higher than expected MOI as determined by confocal microscopy for the in-vitro cytokine assays in bMDM resulted in a significant decrease in cell viability, and no significant differences in cytokine production, cell populations and MAP survival in macrophages were observed between the groups. The high MOI resulted in low viability in cell culture and we are subsequently evaluating the results of a low MOI for in-vitro infection. For the calf trial, some of the young calves succumbed to a rotavirus outbreak and pneumonia leaving the groups with the mutant and the complemented strain with enough calves to continue with a comparative study. At 2 weeks post-challenge, the mutant and the complemented strains showed no differences in cell proliferation or cytokine stimulation.

Conclusions: The in-vitro assays performed need more optimization as PBMC cell numbers were low due to viability issues and high MOI. The animal trial will become a pilot study to determine if the mutant is more attenuated than the complemented strain.

Financial Support: This project was supported by Agriculture and Food Research Initiative Competitive Grant no. 2020-67015-31416 from the USDA National Institute of Food and Agriculture.

Notes:

P023 - Characterization of *Mycobacterium avium* subsp. *paratuberculosis* mutants generated with CRISPRi system

Jun Ho Lee[1], Su Min Kyung[1], Eun-seo Lee[1], Han Sang Yoo[1]

[1]Department of Infectious Disease, Seoul National University. lejh96@snu.ac.kr

Session: Bacteriology, **2023-01-22, 6:00 - 8:00**

Objective: *Mycobacterium avium* subsp. *paratuberculosis* (MAP) causes a chronic emaciating disease of ruminants characterized by diarrhea, severe weight loss, cachexia, resulting in enormous economic losses in the bovine industry. These phenomena might be closely related to the intracellular survival of MAP. However, the precise mechanism of survival is not fully understood. Therefore, we tried to reveal the mechanism by characterization of phenotypes of mutants, generated using the CRISPRi system on intracellular survival related genes.

Methods: Four genes (PknG, Icl, MAP1981c, and Mdh), known as related to mycobacterial virulence, were mutated with CRISPRi system and expression inhibiting mutants were generated. The optimal concentration of CRISPRi-inducer, anhydrotetracyline (ATc), was determined after culture of the mutants to the mid-log phase in kanamycin-7H9 broth for 7 days. Growth patterns of each mutant were determined by measuring OD_{600} and CFU counting when the mutants were cultured with or without ATc. The survival of those mutants was determined under stress conditions (nutrient starvation, oxidative and acidic stress) by triplicates. Also, colony morphology, cell aggregation and envelope alteration were observed using a binocular optical microscope and field emission scanning electron microscopy (FE-SEM). The statistical significance was calculated by two-way ANOVA and Tukey test for multiple comparisons.

Results: The optimal gene expression inhibition concentration of the MAP mutants was 30 μg/ml of ATc and the concentration was confirmed at the mutants of Mdh, Icl, 1981c ($P<0.05$), and PknG ($P<0.01$) after the culture for 7 days. Growth of Mdh gene mutant was decreased by the inhibition of the gene expression. Only mutants on the PknG gene survived through inhibition of gene expression using ATc under all stress conditions. The survival of MAP mutants on Icl and MAP1981c genes was gradually decreased in nutrient starvation and oxidative stress, respectively and both in acidic stress when inhibited the gene expression with ATc. Morphological changes of colony were observed with PknG and MAP1981c gene mutants in the nutrient starvation and oxidative stress conditions, respectively. Similar morphological changes in the colony and aggregation of the Icl gene mutant were also observed under nutrient starvation and oxidative stress conditions. Morphological changes of the above mutants were irregular forms on the margin of MAP colony. The changes might be due to the envelope changes, which were short and shrink observed under the FE-SEM.

Conclusions: Our study indicates that the growth and survival of the mutants in the stress conditions by observing phenotypic alterations might be closely related to the pathogenesis of MAP infection. Changes in colony morphology, aggregation, and envelope under each stress condition when inhibiting gene expression confirmed that the gene could cause changes in the physiological process of MAP related to intracellular survival mechanisms. These phenotypic and physiological characteristics observed in our study might give important insights to reveal the involvement of those genes in MAP pathogenesis in the stages of infection and intracellular survival.

Financial Support: This study was supported by the Food and Rural Affairs Convergence Technologies Program for Educating Creative Global Leader (No. 320005-4), BK21 FOUR and Research Center and Research Institute for Veterinary Science, Seoul National University, Seoul, Republic of Korea.

Notes:

P024 - A search for unique genomic signatures among elk isolates of *Mycobacterium tuberculosis* variant bovis

Muhammad Yasir Zahoor[1], Khelsea Bahr[1], Seulee Robbe-Astermann[2], Tyler Thacker[2], Evan Brenner[1], Joe Darish[1], Srinand Sreevatsan[1]

[1]Department of Pathobiology and Diagnostic Investigation, College of Veterinary Medicine, Michigan State University, [2]National Veterinary Services Laboratory. zahoormu@msu.edu

Session: Bacteriology, **2023-01-22, 6:00 - 8:00**

Objective: Tuberculosis is a disease of multiple host species, caused by members of the *Mycobacterium tuberculosis Complex (MTBC)* and remains a leading cause of death among people (caused by *Mycobacterium tuberculosis* variant *tuberculosis)*and devastating economic losses to animal agriculture and wildlife conservation (*Mycobacterium bovis* or MBO). The purpose of this study is to index genomic signatures among elk isolates of MBO and provide an understanding of their divergence from other strains infecting animals and humans. Previous research on a known set of single nucleotide polymorphisms (SNPs) using MassArray™ technology showed that all elk isolates from different geographic locations, in the US, phylogenetically clustered into a unique clade separated from related *M. bovis* isolates. Thus, we hypothesized that genome wide analysis for unique single nucleotide polymorphisms and/or insertion-deletion events would provide strong scientific foundations to understand host adaptation, host range, and zoonotic potential.

Methods: Genomic DNA from elkisolates of MBO were obtained from USDA and sequenced with Illumina (NovaSeq) technology. Whole genomic sequences were de novo assembled and analyzed with snippy (a program that is used for rapid haploid variant calling and core genome alignment) and compared. Genomes were assembled *de novo* and phylogenetic analysis revealed the elk genomes belonged to a separate clade within MTBC. SNPs Extracted from genomes were compared against the other strains of MBO to define the evolutionary trajectory of elk isolates.

Results: Comparisons between strains AF2122/97, 10-7428, 95-1315, 99-0745, 61-09 and isolated elk strains revealed significant SNPs in the elk genome compared to the AF2122/97 genome. A total of 520 single nucleotide polymorphisms (SNPs) were extracted from all these strains against the AF2122/97 reference. Out of 520 SNPs in the set, 95 were present in and unique to MBO of elk genomes. Of these, 54 were missense, and 25/54 missense mutations were in genes with a putative function assigned. The remaining 32 were synonymous, 1 stop gained and 8 blanks. Further, phylogenetic analysis of the elk genomes revealed that they were clustered in a separate single clade.

Conclusions: We discovered unique genomic signatures in mycobacterium that allows it to adapt in other hosts, especially in elk and to find if there are any unique differences between these variants. Genome analysis of this bacterium will establish within host evolution and provide a repertoire of targets that can be used in diagnostics and/or as targets for subunit vaccine development based on conserved epitope domains.

Notes:

P025 - Circulating foamy macrophages and other features of *Mycobacterium bovis* BCG challenge in Golden Syrian hamsters

Ellie Putz[1], Claire Andreasen[2], Judith Stasko[1], Camila Hamond[3], Steven Olsen[1], Jarlath Nally[1], Mitchell Palmer[1]

[1]USDA Agriculture Research Service, [2]Department of Veterinary Pathology, Iowa State University, [3]National Veterinary Services Laboratories, APHIS. steven.olsen@usda.gov

Session: Bacteriology, **2023-01-22, 6:00 - 8:00**

Objective: Golden Syrian Hamsters are utilized as rodent research models for various bacterial and viral diseases. They are highly susceptible to leptospirosis, making hamsters the most common model for testing and maintaining virulence of laboratory *Leptospira* strains as well as for bacterin vaccine efficiency testing. Hamsters are also used for modeling features of Bacillus Calmette-Guérin (BCG) and tuberculosis, as they consistently develop granulomas, differing from some other BCG animal models. While circulating foamy macrophages can be found in the blood of hamsters following *Leptospira* challenge but not in controls, it has been unknown whether this phenomenon is specific to *Leptospira*/hamster interactions, or whether hamsters will produce circulating foamy macrophages in response to other bacterial infections.

Methods: Hamsters were challenged by intraperitoneal injection with BCG (Danish 1331 strain) or *Leptospira borgpetersenii* strains HB203 and LR131 as comparative positive controls along with media only negative controls. Hamsters challenged with LR131 develop acute clinical signs of leptospirosis and were euthanized within 5 days post challenge, at which time an asymptomatic group of BCG hamsters was additionally euthanized for comparative sampling. Forty days post challenge, asymptomatic groups of BCG and chronic HB203 hamsters were euthanized for sampling. Blood smears were used to evaluate manual differential cell counts and determine the presence of circulating foamy macrophages. Tissues of interest were collected and evaluated by pathologists.

Results: Blood from both acute and chronic BCG injected hamster groups developed circulating foamy macrophages while media alone controls did not. As reported previously, *Leptospira* infected hamsters also developed circulating foamy macrophages. While acute time frame BCG hamsters did not present with gross lesions, microscopic multifocal granulomas were identified. In contrast, chronic time frame BCG hamsters all presented with identifiable gross lesions and microscopic granulomas in the lungs as well in select additional locations such as spleen, liver, mesenteric lymph nodes, and site of BCG intraperitoneal injection. In these animals, granulomas within the lung, liver, spleen, and mesenteric lymph nodes were highly cellular without areas of necrosis. Typical of mycobacteria induced granulomas, large macrophages, many of which contained numerous variably sized vacuoles (foamy macrophages) and low numbers of multinucleated giant cells were readily identifiable. Numerous small, mineralized bodies, consistent with Schaumann bodies, were seen extracellularly as well as within multinucleated giant cells. Some Schaumann bodies contained one to several acid-fast bacilli.

Conclusions: We established that hamsters develop circulating foamy macrophages when challenged intraperitoneally with BCG or *Leptospira*. In addition to circulating foamy macrophages, hamsters infected with BCG had widespread non-necrotizing granuloma formation. Granulomas were found in major organs and at the site of injection, which also contained resident foamy macrophages, as well as Schaumann bodies, sometimes containing acid fast bacteria. Future work is planned to determine functionality and of foamy macrophages and how they may impact pathogenesis.

Financial Support: This research was funded solely by the USDA

Notes:

P026 - Development of an inducible expression cassette for *Leptospira* spp. and optimization of current CRISPR/Cas9 mutagenesis strategies

Luis G.V. Fernandes[1], Ana L.T.O. Nascimento[2], Jarlath E. Nally[1]

[1]USDA-ARS, [2]Butantan Institute. luis.fernandes@usda.gov

Session: Bacteriology, **2023-01-22, 6:00 - 8:00**

Objective: Develop, validate, and apply an isopropyl ß-D-1-thiogalactopyranoside (IPTG) inducible cassette for controlled protein expression in *Leptospira* spp.

Methods: An inducible cassette was tailored using the strong and constitutive *lipL21* promoter from *L. interrogans* to express the codon optimized Lac repressor (LacI), followed by a transcription terminator. For heterologous protein expression, the *lipL41* promoter was used; immediately after the TSS, the Lac operator (lacO) sequence was included, followed by the 5' UTR from *lipL41*, including the putative Shine-Delgarno (SD) sequence. The *lipL32* gene CDS was used as a reporter gene to evaluate controlled protein expression in the surrogate *L. biflexa*. The inducible cassette was then validated in our CRISPRi (interference) gene silencing strategies by substituting the promoter driving dCas9 (deactivated nuclease Cas9), expression in the plasmids pMaOri.dCas9, by PCR and Gibson assembly reactions. Recombinant plasmids were delivered to *Leptospira* by conjugation, and transconjugants were grown with or without IPTG for evaluation by immunoblotting. For further phenotype validation, distinct colonies were selected and grown in liquid media with or without IPTG.

Results: Saprophytic *L. biflexa* cells containing the plasmid for inducible expression of LipL32 were recovered after growth to mid-log phase ($2x10^8$ cells/mL) in the presence or absence of IPTG. Heterologous LipL32 expression was detected only in the presence of IPTG, with no expression detected in the absence of the inducer, indicating a tightly regulated cassette. Plasmids for inducible expression of dCas9 were successfully obtained, and protein expression validated by immunoblotting with anti-Cas9 antibodies, confirming expression only in the presence of IPTG. Controlled dCas9 expression plasmids were then used to validate the classic essential *dnaK* (chaperone) gene in *L. biflexa* by CRISPRi. After conjugation and plating, colonies could only be recovered in the absence of IPTG, corroborating that silencing of *dnaK* is not tolerated in *Leptospira* spp. For phenotype confirmation, colonies grown in plates without IPTG were selected and seeded into liquid media with and without IPTG. Conditional mutants for chaperone DnaK could not grow in the presence of IPTG at 37 ºC. However, at 29 ºC, cells reached lower densities (below 10^8/mL) and presented anomalous morphology. Control cells containing plasmids with no sgRNA displayed no significant difference in growth curves in the conditions tested, reaching cell densities above 10^9/mL.

Conclusions: An IPTG-inducible cassette has been effectively engineered for controlled protein expression in *Leptospira*. This newly described cassette was validated for controlled expression of dCas9 which will optimize the gene silencing CRISPR-interference tool for *Leptospira*. Controlled gene silencing approaches hold promise to investigate the essential nature of genes, thereby advancing our understanding of leptospiral biology and virulence. Further controlled Cas9 expression can be applied to facilitate knockout recovery, which paves the way for optimized and efficacious leptospirosis vaccines and bacterins.

Financial Support: Financial support by USDA-ARS (US), Oak Ridge Institute for Science and Education (ORISE) and FAPESP (Brazil).

Notes:

P027 - Isolation, identification and development of lactic acid bacteria and yeast as starter cultures for the production of "teff" (*Eragrostis tef*) injera

Ayenew Meresa[1], Hirut Assaye[2], Takele Habitu[2], Demelash Areda[3]

[1]Food Science and Postharvest Handling Research Directorate, Amhara Agricultural Research Institute, Ethiopia., [2]Faculty of Chemical and Food Engineering, Bahir Dar University, Ethiopia, [3]Ottawa University. demelash.biffa@gmail.com

Session: Bacteriology, **2023-01-22, 6:00 - 8:00**

Objective: To isolate and identify starter cultures and assess their influence on the fermentation process of teff dough and the sensory attributes of injera.

Methods: Lactic acid bacteria (LAB) and yeast were isolated from traditional teff dough. Samples were collected in duplicate every 24 hours over three consecutive fermentation days and were subsequently plated on suitable media. Approximately three representative colonies of LAB and yeast were randomly selected every 24 hours from higher dilution plates, totaling nine colonies for LAB and five for yeast. Further purification and sub-culturing were conducted using MRS (DeMan, Rogosa, and Sharpe) and PDA (Potato Dextrose Agar) agar for LAB and yeast, respectively. The isolates were subjected to various techniques, including assessments of cell and colony morphology, Gram staining (for LAB), and a range of physiological and biochemical tests.

Results: The results of this investigation revealed the presence of five distinct LAB species, specifically Lactobacillus delbrukii subsp. Delbrukii, L. acidophilus, L. amylolyticus, L. fermentum, and Pediococcus stilesii. Additionally, three yeast species were identified as Saccharomyces cerevisiae, S. kluyveri, and S. exiguus. These isolates were subsequently propagated and freeze-dried to create starter cultures. The combinations of LAB and yeast were employed to evaluate the fermentation process and sensory characteristics of injera. Selection of L. fermentum and L. delbrukii subsp. Delbruk was based on their acid production capabilities, while the yeasts S. cerevisiae and S. exiguous were chosen for their efficiency in CO2 production. The study yielded significant differences ($p<0.05$) in mean pH and total titratable acidity (TTA) across various combinations of LAB, yeast, and fermentation time. Mean pH ranged from 3.57 to 5.60 (with the control group at pH 3.47), while TTA varied from 2.13 to 11.33 ml 0.1 N NaOH/10 g, compared to the control at 12.53 ml 0.1 N NaOH/10 g. Notably, the combination of S. cerevisiae and L. fermentum, which achieved a pH of 3.57 and TTA of 11.33 at the 72-hour fermentation mark, emerged as the preferred starter culture, closely following the naturally fermented teff dough control group. Furthermore, significant differences ($p<0.05$) were observed in all sensory attributes among the treatment combinations. Taste ranged from 1.73 to 6.47 (control=6.73), flavor from 1.60 to 5.87 (control=6.80), appearance from 1.47 to 6.33 (control=6.87), texture from 1.53 to 6.13 (control=6.60), and overall acceptability from 1.53 to 6.20 (control=6.73).

Conclusions: In conclusion, the combination of S. cerevisiae and L. fermentum, which achieved the highest mean value on the hedonic scale, was selected for all sensory attributes at the 72-hour fermentation mark, closely trailing the control group. This combination also demonstrated acceptability at 24 and 48 hours of fermentation. These characteristics, including the ability to attain lower pH and higher TTA in a shorter time frame, offer promising prospects for the potential application of genetic engineering tools in the commercial-scale production of injera.

Notes:

P028 - Detection of *Salmonella* in turkeys by cloacal swab in comparison to intestinal tissue prevalence and colonization

Melissa S. Monson[1], Bradley L. Bearson[2], Samuel Whelan[1, 3], Selma Burciaga[1, 3], Shawn M.D. Bearson[1]

[1]USDA-ARS, National Animal Disease Center, [2]USDA-ARS, National Laboratory for Agriculture and the Environment, [3]Oak Ridge Institute for Science and Education, ARS Research Participation Program. melissa.monson@usda.gov

Session: Bacteriology, **2023-01-22, 6:00 - 8:00**

Objective: Human foodborne outbreaks of salmonellosis are often associated with *Salmonella enterica* contamination of poultry products. A non-lethal, on-farm turkey sampling method for detection of *Salmonella* in turkeys is desired by poultry producers for pre-harvest monitoring. Environmental sampling, such as litter grabs or boot socks, can be used for *Salmonella* detection at the flock level, but these methods do not assess *Salmonella* prevalence or load on an individual bird basis. Cloacal swabs could have utility as an antemortem sample that can be taken individually from group-housed turkeys; however, the efficacy of swabs as a predictive tool for *Salmonella* intestinal load needs to be further assessed in turkeys.

Methods: At six weeks of age, male turkey poults were inoculated by oral gavage with 1 x 10^9 CFU of either *Salmonella enterica* serovar Infantis (*S.* Infantis) or *Salmonella enterica* serovar Hadar (*S.* Hadar); these serovars have been involved in recent foodborne outbreaks in the U.S. Randomly selected turkeys from each group were necropsied at 7- or 14-days post-inoculation with *Salmonella*. From each turkey, a cloacal swab was collected for *Salmonella* prevalence determination and *Salmonella* colonization levels were quantitatively assessed in cecal contents, cecum, cecal tonsil, and cloaca. Statistical analyses were performed to establish whether concurrence of *Salmonella* prevalence between swab and tissue samples was significantly greater than chance (i.e., >50%) and whether there was a significant relationship between *Salmonella* positive swabs and load in intestinal tissues.

Results: While *Salmonella*-positive cloacal swabs were likely to co-occur with *Salmonella*-positive cecum, cecal tonsils, or cecal contents within a given turkey (p-value <0.001 for all three sample types), many of the turkeys with *Salmonella*-positive intestinal tissues tested negative for *Salmonella* by cloacal swab (only 29-35% concurrence). Analysis of samples collected in our research study suggested that cloacal swab testing was a poor indicator of *Salmonella* positivity in turkeys (p-value >0.969 in all four intestinal samples). The relationship between *Salmonella* load in the tissues and *Salmonella* prevalence by cloacal swabbing trended (p-value =0.057) towards higher colonization in turkeys with *Salmonella*-positive cloacal swabs.

Conclusions: Additional studies in both research and production settings are warranted to expand the number of matched cloacal swabs/tissue samples compared (including from turkeys naturally colonized with *Salmonella*) to validate the utilization of cloacal swabbing as a predictive tool for *Salmonella* intestinal load in turkeys as a risk indicator for product contamination.

Financial Support: Supported by USDA, ARS appropriated funds.

Notes:

P029 - Competitive exclusion of *Salmonella* colonization in chickens using a defined community of bacteria

Carmen L. Wickware[1], Dylan Harris[1], Briony Atkinson[2], Shawn Bearson[1], Melissa Monson[1], Adelumola Oladeinde[1], Christopher Anderson[1], Torey Looft[1]

[1]USDA-Agricultural Research Service, [2]Iowa State University. carmen.wickware@usda.gov

Session: Bacteriology, **2023-01-22, 6:00 - 8:00**

Objective: Non-antimicrobial intervention strategies to decrease *Salmonella* load in pre-harvest poultry production are important to improve food safety and protect public health. Previous use of cecal contents from mature chickens has shown protection against *Salmonella* through competitive exclusion, an antagonistic relationship between two or more species. However, the composition of cecal contents is undefined and direct introduction to chicks could lead to variable results and safety concerns. The objective of this study was to determine if *Salmonella* colonization of chickens could be reduced through competitive exclusion using a defined community of chicken commensal bacteria.

Methods: One-day old White Leghorn chicks, hatched on-site, were randomly divided into experimental groups and given an oral gavage of either a defined community of 15 bacterial species (DC), cecal contents (CC), or sterile PBS (control; CT). After one week, birds were euthanized for cecal content collection (pre-*Salmonella* sample) while the remaining birds were orally gavaged 1 X 10^8 colony forming units (CFU) of *Salmonella enterica* ser. Heidelberg strain 2813 (SH2813). Bacterial counts for three post-*Salmonella* timepoints (3, 14, and 28 days post inoculation; dpi) were evaluated. Bacteriological enumeration was performed by plating cecal contents onto *Salmonella* selective agar to determine CFU/g in each group for all collection days. Cecal contents were also used for bacterial community analysis. Briefly, DNA was extracted from cecal contents and the V4 region of the 16S rRNA gene was amplified and sequenced. Sequences were used to examine the bacterial diversity as well as differences in abundance for each group. Significance was tested using Kruskal-Wallis for bacterial counts and alpha diversity, and perMANOVA for beta diversity. Multiple-test corrected p-values (adj.p) were used for all statistical tests.

Results: A 2 $\log_{10}$ reduction in SH2813 was observed in DC compared to CT at 28 dpi (adj.p < 0.05). SH2813 counts from the CC group were below 10 CFU/g for all timepoints. The bacterial community of CC showed significant differences in alpha and beta diversity compared to other groups for all timepoints (adj.p < 0.05). The bacterial community in DC birds had lower diversity and abundance compared to CT birds 14 dpi but diversity in DC was closer to CT 28 dpi. In examining the relative abundances of the top genus-level operational taxonomic units (OTU; 97% sequence similarity) shared across the three groups, CC had high relative abundances of several OTUs for all sample times (> 10% each OTU). In comparison, during the first two sample points DC samples had high relative abundances of OTUs belonging to the same genera as members contained within the initial treatment gavage, though there were fewer overall OTUs.

Conclusions: Though birds receiving cecal contents had improved protection against SH2813 colonization, DC showed a reduction in SH2813 when compared to the control. The reduction shows the potential of defined microbial communities to reduce *Salmonella* colonization. Moving forward, studies to establish the optimal community should include load of DC given, timing of *Salmonella* exposure, and potential cross-protection.

Financial Support: Oak Ridge Institute for Science and Education (ORISE) - Postdoctoral Fellowship

Notes:

P030 - Prevalence of brucellosis in milk from livestock in Nyagatare district, Rwanda

Emmanuel Kabalisa[1], Agrippine Mukarurangwa[1], Immaculée Nyampinga[1], Claude Muvunyi Mambo[2], Jean Bosco Ntivuguruzwa[3], Maurice Byukusenge[4], Martin Ntawubizi[3], Nadine Rujeni[3], Joram Buza[5], Robab Katani[4]

[1]National Reference Laboratory, Rwanda, [2]Rwanda Biomedical Centre, [3]University of Rwanda, [4]The Huck Institute of Life Sciences, Pennsylvania State University, [5]Nelson Mandela African Institute for Science and Technology, Tanzania. emmanuel.kabalisa@rbc.gov.rw

Session: Bacteriology, **2023-01-22, 6:00 - 8:00**

Objective: *Brucella* is a zoonotic pathogen that could infect animals, particularly livestock such as cattle, sheep, and goats, and poses a significant public health concern. Livestock can serve as a reservoir for *Brucella*, and humans can become infected through direct contact with infected animals or their products, such as unpasteurized milk or undercooked meat. Brucellosis in humans can lead to a range of symptoms, including fever, joint pain, fatigue, and gastrointestinal issues, and in severe cases, it can become a chronic and debilitating illness.

Methods: Brucellosis is endemic in numerous low- and middle-income countries, including Rwanda. One of the predominant modes of transmission in humans is via the ingestion of unpasteurized milk. *Brucella* species can localize in the mammary lymph nodes and glands of infected dairy animals that may shed the pathogen in milk for extended periods of time and present a significant health risk to consumers of unpasteurized dairy products and individuals in direct contact with infected animals. Since the sale and consumption of raw and unprocessed milk is common amongst pastoral and agropastoral communities in Rwanda, large proportions of the population are susceptible to milk-borne and zoonotic diseases, including brucellosis. Currently, the prevalence of brucellosis in the Nyagatare district of Rwanda, where majority of the population are pastoralists or agropastoralists, and its implications for public health are unknown. The primary objective of this study was to ascertain the prevalence of *Brucella* among livestock during the lactation period in the Nyagatare district. Additionally, our research endeavors to disseminate critical findings to both public health and livestock authorities, shedding light on the implications of *Brucella* in the livestock population.

Results: To achieve our research objective, we aseptically collected 418 milk samples from livestock between March - July 2023. Of these, 332 (79.4%) were sourced from cattle, 82 (19.6%) originated from sheep, and the remaining four samples (1%) were collected from sheep. DNA extraction was performed on all the milk samples using a magnetic-based extraction method, followed by Real-time PCR (qPCR) to detect the IS*711* insertion sequence. Our preliminary data analysis indicated that the overall *Brucella* prevalence in milk samples was 20.6%. A differential diagnosis was conducted on the positive milk samples. Among these cases, 10.5% were attributed to *B. abortus*, 1.2% to *B. melitensis*, while 88.3% of the positive samples were un-speciated.

Conclusions: The observed prevalence of *Brucella* in livestock within the Nyagatare district is notably high, reaching 20.6%. This underscores the urgent need for public health and livestock authorities to implement essential control measures. These measures are essential to mitigate the risk of *Brucella* transmission from milk to humans and enhance livestock animals' welfare and health.

Financial Support: The Huck Institute of Life Sciences, Penn State, University Park, Pennsylvania, USA

Notes:

P031 - Molecular epidemiology of *Mycoplasma capricolum* subsp. *capripneumoniae*; causative agent of contagious caprine pleuropneumonia in Pakistan

Faisal Ahmad[1], Farhan Anwar Khan[2], Muhammad Saeed[2], Hayatullah Khan[3]

[1]Disease Investigation Laboratory, Directorate of Livestock and Dairy Development (Extension), Pakistan, [2]College of Veterinary Sciences, The University of Agriculture Peshawar, [3]Veterinary Research Institute, Peshawar. drfaisal.vet31@gmail.com

Session: Bacteriology, **2023-01-22, 6:00 - 8:00**

Objective: Contagious caprine pleuropneumonia (CCPP) is a classical transboundary and dreadful disease of small ruminants enlisted by World Organization for Animal Health (WOAH) as a notifiable disease. CCPP is caused by *Mycoplasma capricolum* subsp. *capripneumoniae* (Mccp). The present study was designed for the molecular epidemiology of CCPP caused by Mccp strain endemic in Pakistan. For this purpose, the study area was divided in four different zones, namely Northern zone, Central zone, Southern zone, and Tribal zones according to their geographical and climatic condition.

Methods: After successful isolation, nine Mccp isolates, three from the northern zone and two each from the tribal zone, central zone, and southern zone respectively were sequenced by next-generation sequencing technology (NGS). Taxonomic recognition of the isolates was confirmed as Mccp genome on Kraken2 method. Phylogenetic tree deduced on H2 locus sequences of the nine isolates of the present study with available H2 locus sequences at NCBI. The improved molecular epidemiological tool for CCPP, Multi Locus Sequencing Analysis (MLSA), was applied to all samples.

Results: The H2 analysis revealed all current isolates fall at a distant place at the base of the clade, however the isolate no. A4 from the northern zone showed close similarity to a strain of Mccp isolated in Tajikistan and China. The MLSA results indicate a significant difference, and all isolates form a separate position in the phylogenetic tree compared to the available sequences at NCBI.

Conclusions: The genotyping of Mccp isolates recovered in the studied region indicates that the disease has been present in the vicinity long way back. Furthermore, we suggest MLSA for molecular epidemiology of CCPP as the H2 locus represents a small genome fragment size and shows some flawless connection among the Mccp isolates and their geographical location.

Financial Support: This project was financially supported by the Pak-US Science and Technology Cooperation Program, Phase 7, 2017 under the Higher Education Commission (HEC) of Pakistan, and joint Research work with Sandia National Laboratories, New Mexico, USA.

Notes

P032 - Goblet cells in swine enteroids are not infected by *Lawsonia intracellularis* during the first 24 hours of exposure

Yvonne Peng[1], Caitlin Klaeui[1], Milena Saqui-Salces[1], Connie J. Gebhart[1]

[1]University of Minnesota. peng0437@umn.edu

Session: Bacteriology, **2023-01-22, 6:00 - 8:00**

Objective: *Lawsonia intracellularis* is the causative agent of proliferative enteropathy (PE) in weaned pigs which is characterized by thickening of the ileum mucosa leading to pig poor performance. Reduced goblet cell numbers and mucin 2 (*MUC2*) expression have been reported in intestine samples of pig with PE. To define whether loss of goblet cells is due to direct infection by *L. intracellularis*, we evaluated the co-localization of *L. intracellularis* and goblet cell marker Muc 2 within 24 hours of exposing enteroids from porcine ileum to the bacterium.

Methods: Enteroids were cultured as tridimensional structures suspended in commercial organoid (Intesticult) media for 48 hours to allow for the cell apexes to face the media (inversion) before exposure to *L. intracellularis*. Bacteria inoculum was prepared by collecting *L. intracellularis* from infected McCoy cells, and suspending them in IntestiCult (STEMCELL Technologies) media. Enteroids were fixed in formalin and suspended in Histogel (Fisher Scientific) at 1, 2, 3, 8, 16, and 24 hours post-exposure (n=3 wells at each time) along with negative (non-infected) controls at 1 and 24 hours. Enteroids in Histogel were processed and embedded in paraffin for histology. Five µm thick sections were mounted on slides and deparaffinized and rehydrated in a series of xylene and ethanol baths. Heat-induced antigen retrieval was performed in 10 mM sodium citrate (pH = 6) and permeabilization in PBS containing 0.3%Triton before dual immunofluorescence staining. Nonspecific binding was blocked with 10% donkey serum in PBS. Slides were incubated with anti-*L. intracellularis* antibody (1:10,000, rabbit polyclonal developed by Guedes and Gebhart) for 2 hours at room temperature. Subsequently, slides were incubated with TRITC-conjugated Alexa 594 donkey anti-rabbit antibody (1:500, Biolegend 406418) in the dark for 30 minutes. Slides were then blocked with 10% goat serum in PBS before incubation with anti-Muc 2 (1:800, rabbit, Ab134119) antibody overnight at 4 °C followed by FITC-conjugated goat anti-rabbit antibody (1:500, AB7086). After washing, slides were mounted with ProLong™ Gold Antifade Mountant with 4′,6-diamidino-2-phenylindole (DAPI, Invitrogen) to visualize nuclei. Fluorescent images were taken at 40x and 60x magnification and merged in Image J. Two slides per replicate with a minimum of 5 intact enteroids were chosen and examined for all collection time points.

Results: Goblet cells identified by Muc2 staining were present in all the sections analyzed. *L. intracellularis* was identified only in inoculated samples, as expected. Although both signals were present in the infected samples, there was no co-localization in any of the slides evaluated.

Conclusions: Our results suggest that *L. intracellularis* does not infect goblet cells as initial target, suggesting an indirect mechanism for reduction of goblet cells during *L. intracellularis* infection.

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

Notes:

P033 - Intramammary infections in primigravid heifer mammary glands during progressing stages of gestation

B. D. Enger[1], M. X. S. Oliveira[1], P. H. Baker[1], C. S. Gammariello[1], K. M. Enger[1], M. A. McGuire[2], J. Williams[2]

[1]Dept. of Animal Sciences, The Ohio State University, [2]Dept. of Animal, Veterinary and Food Sciences, University of Idaho. enger.5@osu.edu

Session: Bacteriology, **2023-01-22, 6:00 - 8:00**

Objective: Intramammary infections are common in primigravid dairy heifers and occur during periods of marked mammary gland growth and development. Such infections are expected to impair mammary gland morphogenesis and microstructure, thereby reducing future milk yields. The objective of this study was to assess how *Staphylococcus aureus* infections affect mammary glands during progressive stages of gestation using histological analysis.

Methods: A total of 21 pregnant Holstein dairy heifers divided across gestational ages (5.75, 6.75, and 7.75 months pregnant) were used. A randomly selected mammary gland of each heifer was infused with *Staphylococcus aureus* (STAPH) and another mammary gland was infused with saline (SAL), serving as an uninfected control. Mammary secretions were collected on day 0, 1, 2, 8, 14, and 20 d relative to mammary infusions. Mammary tissues were collected 21 d later from mammary parenchyma that was in the center of the mammary gland and distal regions, near the abdominal wall. Mammary secretions were evaluated for bacteriology, somatic cell count, and somatic cell differentials. Tissues were fixed, embedded, and subject to microscopic analysis.

Results: Intramammary infections were maintained in 20 STAPH mammary glands throughout the study and all 21 SAL mammary glands remained uninfected. Somatic cell counts of STAPH mammary secretion were consistently greater than for SAL mammary glands, with neutrophils being the most abundant cell type. Tissue morphometric analyses are in progress.

Conclusions: These in progress results indicate that *Staphylococcus aureus* infections can be maintained during first pregnancy, and they elicit sustained increases in somatic cell counts. Complete evaluation of the mammary tissue microstructure will delineate how mammary gland epithelial growth (i.e., cellular proliferation) and mammary gland morphogenesis (i.e., regression of stromal tissue areas and increases in luminal space) are affected by *Staphylococcus aureus* infection during first pregnancy. The results will indicate when and how mammary tissue architecture is altered by intramammary infection during first pregnancy.

Financial Support: This work was supported by a competitive USDA NIFA grant (no. 2020-67015-31677) awarded to B. D. Enger and M. A. McGuire.

Notes:

P034 - Microbiota-metabolizing chenodeoxycholic acid inhibits *Clostridium perfringens* growth and virulence

Tahrir Alenezi[1], Ying Fu[1], Bilal Alrubaye[1], Thamer Alanazi[1], Hong Wang[1], Xiaolun Sun[1]

[1]University of Arkansas. xiaoluns@uark.edu

Session: Bacteriology, **2023-01-22, 6:00 - 8:00**

Objective: *Clostridium perfringens*-induced chicken necrotic enteritis (NE) has reemerged as a prevalent chicken disease due to restricting antibiotic growth promoters. Microbiota-derived secondary bile acid deoxycholic acid prevents NE.We aimed to examine the hypothesis that microbiota-metabolizing chenodeoxycholic acid (CDCA) reduced *C. perfringens* growth and virulence.

Methods: To examine this hypothesis, cholic acid (CA) or CDCA was cultured for 72 hr with bile acid metabolizing bacteria *Parabacteroides merdae*, *Eggerthella lenta*, *Clostridium sardiniense* or *Clostridium paraputrificum*. After autoclave, *C. perfringens* was inoculated into the media and cultured for 24 hr. The growth of *C. perfringens* was enumerated with serial dilution and plating. *C. perfringens* was also cultured with CDCA microbiota-derivative lithocholic acid (LCA) and isoalloLCA for 24 hr. Virulence gene expression was quantified.

Results: *C. perfringens* growth reached 7.38 log10 CFU after 24 h culture. Consistent with previous reports, CDCA at 1.5 mM mildly reduced *C. perfringens* growth. Similarly, media pre-grown with *P. merdae*, *E. lenta*, or *C. sardiniense* or *C. paraputrificum* mildly reduced *C. perfringens* growth. Notably, 1.5 mM CDCA cultured with *P. merdae* or *E. lenta* reduced *C. perfringens* growth. Notably, isoalloLCA at 0.001 and 0.01 mM completely inhibited *C. perfringens* growth, while LCA at 1 mM reduced the growth by 1.28 log10 CFU/ml. The gene expression of *asrA1* was reduced by 100% with 0.01 mM isoalloLCA, while CDCA or LCA reduced *asrA1* by less than 30%. Master regulatory gene *spoOA* was reduced by 1 mM LCA (73%) and 0.01 mM isoalloLCA (73%), while 1 mM CDCA increased the gene by 0.68-fold.

Conclusions: Together, these results suggest that CDCA metabolized by microbiota could reduce *C. perfringens* virulence. The findings could be used for designing new interventions against chicken NE.

Financial Support: Arkansas Biosciences Institute, NIFA Hatch/Multi State project 1018699, NIFA SAS 2019-69012-29905, and NIFA project 2020-67016-31346

Notes:

P035 - Assay demonstrates the efficacy of direct-fed microbials against potentially harmful bacteria

Jennifer Schutz[1], Octavio Guimaraes[1], Bruno Cappellozza[1], Lena Capern[1], Giuseppe Copani[1]

[1]Chr Hansen. USJESC@chr-hansen.com

Session: Bacteriology, **2023-01-22, 6:00 - 8:00**

Objective: To evaluate the inhibitory efficacy of specific strains of direct-fed microbials (DFM) against potentially harmful bacteria in a qualitative agar-diffusion assay.

Methods: The agar diffusion assay was performed by evaluating *Fusobacterium necrophorum* (CHCC #28517), *Trueperella pyogenes* (CHCC #48578), and *Mannheimia haemolytica* (CHCC #48581) following the methodology described by Santano et al. (2020) with adjustments depending on the pathogen being evaluated. Briefly, pathogenic bacteria, described above, were inoculated on trypticase soy agar with or without sheep blood medium and incubated, separately, at 37°C for up to 24-h. On the day of DFMs preparation, independent cultures in brain heart infusion broth of *Bacillus licheniformis 509*, *Lactobacillus animalis* 506, and *Propionibacterium freudenreichii* 507, or the combination of the three with *B. subtilis* 597 were prepared and incubated for up to 48-h between 30-37°C, depending on the bacterial DFM strain. On the day of assay setup, pathogenic bacterial cultures were suspended by using a cotton swap with a 0,5 McFarland suspension in a maximum recovery diluent medium. Following this step, a 35-mL melted Wilkins Chalgren (Oxoid; for *F. necrophorum and T. pyogenes*) or LB agar (for *M. haemolytica*) agar and 10 µL of pathogen suspension were mixed in a 50-mL Falcon tube and the mixture was cast in Omnitray plates with an immediate application of the Nunc™Immuno TSP (Thermo Fisher Scientific, Waltham, MA). Then, the plates were left to solidify for 20 min before lid removal. Thereafter, plates were allowed to dry with lid on for additional 20 min. Lastly, 5-10 µL of the DFM strains, alone or in combination, following overnight culture were applied to the selected wells in the agar. Samples were analyzed in triplicates. Depending on the strain and the pathogen, anaerobic (*F. necrophorum* and *T. pyogenes*) or aerobic (*M. haemolytica*) incubation lasted up to 72 h and after that the zone of inhibition was measured (in mm) via photoshop from full growth to full growth, with a lower limit of 3.5 mm (width of each well in the plate).

Results: Combining the selected DFM strains yielded a numerically greater zone of inhibition than adding the *B. licheniformis 509* by itself in *T. pyogenes* (16.0 vs. 8.5 mm). On the other hand, the same inhibition was observed for *M. haemolytica* when B. licheniformis 509 was added by itself or combined with the other bacterial strains (10.1 vs. 10.4 mm). Lastly, adding *L. animalis* 506 by itself, with *P. freudenreichii* 507, or with all the other bacterial DFM yielded the same inhibition efficacy against *F. necrophorum* (16.0, 15.3, and 16.0 mm, respectively).

Conclusions: Our results demonstrate that adding the selected strains of bacterial DFM with different known modes of action provided satisfactory zones of inhibition against potentially harmful bacteria when compared with single selected DFM strains in a qualitative agar-diffusion assay.

Financial Support: Research was sponsored by Chr-Hansen.

Notes:

P036 - Evidence of a new protein-coding gene in bovine herpesvirus 1.

Victoria A. Jefferson[1], Hannah Bostick[1], Darby Oldenburg[2], Florencia Meyer[1]

[1]Mississippi State University, [2]Gundersen Medical Foundation. florencia.meyer@msstate.edu

Session: Virology, **2023-01-22, 6:00 - 8:00**

Objective: Bovine Herpesvirus type 1 (BoHV-1) is a respiratory and genital pathogen in cattle. Its genome was originally sequenced using a variety of strains and subtypes. The Cooper strain was fully sequenced in 2012, retaining the original gene annotation. However, the viral coding potential appears to be much larger than originally established. In this work we characterize a previously unrecognized protein-coding gene encoded antisense to a major viral gene.

Methods: A previously generated mass spectrometry dataset obtained from BoHV-1 infected cells was used to map MS/MS peptides to specific viral genomic locations in-silico. Characterization of the transcript generated from a particular genomic. Rapid amplification of cDNA ends (RACE) and primer walking was used to elucidate the boundaries of the transcript. Expression kinetics of the transcript and protein were carried out via strand-specific RT-PCR and western blot, respectively. Western blot and immunofluorescence was accomplished using a custom-generated antibody raised against the original MS/MS peptide.

Results: Transcript mapping confirmed there is active transcription occurring antisense to the essential UL5 gene from genomic position 95279 to 94212. We further detected a protein of about 30 KDa in protein blots from infected bovine kidney and turbinate cells, starting at at 8 hours post infection. However, the transcript can be detected from earlier time points by quantitative RT-PCR. Immunofluorescence imaging detected a protein in infected cells with no clear localization. Sequence analysis reveals 61% sequence identity with closely related alphaherpesviruses and betaherpesviruses in a similar location in their genomes.

Conclusions: We provide evidence that BoHV-1 encodes a protein-producing gene across from a major viral gene, UL5. The newly described gene is named ORF M. The protein's function is the target of ongoing studies. Our work adds to the ever-expanding genetic potential within these relatively large DNA viral genomes.

Financial Support: HB was funded in part by the CALS/MAFES Undergraduate Research Scholars Program.

Notes:

P037 - Effects of temperature and relative humidity on the survival of ASFV dried on porous and non-porous surfaces

Janine Simmons[1], Jessica Mason[1], Lauren Martignette[1], Mariceny Zurita[1], Jose Barrera[1], John Neilan[2], Lindsay Gabbert[2]

[1]SAIC, Plum Island Animal Disease Center. [2]U. S. Department of Homeland Security Science and Technology Directorate, Plum Island Animal Disease Center. Janine.Simmons@ST.DHS.GOV

Session: Virology, **2023-01-22, 6:00 - 8:00**

Objective: African swine fever virus (ASFV) is a highly stable DNA virus and causative agent of African swine fever (ASF), a lethal hemorrhagic disease that inflicts up to 100% mortality in domestic pigs. While ASF transmission may occur by direct contact between infected swine, new outbreaks often result from ASFV-contaminated materials and fomites to ASF-free zones. Stability of ASFV in cool conditions and freezing environments, especially in the presence of organic materials, has been previously reported. However, environmental survival of ASFV dried on surfaces at ambient and elevated temperatures has not been well documented. To address this gap, we sought to determine the effects of temperature and relative humidity (RH) on the viability of ASFV when dried in two soil loads on porous and non-porous surfaces.

Methods: Viral inocula were prepared by combining high titer stocks of the avirulent Vero cell-adapted ASFV strain BA71V with either porcine whole blood (WB) or a 3-part standardized soil load (SL) consisting of bovine serum albumin, yeast extract, and bovine mucin, used to simulate the presence of bodily fluids and secretions. Sterile stainless steel and carbonated concrete testing coupons (1 cm^3) were inoculated with ~5-6.0 log_{10} $TCID_{50}$ of ASFV and allowed to dry in a BINDER environmental chamber (Model KMF240) under the following temperature and humidity combinations: 25°C, 35°C, 55°C, and 95°C with 20% and 70% RH. Coupons were collected in triplicate at timepoints spanning minutes to days depending on the treatment. ASF viral titers were determined by endpoint determination assay on Vero cells, and the $TCID_{50}$ calculated using the Reed-Muench method. When cytopathic effects were no longer observed, sample supernatants were passaged three times in Vero cells to confirm sample negativity and determine the endpoint for inactivation of ASFV at each temperature and RH combination.

Results: For all experiments, temperature proved to be the most important variable impacting ASFV survival and infectivity, with survival up to 21 days (SL) and 10 days (WB) at 25°C (20% RH), dropping to a maximum of 3 days at 35°C, 5 hours at 55°C, and <15 minutes at 95°C. Surface type did not appear to impact recovery or survival of ASFV, as infectivity titers and inactivation endpoints were similar on both stainless steel and carbonated concrete regardless of the soil load type. ASFV was inactivated more quickly at 70% RH compared with 20% RH. This was especially apparent at ambient temperature (25°C) for ASFV dried in both the SL and WB, with endpoint determinations reduced from 21 days to 72 hours in the SL, and 10 days to 3 days in WB at high (70%) RH.

Conclusions: Elevated temperatures rapidly inactivate ASFV regardless of RH, surface type, or soil loading. Our data suggest that extra precautions should be taken when handling and/or transporting potentially contaminated materials at or below 25°C, especially in conditions of low humidity, as materials may remain infectious with ASFV for many days.

Notes:

P038 - Characterization of the role of EHV viral genes for neuropathogenesis and immune regulation in horses

Gisela Soboll Hussey[1], Glorian Berríos-Vázquez [1], Amie Wilson[2], Melissa Esser[1], Lutz Goehring[3], Nikolaus Osterrieder[4], Swati Sharma[1], Trevor Gilbert[1]

[1]Department of Pathobiology and Diagnostic Investigation, Michigan State University, [2]School of Veterinary Sciences, University of Liverpool, [3]Gluck Equine Research Center, University of Kentucky, [4]Freihe Universitaet of Berlin, Germany. husseygi@msu.edu

Session: Virology, **2023-01-22, 6:00 - 8:00**

Objective: Equine herpesvirus 1 (EHV-1) affects horses worldwide and causes respiratory disease, abortions, and equine herpesvirus myeloencephalopathy (EHM). Following initial infection of the nasal epithelium, a cell-associated viremia is established, which is central in the pathogenesis of EHM and abortions. We have previously identified the glycoprotein B (gB) and the US3 genes as important viral genes for transfer of the virus to the CNS endothelium and modulation of immunity *in vitro*. Based on this data, our hypothesis is that replacing EHV-1 gB with EHV-4 gB (Ab4gB4) or deletion of EHV-1 US3 (Ab4DUS3) will alter EHV-1 clinical disease and modulate immune responses compared to infection of horses with wild type EHV-1 (Ab4).

Methods: Three groups of horses were established (n=8) and infected with $5*10^7$ PFU of Ab4, Ab4gB4, or Ab4DUS3. Physical exams and swabs for detection of nasal viral shedding were taken prior to infection, daily on days 1-14 post-infection (p.i.), and every other day until day 21 p.i. Blood for detection of viremia was taken prior to infection and on days 1 to 10 p.i.. In addition, nasal secretions and Paxgene tubes were collected for determination of cytokine/chemokine responses prior to infection and daily until day 7 p.i.. Lastly, CSF was collected by ultrasound guided cervical centhesis prior to infection and on day 11 p.i. for determination of cytokine responses and proteomic/metabolomic analysis.

Results: Compared with wild-type EHV-1 Ab4 infection, infection with the Ab4gB4 or Ab4DUS3 virus resulted in significant attenuation of respiratory disease. In addition, horses in both EHV-1 mutant infected groups did not exhibit secondary fever responses, which typically correlate with the onset of viremia. None of the horses in the mutant infection groups exhibited neurological disease. Analysis of the remaining samples is in process and results will be discussed.

Conclusions: The design of an EHV-1 mutant with multiple deletion/exchanges of viral genes could be used as a vaccine that is safe for mucosal vaccination, can prevent viremia and infection of the CNS, and induce strong mucosal and systemic immune responses. In addition, discoveries made on the function of specific genes could ultimately be used in the rational design of novel mRNA or other subunit vaccines.

Financial Support: This project was supported by Agriculture and Food Research Initiative Competitive Grant no. 2018-67015-28242 from the USDA National Institute of Food and Agriculture.

Notes:

P039 - SARS-CoV-2 pseudovirus infects equine bronchial epithelial cells in vitro

R.M. Legere[1], A.R. Allegro[1], Y. Affram[2], B.P. da Silveira[1], J.L. Fridley[1], K.M. Wells[2], N. Oezguen[3], R.C. Burghardt[4], G.A. Wright[5], J. Pollet[6, 7], A.I. Bordin[1], P. de Figueiredo[2], J.L. Leibowitz[2], N.D. Cohen[1]

[1]Department of Large Animal Clinical Sciences, Texas A&M University, [2]Department of Microbial Pathogenesis & Immunology, Texas A&M University, [3]Texas Children's Microbiome Center, Baylor College of Medicine, [4]Department of Veterinary Integrative Biosciences, Texas A&M University, [5]Department of Veterinary Pathobiology, Texas A&M University, [6]National School of Tropical Medicine, Baylor College of Medicine, [7]Texas Children's Hospital Center for Vaccine Development, Baylor College of Medicine rlegere@cvm.tamu.edu

Session: Virology, **2023-01-22, 6:00 - 8:00**

Objective: To compare the susceptibility to a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pseudovirus of cultured primary equine bronchial epithelial cells (EBECs) with human bronchial epithelial cells (HBECs).

Methods: Primary EBEC cultures were established from healthy adult horses and commercially-sourced human bronchial epithelial cells (HBECs) were used as a positive control. Angiotensin-converting enzyme 2 (ACE2) expression by EBECs was demonstrated using immunofluorescence, western immunoblot, and flow cytometry. EBECs were transduced with a lentivirus pseudotyped with the SARS-CoV-2 spike protein that binds to ACE2 and expresses the enhanced green fluorescent protein (eGFP) as a reporter. Cells were transduced with the pseudovirus at a multiplicity of infection of 0.1 for 6 hours, washed, and maintained in media for 96 hours. After 96 hours, eGFP expression in EBECs was assessed by fluorescence microscopy of cell cultures and quantitative PCR.

Results: ACE2 expression in EBECs detected by immunofluorescence, western immunoblotting, and flow cytometry was lower in EBECs than in HBECs. After 96 hours, eGFP expression in EBECs was demonstrated by fluorescence microscopy. Mean ΔCt values from quantitative PCR were significantly ($P < 0.0001$) higher in EBECs (8.78) than HBECs (3.24) indicating lower infectivity in EBECs.

Conclusions: Equine respiratory tract cells were susceptible to cell entry with a SARS-CoV-2 pseudovirus. Lower replication efficiency in EBECs suggests that horses are unlikely to be an important zoonotic host of SARS-CoV-2, but viral mutations could render some strains more infective to horses. Serological and virological monitoring of horses in contact with persons shedding SARS-CoV-2 is warranted.

Financial Support: The project was funded by the Link Equine Research Endowment, Texas A&M University, the Department of Large Animal Clinical Sciences, School of Veterinary Medicine & Biomedical Sciences, Texas A&M University, and the Judy Calder Foundation.

Notes:

P040 - The effects of RHDV2 3C-like protease in inflammation and cell death

Luija Wijesekara[1], Kyeong-Ok Chang[1], Yunjeong Kim[1]

[1]Department of Diagnostic Medicine and Pathobiology, Kansas State University. luija@ksu.edu

Session: Virology, **2023-01-22, 6:00 - 8:00**

Objective: Rabbit hemorrhagic disease Virus 2 (RHDV2) is a highly contagious and deadly legovirus of the family *caliciviridae*, that affects various lagomorphs, including European rabbits (*Oryctolagus cuniculus*), cottontail rabbits (*Sylvilagus spp*.) and hare (*Lepus)* species. In RHDV2 infection, excessive inflammation induces a procoagulant state, contributing to disseminated intravascular coagulation, characterized by widespread blood clot formation and severe bleeding tendencies. The combination of viral replication, inflammation, and immune responses can cause extensive hepatocellular damage, leading to acute hepatocellular necrosis, which is a defining pathological feature of RHDV2 infection. Therefore, understanding the role of cell death and inflammation in RHDV2 pathology is vital for our understanding of disease progression. RHDV2 3C-like protease (3CLpro) processes most cleavage sites on the expressed polyproteins to produce functional non-structural proteins and is crucial in viral replication. In this study, we investigated the cytotoxic effects of RHDV2 3CLpro, inflammation and mechanism of cell death and the roles of catalytic domain of 3CLpro in mediating cell death and inflammation.

Methods: The full-length codon-optimized RHDV2 3CLpro sequence was cloned into the pLVX-EF1α-IRES-Puro plasmid to generate a functional 3CLpro (pLVX-3CLpro). An inactive RHDV2 3CLpro was also generated by substituting cysteine with alanine in the catalytic site of 3CLpro using site-directed mutagenesis. Empty pLVX-EF1α-IRES-Puro plasmid was used as a control in all experiments. The effect of RHDV2 3CLpro on cell viability and cell death was determined by transfecting HEK293T cells with plasmids encoding active or inactive RHDV2 3CLpro using RealTime-Glo™ MT cell viability assay and RealTime-Glo™ Annexin V necrosis assay, respectively. Caspase 3/7 activity was determined by dual-luciferase assay following co-transfecting HEK293T cells with plasmids encoding active and inactive RHDV2 and pGloSensor™ 30F plasmid containing the caspase 3/7 cleavage site (DEVDG). Expression of cytokine genes including pIFNβ-Luc, pGAS-TA-Luc, pISRE-Luc-TA, pNFkβ-Luc, pSTAT3-TA-Luc, and pAP1-Luc, as well as pRL-CMV (transfection control), were determined by dual-luciferase assay after co-transfecting HEK293T cells with plasmids encoding active or inactive RHDV2.

Results: The viability test results showed a significant reduction in live cells in HEK293T cells expressing active RHDV2 3CLpro in comparison to those expressing the inactive RHDV2 3CLpro and empty vector, while there is no significant difference in live cells between inactive RHDV2 3CLpro and empty vector. Real time necrosis assay results showed an increased fluorescence indicative of dead cells in the presence of active RHDV2 3CLpro compared to inactive RHDV2 3CLpro and empty vector. Moreover, RHDV2 3CLpro-expressing cells demonstrated an upregulation in caspase 3/7 activity compared to inactive RHDV2 3CLpro and empty vector along with a significant increase in the expression of pGAS-TA-Luc and pIFNβ-Luc cytokine genes compared to inactive RHDV2 3CLpro and empty vector.

Conclusions: Active RHDV2 3CLpro induces cell death through its catalytic activity and this process is associated with the activation of caspase 3/7. Elevated expression of pGAS-TA-Luc and pIFNβ-Luc cytokine genes suggest that active RHDV2 3CLpro induces inflammatory responses associated with interferon signalling.

Financial Support: USDA-NIFA AFRI 2019-67015-29864

Notes:

P041 - Development of a multispecies double-antigen ELISA using RBD and N proteins to detect antibodies against SARS-CoV-2

Maritza Alejandra Cordero Ortiz[1], Mónica Reséndiz-Sandoval[1], Freddy Dehesa-Canseco[2], Mario Solís-Hernández[2], Jahir Pérez-Sánchez[3], Carlos Martínez-Borges[4], Verónica Mata-Haro[5], Jesús Hernández[1]

[1]Laboratorio de Inmunología, Centro de Investigación en Alimentación y Desarrollo, A.C. Hermosillo, [2]Comisión México-Estados Unidos para la Prevención de la Fiebre Aftosa y otras Enfermedades Exóticas de los Animales (CPA), SENASICA, SADER, [3]Centro de Biotecnología Genómica, Instituto Politécnico Nacional, Ciudad Reynosa, [4]Hospital Veterinario Borges, [5]Laboratorio de Microbiología e Inmunología, Centro de Investigación en Alimentación y Desarrollo, A.C. maritzacoordero@gmail.com

Session: Virology, **2023-01-22, 6:00 - 8:00**

Objective: SARS-CoV-2 infects humans and a broad spectrum of animal species, including pets (cats, dogs, and guinea pigs), zoo animals (tigers and lions), and nondomestic animals (deer, fox, and rats). Constant monitoring of the infection in animals is important for the risk of interspecies transmission and the emergence of new viral variants. Economical, fast, efficient, and sensitive diagnostic tests are required to analyze animal infection. Double-antigen sandwich ELISA adds the advantage of being multispecies, ideal in zoonotic infections. In addition to being a multispecies test, we intended to improve the sensitivity and overall performance of the test by using two proteins of the virus in a single test. Based on this, the study aimed to develop a double-antigen sandwich ELISA using two SARS-CoV-2 proteins, N and RBD.

Methods: We compared the performance of double-antigen sandwich ELISA with RBD and N proteins with double-antigen sandwich ELISA using only RBD or N protein, with indirect ELISA using only RBD, N, or S1 protein, as well as with a surrogate virus neutralization test (sVNT). Positive and negative controls from a cat population (n=31) were evaluated with all the tests and used to compare their performance according to the diagnostic sensitivity and specificity, and area under the curve (AUC) values obtained from ROC curves. These parameters were re-evaluated using positive and negative samples from humans (n=32) and guinea pigs (n=3) to adjust the cutoff for more species in the double-antigen sandwich ELISA with RBD and N proteins. Sera from 2 tigers, 51 rats, and 45 dogs were also evaluated. Additionally, intralaboratory repeatability was evaluated by eleven runs of the test on different days, and interlaboratory reproducibility was determined by correlating the results of two different laboratories.

Results: The highest AUC (88%) was obtained with the double-antigen sandwich ELISA with RBD and N proteins in comparison to using only RBD (86.4%) or N (67.4%) proteins separately. This AUC was also higher than the ones from indirect ELISA with S1 (75.4%), RBD (85.7%), or N (53.5%) proteins, and higher than the sVNT AUC (82.3%). The cut-off was adjusted to 0.3615 by using humans' and guinea pigs' positive and negative controls, and diagnostic sensitivity improved from 80% to 83.87%, and AUC improved from 88% to 92.5% while maintaining a diagnostic specificity of 100%. Samples of tigers and rats were evaluated, and good agreement was found with the microneutralization test results from these species. Also, good discrimination was observed with dog samples.

Conclusions: These results show that using RBD and N SARS-CoV-2 proteins in the double-antigen sandwich ELISA increases its performance and turns it into a valuable assay to monitor infection in different animal species.

Notes:

P042 - Assessment of spike processing by host proteases in the entry of SARS-CoV, SARS-CoV-2, and MERS-CoV

Alexandria Zabiegala[1], Yunjeong Kim[1], Kyeong-Ok Chang[1]

[1]Department of Diagnostic Medicine and Pathobiology, Kansas State University. zabiegalaa@vet.ksu.edu

Session: Virology, **2023-01-22, 6:00 - 8:00**

Objective: Host proteases, specifically furin and transmembrane serene protease 2 (TMPRSS2), have been shown to directly influence the entry of coronaviruses through processing of the spike protein (S). Coronavirus spike proteins contain two major cleavage sites: S1/S2 junction, which is variable among coronaviruses, and S2', which is highly conserved among coronaviruses. When processed by the appropriate host proteases, a direct membrane fusion route is preferred over an indirect endosomal route where S is processed by endosomal cathepsins. This direct fusion route is reported to be more efficient than endosomal route. In this study, we aimed to assess the entry efficiency of SARS-CoV, SARS-CoV-2 and MERS-CoV when key amino acids at the cleavage sites are mutated. We also evaluated the role of other membrane bound proteases, human airway trypsin-like protease (HAT), matriptase-2 and corin, for their roles in efficient entry.

Methods: We created lentivirus-based pseudoviruses expressing coronavirus S protein that carry various mutations focusing on basic amino acids at the S1/S2 and S2'. The mutations included a deletion of ΔP681-A684 at S1/S2 junction, and substitution mutations of S2' at R815A, K814A-R815A, and S816A-F817A of SARS-CoV-2 S, R797A, K796A-R797A, and S798A-F799A of SARS-CoV S, and R887A and S888A of MERS-CoV S. Using a luciferase reporter system, we assessed the entry of the pseudoviruses expressing the mutated S into the HEK293T cells with or without TMPRSS2. Additionally, we expressed HAT, matriptase-2, or corin in HEK293T cells expressing DPP4 or ACE2 to determine their effects on the entry of SARS-CoV, SARS-CoV-2 and MERS-CoV. All HEK293T cells used in this study express the dipeptidyl peptidase 4 (DPP-4) or angiotensin-converting enzyme 2 (ACE2) for MERS-CoV or SARS-CoV(and 2), respectively, as a cellular receptor.

Results: We found that most mutations at S1/S2 junction of SARS-CoV-2 S significantly decreased entry in cells regardless of TMPRSS2 expression. Substitutions at the highly conserved S2' site significantly decreased the entry of SARS-CoV and SARS-CoV-2, but not MERS-CoV, with or without TMPRSS2 expression. We also found that the presence of HAT and corin yielded little to no increase in viral entry, while the presence of matriptase-2 greatly increased the entry of the SARS-CoV, SARS-CoV-2, and MERS-CoV pseudoviruses.

Conclusions: The results indicate important roles of basic amino acids at the S1/S2 junction of SARS-CoV-2 S, and conserved amino acids at S2' of SARS-CoV and SARS-CoV-2 in the entry. The results also show that in addition to TMPRSS2, matriptase-2 could also facilitate efficient entry of SARS-CoV, SARS-CoV-2 and MERS-CoV.

Financial Support: National Institutes of Health (NIH) (grants R01 AI130092 and AI161085).

Notes:

P043 - Molecular characterization of canine parvovirus in domestic dogs presented to veterinary clinics in Botswana.

K. Tlotleng[1, 2], A. Abdallah[1], D.M. Pfukenyi[1], S.S. Ramabu[1], E.A.M. Anis[2]

[1]Department of Veterinary Sciences, Botswana University of Agriculture & Natural Resources, [2]Department of Pathobiology School of Veterinary Medicine, University of Pennsylvania. tlotleng@upenn.edu

Session: Virology, **2023-01-22, 6:00 - 8:00**

Objective: Canine Parvovirus 2 (CPV 2) is a highly contagious viral disease characterized by vomiting, diarrhea and death in naive and some vaccinated dogs. CPV2 undergoes a high antigenic variation giving rise to emergence of new variants among Canidae species. In Botswana, several cases of the disease have been reported from Veterinary clinics on canine species, but no detailed studies have been undertaken to document and characterize the disease. The aim of the study is to provide the first genetic characterization of the virus and establish the dominant variant and the genealogy of circulating virus in Botswana.

Methods: A total of 182 fecal samples were collected from dogs presenting with suspected CPV infections across eight Veterinary clinics in Botswana. The cases were tested at point of care using ELISA SNAP Parvo Antigen Test (IDEXX) and a questionnaire administered alongside to capture demographic data for each case. Samples were subjected to further testing by Real Time PCR assay (qPCR) for confirmation and genotyping based on the amino acid change at position 426 of the VP2 protein of the virus. Additionally, 38 samples were sequenced using either targeted Next Generation Sequencing (NGS) or Sanger sequencing to obtain and analyze the entire CPV2 genome.

Results: Out of 182 tested samples, 110 (60.4%) and 182 (100%) were positive for CPV 2 using the ELISA SNAP Parvo Antigen Test kit and CPV qPCR, respectively. These results align with other studies that have shown that PCR is more sensitive than the rapid ELISA SNAP test. Based on the capsid protein residue at position 426, 180 samples were typed as CPV2c (98.90%), 1 sample was typed as CPV2a (0.55%) and 1 sample typed as CPV2b (0.55%). CPV2a variant was detected from a dog with an unknown vaccination history while CPV2b variant was detected from a vaccinated dog and very low viral load was recorded from the latter sample by qPCR. Demographic data recorded 110 (60.4%) cases of puppies less than 3 months and 61 (33.5%) cases between 3-6 months and 11 (6.0%) cases over 6 months of age. From the study, 88 (48.4%) cases were females while 94 (51.6%) cases were males. Analysis of the entire VPV2 genome and VP2 gene sequences from representative samples and phylogenetic studies are ongoing.

Conclusions: The major predominant variant of CPV2 causing disease among dog populations in Botswana has been characterized as CPV 2c. This is the first study to report the presence and circulation of CPV 2c among dog population in Botswana.

Financial Support: Martin and Pamela Winter Infectious Disease Fellowship under Institute for Infectious and Zoonotic Diseases, University of Pennsylvania. USA. Botswana University of Agriculture & Natural Resources, Botswana.

Notes:

P044 - US-UK Collab: Influence of vaccines, host genetics, and mutation rates on the evolution of infectious diseases

John Dunn[1], Jody Mays[1], Cari Hearn[1], Hans Cheng[1], Margo Chase-Topping[2], Samantha Lycett[2], Andrea Doeschl-Wilson[2]

[1]USDA, Agricultural Research Service, [2]Roslin Institute. john.dunn@usda.gov

Session: Virology, **2023-01-22, 6:00 - 8:00**

Objective: Imperfect vaccines or host genetic resistance may alter the balance of selection between pathogen transmission and virulence by allowing more divergent but still virulent strains to be transmitted at reduced cost. Our objectives are 1) determine the influence of imperfect vaccines and host genetics on viral transmission and evolution; 2) validate viral genome polymorphisms associated with increased virulence; 3) build models to develop strategies to control the ecology, evolution and economic burden of Marek's disease (MD); and 4) disseminate information on Marek's disease virus (MDV) and infectious bronchitis virus (IBV), and the impact of vaccination to the public using various tools.

Methods: Efforts on Objective 1 are provided. We used a shedder-sentinel challenge model to naturally passage MDV through 10 successive groups. Each group consists of 10 birds kept in an individual isolator and replicated 3-6x. Viral replication and transmission are assessed by sampling shedder (donor) birds that transmit infectious virions prior to, at, and following co-housing with the contact (recipient) birds. Birds infected in Passage 1 transmit virus to recipients in Passage 2, and so on. Variables include host genetics, vaccination status and dosage.

Results: Statistical analyses of the experimental data showed that the infection and transmission dynamics of birds that have been inoculated with MDV differ substantially from those of birds that have become naturally infected through contact with infected shedder birds, highlighting the importance of mimicking modes of transmissions representative of field conditions in vaccination and other MDV challenge experiments. Furthermore, experiment 1 demonstrated that HVT vaccination does not prevent MDV transmission within all 10 subsequent passages. However, vaccination with the full recommended HVT dose was found to not only provide direct protection from MD and death to the vaccinated birds, but also indirect protection for non-vaccinated contact birds.

Conclusions: MDV is being transmitted through serial passage in all groups of chickens, however, without clinically observable increase in virulence. Additional experiments are underway designed to increase pressure on virus evolution as well as compare methods with an avian coronavirus, infectious bronchitis virus.

Financial Support: This project was supported by Agriculture and Food Research Initiative Competitive Grant no. 2021-67015-33408 from the USDA National Institute of Food and Agriculture.

Notes:

P045 - Genotyping avian reovirus genomic segments

James B. Stanton[1], Kelsey T. Young[1], Holly S. Sellers[2], Vicente A. Avila Reyes[1], Morgan L. Cunningham[1], JoAnna D. Bowers[1], Crosby S. Casto[1]

[1]Department of Pathology, University of Georgia, [2]Poultry Diagnostic and Research Center, University of Georgia. jbs@uga.edu

Session: Virology, **2023-01-22, 6:00 - 8:00**

Objective: Avian reovirus (ARV) infections are a significant problem to the poultry industry. ARV infections are associated with arthritis, tenosynovitis, as well as myocarditis, enteric disease, and immunosuppression. Diagnosis of ARV is difficult due to the genomic reassortment permitted by its segmented genome. The lack of full-genome sequencing and characterization has also hindered understanding the genomic determinants of pathogenesis. While genotypic classification is currently based on the sC gene (on the S1 segment) and this gene's product is an important determinant of pathogenicity, data suggest other segments (e.g., M2) may contribute to pathogenicity. Thus, expanding the genetics that are analyzed will improve the understanding of ARV disease. The objective of this study was to create a genotyping scheme for all non-S1 ARV segments using available ARV genome data.

Methods: Non-S1 ARV genome segments were downloaded from GenBank. RDP4 was used to detect and remove likely recombinant sequences. Remaining sequences were aligned (ClustalW) by segment. The lowest BIC score was used to determine the best substitution model, which was then used to create a maximum likelihood tree based on nucleotide sequence (1000 bootstraps). Pairwise distance tables were also constructed. Previously published genotyping schemes were applied to segments M1-M3. Genotypes were identified for segments L1-L3 and S2-S4 using similar criteria.

Results: For S2-S4 segments, 3-6 genotypes were identified. For each L segment, 4-5 genotypes were identified. The previously developed genotyping criteria for the M segments was sufficient for this larger data set. For all segments, occasional sequences failed to fall within genotypic clusters, representing potential additional genotypes. Turkey and duck isolates frequently clustered within the same genotype.

Conclusions: The results demonstrate the ability to genotype all segments of the ARV genome, which can then be used in conjunction with full genome sequencing to better understand reassortment and genetic determinants of pathogenicity within ARV.

Financial Support: This project was supported by Agriculture and Food Research Initiative Competitive Grant no. 2023-67015-39735 from the USDA National Institute of Food and Agriculture. Additional support provided by US Poultry & Egg Association Grant #730 and NIH NIH T35 OD 010433.

Notes:

P046 - Pathogenesis of reoviral arthritis in turkeys derived from vaccinated breeder hens

Saroj Khatiwada[1], John Ngunjiri[2], Patricia A. Boley[1], Kush Kumar Yadav[1], Carolyn M. Lee[1], Chang-Won Lee[3], Saad Gharaibeh[4], Gireesh Rajashekara[1], Scott P. Kenney[1]

[1]Department of Animal Sciences, The Ohio State University, [2]Targan Inc., [3]Southeast Poultry Research Laboratory, US National Poultry Research Center, USDA, [4]Department of Veterinary Population Medicine, University of Minnesota. khatiwada.20@osu.edu

Session: Virology, **2023-01-22, 6:00 - 8:00**

Objective: Turkey reoviral arthritis is a viral disease in turkeys, caused by turkey arthritis reovirus (TARV), manifesting joint and tendon swelling mainly affecting hock joints. While TARV is found in the GI tract of turkeys, in some instances it travels to joints resulting in a disease which causes symptoms such as reluctance to move and eat, decreased feed intake, and decreased body weight in market age turkeys. It caused an estimated loss of $33.7 million in 2019 to the turkey industry mainly due to bird lameness resulting in decreased production, increased production costs, and culling of birds prior to market. There are currently no effective control strategies for TARV induced arthritis in turkeys. Factors mediating pathogenesis and transmission of TARV infection are not well defined. Our long-term goal is to understand the factors that may contribute to TARV-mediated arthritis such as age at time of initial infection, immune or vaccine status, co-infection by turkey enteric reovirus, and commensal microbiota and probiotics. In this study, we investigated effects of bird age on TARV infection and pathogenesis using turkeys derived from vaccinated breeder hens.

Methods: Commercial turkey poults, derived from breeder hens vaccinated with autogenous vaccines and with known reovirus antibody endpoint titers, were orally inoculated with 4×10^6 TCID50 of TARV O'Neil at 1, 3, and 7 weeks of age (WOA) and euthanized at 3-, 7-, and 28-days post infection (dpi). Serum, cloacal swabs, hock joint, ileal and cecal content were collected. The poults were weighed weekly to determine weight gain suppression. Viral RNA was extracted from tendon homogenates and real-time RT-PCR was done to quantify reoviral RNA. Cloacal virus shedding, virus isolation from tendons, and virus migration to tendons was measured. Histological slides were prepared from formalin fixed hock joints and scored severity of tendon inflammation.

Results: TARV induced weight gain suppression was pronounced when infected at 1 week of age compared with poults infected at 3 and 7 weeks of age. The average hock joint inflammation score was significantly higher compared to mock group at 4 weeks post infection, when poults were infected at 1 and 3 weeks of age. Cloacal viral shedding and virus isolation from tendons correlated with age dependent severity of inflammation of tendons associated with infection.

Conclusions:
Maternally derived antibodies, derived from breeder hens vaccinated with autogenous vaccines, is likely insufficient to prevent same strain TARV infection and associated pathology at 4 weeks post infection. In addition to passive immunity, the effect of acquired immunity on the development of disease in turkeys should be further investigated. In future experiments, we will also define how viral, host and intrinsic microbial factors contribute toward the onset, development, and severity of reoviral arthritis in turkeys. Additionally, we will investigate whether probiotic interventions modulating the gut microbiome, with focus on gut-joint axis interplay, can alleviate symptoms of arthritis in turkeys.

Financial Support: This project was supported by Agriculture and Food Research Initiative Competitive Grant no. 2021-67015-34465 from the USDA National Institute of Food and Agriculture.

Notes:

P047 - A zoonotic strain of Rocahepevirus ratti experimentally infects chickens

Kush Kumar Yadav[1, 2], Patricia Boley[2], Carolyn Lee[1, 2], Saroj Khatiwada[2], Scott Kenney[1, 2]

[1]Department of Veterinary Preventive Medicine, The Ohio State Uiversity, [2]Department of Animal Sciences, The Ohio State University. kenney.157@osu.edu

Session: Virology, **2023-01-22, 6:00 - 8:00**

Objective: Rocahepevirus ratti [rat hepatitis E virus (HEV)] was originally isolated from rats and found to be non-infectious to pigs and nonhuman primates, suggesting humans were not a susceptible host. More recently, rat HEV cases have been identified in people through unknown transmission sources. A high seroprevalence of rat HEV in rats in the United States necessitates studying this emerging zoonotic strain. Lack of an infectious clone, cell culture systems, and animal models have hindered this effort. In response to the increase in human infections by rat HEV, we sought to develop an infectious clone of a zoonotic rat HEV strain and to experimentally test susceptibility of food animals such as chickens to help identify potential transmission vectors and produce an animal model for future studies.

Methods: We constructed a full-length rat HEV cDNA clone of genotype 1 LCK-3110 strain of rat HEV. Replication was tested in mouse fibroblasts and myeloma cells, human lung and liver cells, baby hamster kidney, and chicken liver cell lines (LMH) via RNA transfection in triplicate with *in vitro* transcribed viral RNA. To test whether rat HEV transcripts productively replicate in the target cells, we assessed the presence of HEV ORF2 protein at the single-cell level using immunofluorescence and flow cytometry. Furthermore, 10 chickens were intrahepatically inoculated with RNA transcripts of rat HEV. RT-qPCR for rat HEV RNA was performed weekly in serum, fecal, and tissue (liver, spleen, pancreas, jejunum) of chickens. Tissue samples from cohoused sentinel birds were also screened for virus. GraphPad Prism Software 9.0 was used for data analysis using Student's t-test to assess significance. $p < 0.05$ was considered significant.

Results: The RNA transcripts of LCK-3110 strain of rat HEV are replication-competent in mouse subcutaneous tissue, human lung, human liver cells, and baby hamster kidney cells. Importantly, chickens inoculated with RNA transcripts of LCK-3110 strain developed infection as shown by virus shedding in feces, detectable HEV RNA in the liver, spleen, pancreas, and jejunum. Cohoused naïve animals also became infected as indicated by fecal RNA shedding, seroconversion, and histopathological lesions.

Conclusions: Rat HEV is an emerging zoonotic virus with an ability to spillover across species. Chickens have the potential to serve as intermediary hosts for the LCK-3110 strain and possibly other zoonotic rat HEV strains. If infected by the LCK-3110 strain, chickens have the ability to transmit the virus within a flock and potentially to humans if safe food preparation is not performed.

Notes:

P048 - Genetic and serological comparison between current highly pathogenic avian influenza H5Nx virus clade 2.3.4.4

Darrell Kapczynski[1], Kelsey Briggs[1], Ryan Sweeney[1], Karen Segovia[1], Klaudia Chrzastek[1], Samantha Lycett[2], Lonneke Vervelde[2], Daniel Perez[3], Lu Lu[2], Wenjun Liu[4], Paul Digard[2]

[1]USDA-ARS, [2]University of Edinburgh, [3]University of Georgia, [4]Chinese Academy of Sciences. darrell.kapczynski@usda.gov

Session: Virology, **2023-01-22, 6:00 - 8:00**

Objective: Avian influenza (AI) is a highly contagious, agriculturally relevant disease that has spread globally to most countries. A highly pathogenic avian influenza (HPAI) virus lineage of H5N1 subtype emerged in China in 1996 (Gs/Gd/96), which has caused severe disease in poultry and wild bird species. Viruses in this lineage have evolved into several different clades, with 2.3.4.4 becoming the most relevant to date. The objectives of this USDA-NIFA research are to develop mathematical models of AI virus evolution that can be used to inform vaccination and other control strategies.

Methods: Using phylogenetic methods, clade 2.3.4.4 (a-h) H5Nx HPAI genetic composition and spreading pattern across continents has been reconstructed. For vaccine studies, birds were vaccinated with various H5-based inactivated or recombinant vaccines (85-98% relatedness to hemagglutinin) and challenged against the 2.3.4.4c lineage viruses. Using serum from these birds, we examined the antigenic relatedness based on cross reactivity using the hemagglutinin-inhibition (HI) assay.

Results: Compared to the original Gs/Gd/96 virus, the clade 2.3.4.4 viruses share approximately 95% sequence identity in the hemagglutinin (HA) protein. Within the subclades of 2.3.4.4 (a-h) which are approximately 98 % similar, between 13 and 42 amino acid differences in the HA protein were determined from the isolates used in these studies. Serum was tested against homologous vaccine virus, clade 2.3.4.4b virus, or clade 2.3.4.4c virus. As expected, birds vaccinated with inactivated 2.3.4.4c viruses maintained the highest level of cross reactive HI titers to the current 2.3.4.4b viruses, with an average of 1$\log_2$ drop in titer using prechallenge serum. When birds in these groups were challenged with 2.3.4.4b HPAIV, the serological cross reactivity between 2.3.4.4b and c viruses was near 100% in titer. Conversely, when birds received non-Gs/Gd/96 lineage H5 vaccine, the serum titers were generally 7$\log_2$ lower against the clade 2.3.4.4 virus. Serum from birds that received vaccination with a clade 2.3.2 vaccine demonstrated reduced HI titers of approximately 4-5$\log_2$.

Conclusions: Taken together, the data demonstrate there is limited serum cross reactivity from birds not vaccinated with 2.3.4.4 antigen, further strengthening the concept of matching the vaccine antigen to the field strain.

Financial Support: This study was supported by a US-UK BBSRC-NIFA Collaboration grant (no. 2015-67015-22968 (NIFA) and BB/M027163/1 (BBSRC)) to DRK, LV and PD. Additional funding was provided by USDA-ARS CRIS # 6040-32000-081-00D to DRK.

Notes:

P049 - Protective antigens of ASFV Georgia-07

David Doub[1], Jehadi Osei-Bonsu[1], Herman Chambaro[1], Gustavo Delhon[2], Edan Tulman[3], Daniel L. Rock[1]

[1]Department of Pathobiology, University of Illinois, [2]School of Veterinary Medicine & Biomedical Sciences, University of Nebraska, [3]Department of Pathobiology and Veterinary Science, University of Connecticut. jehadio2@illinois.edu

Session: Virology, **2023-01-22, 6:00 - 8:00**

Objective: African Swine Fever (ASF) is a viral hemorrhagic disease of swine causing nearly 100% mortality in infected animals and has devastated pork producers worldwide. There is no vaccine for ASF available. Although live attenuated vaccines currently under development might be helpful in endemic regions, it is hard to imagine a scenario where they would be suitable for use in countries with highly developed swine industries like the U.S.; issues of efficacy, residual pathogenicity with immunopathologic sequelae, and potential for long-term viral persistence raise significant safety concerns. Efficacious subunit/vectored ASF vaccines with significantly enhanced safety profiles are needed for use in the US. Our work will: 1) Evaluate protective efficacy of DNA virus vectors-expressing putative ASFV protective antigens (PA) of epidemic strain Georgia-07 using vaccination challenge experiments in pigs and 2) Identify PA epitopes and host responses associated with protection.

Methods: To identify potential PAs, recombinant virus vectors containing ASFV proteins will be constructed and evaluated using vaccination/challenge experiments in pigs.

Results: Data including antibody and cellular immune response obtained following vaccination (ASFV-specific immune assays, including serum neutralization assays, hemadsorption inhibition (HAI) assays, Macrophage Infection and Inhibition assays (M-II), and ASFV PA-specific IFNγ flow cytometry and ELISPOT assays) will be evaluated and correlated with various aspects of protection: mortality rate, time-to-death, clinical disease, viremia (onset, magnitude, and duration) and necropsy findings (gross/histopathologic).

Conclusions: Project success will provide critical foundational information necessary for design/development of safe and efficacious Differentiate Infected from Vaccinated Animal (DIVA) compatible subunit or vectored ASF vaccines that would be of considerable value for emergency use in the US should ASF be introduced.

Financial Support: This research was supported in part by the U.S. Department of Agriculture, National Institute of Food and Agriculture, award number 2023-67915-39737, as well funding from the National Pork Board, grant numbers 13-102 and NPB PR-005526.

Notes:

P050 - Cytoplasmic tail truncation of SADS-CoV spike protein facilitates cell surface expression and cell-cell fusion

Bac Tran Le[1], Jung-Eun Park[1]

[1]Chungnam National University. jepark@cnu.ac.kr

Session: Virology, **2023-01-22, 6:00 - 8:00**

Objective: Swine acute diarrhea syndrome coronavirus (SADS-CoV), also known as swine enteric alphacoronavirus, was identified in piglets with diarrhea in 2017. The spike (S) protein of SADS-CoV plays a key role in the receptor recognition and cell membrane fusion processes. The cytoplasmic tail (CT) of S protein is a small part consisting of a cysteine-rich motifs and an endoplasmic reticulum retention signal at C-terminus. The distribution of S protein in host cells is likely related to CT regulation.

Methods: In the present study, we used the S gene with CT truncation mutants (S-dCTs) to examine the effects of CT on the intracellular localization of S proteins and cell-to-cell fusion.

Results: Compared to wild-type (WT) S proteins, S proteins lacking 5 or 16 amino acids at the C-terminus (dCT5 and dCT16) showed higher colocalization with the cell surface marker Zo-1 and lower colocalization with intracellular organelle markers. Additionally, dCT5 and dCT16 S proteins enhanced cell-cell fusion in HEK293T cells.

Conclusions: Our results indicate that CT of SADS-CoV S proteins promotes subcellular localization of the S proteins at or near the ERGIC.

Notes:

P051 - Detection of multiple influenza A virus subtypes at the single pig level under field conditions

Joaquin Alvarez-Norambuena[1], Chong Li[1], My Yang[1], Marie Culhane[1], Montse Torremorell[1]

[1]University of Minnesota. jalvare@umn.edu

Session: Virology, **2023-01-22, 6:00 - 8:00**

Objective: The aim of this study was to describe the detection of co-circulating IAV subtypes at the pig level as a first step to evaluate farm factors involved in the emergence of IAV reassortment in pigs.

Methods: We identified one production flow consisting of three sow herds, three nursery sites and three finishing sites. Pigs from the three sow farms were commingled at weaning into all-in/all-out nurseries with two of the cohorts having pigs commingled from all three sow herds, and one cohort having pigs from two sow herds. Afterwards, pigs in the nursery were transported to all-in/all-out finishers. Herd enrollment criteria consisted of: (a) a production flow with nursery sites with history of IAV co-infections within the previous 3 months of starting the study, (b) sow herds with an external source of replacement gilts, (c) sow herds not undergoing IAV elimination, and d) researchers ability to trace the pigs from birth to market. Sixty pigs were identified at 4, 8 and 16 weeks of age approximately for a total of 240 pigs per cohort (720 pigs total). Pigs were sampled by collecting nasal swabs (n= 720) and tested by RT-qPCR to detect the IAV matrix gene. A subset of nasal swabs from pigs at weaning (n= 272) that tested RT-qPCR matrix gene positive, as well as cohort matrix gene RT-qPCR positives samples were further tested for IAV subtyping RT-qPCR to detect the IAV H1 and H3, and N1 and N2 genes.

Results: Overall, 261 out of 720 pigs tested IAV RT-PCR positive, and these were distributed in 9.3% (67/720) pigs at weaning (sow farms), 14.6% (105/720) nursery pigs between 3 to 5 weeks of age and 8.5% (61/720) between 7 and 8 weeks of age, and 3.9% (28/720) corresponding to finishing pigs of 16 weeks of age approximately. There was evidence of more than one IAV subtype circulating in all three cohorts. Among the samples tested, 18.4% (50/272) tested positive for H1 and H3, or N1 and N2 or H1, H3, N1, and N2, which indicated co-detection of multiple IAV subtypes in the same animal.

Conclusions: This study presents evidence of co-infection of multiple IAV subtypes in pigs from weaning to market. The highest percentage of co-infecting IAV subtypes was observed at weaning and throughout the nursery phase creating crucial conditions for reassortment to occur.

Financial Support: This project was supported by the Agriculture and Food Research Initiative Competitive Grant no. 2022-67015-36660 from the USDA's National Institute of Food and Agriculture.

Notes:

P052 - A CRISPR screen identifies host factors involved in PRRSV replication in a pig macrophage cell line

Jiyoun Kim[1], Christopher J. Davies[1], Brian B. Gowen[1], Dongwan Yoo[2], Young-Min Lee[1]

[1]Department of Animal, Dairy, and Veterinary Sciences, Utah State University, [2]Department of Pathobiology, University of Illinois at Urbana-Champaign. jiyoun.kim@usu.edu

Session: Virology, **2023-01-22, 6:00 - 8:00**

Objective: Porcine reproductive and respiratory syndrome virus (PRRSV) is the causative agent of PRRS, an economically devastating swine disease that is characterized by reproductive failure in pregnant sows and respiratory problems in young piglets. Since the discovery of PRRSV, significant progress has been made in understanding its epidemiology and transmission; however, no adequate control measures are yet available to eliminate infection with this pathogen. The replication of PRRSV is initiated by binding of the virion to an as-yet-unknown cell-surface receptor(s), followed by viral internalization, membrane fusion, gene expression, genome replication, assembly, and release. In this multistep process, a collection of host factors are likely involved. Despite considerable efforts, however, these important host factors remain largely unknown. The present study aims to identify porcine cellular factors involved in PRRSV replication.

Methods: We are using a multiplexed CRISPR screen strategy with a lentiviral porcine sgRNA library in a newly developed, highly PRRSV-susceptible pig macrophage cell line.

Results: We expect to discover the porcine cellular factors that are crucial for PRRSV entry and dissect the discrete entry steps that are regulated by specific host factors.

Conclusions: The outcomes of this study will not only shed new light on the cell/tissue tropism and pathogenesis of PRRSV, but also significantly advance the development of new control strategies for the prevention of PRRSV infection.

Notes:

P053 - Virus replication kinetics and risk of spillback of cervid-adapted SARS-CoV-2 variants in deer and wildlife

Mariano Carossino[1], Wellesley Dittmar[1], Udeni Balasuriya[1], Juergen Richt[2], Natasha Gaudreault[2], Côme Thieulent[1], Igor Morozov[2], Jeffrey Hall[3], Ganwu Li[4]

[1]Department of Pathobiological Sciences & Louisiana Animal Disease Diagnostic Laboratory, Louisiana State University, [2]Department of Diagnostic Medicine/Pathobiology, Kansas State University, [3]USGS National Wildlife Health Center, [4]Department of Veterinary Diagnostic and Population Animal Medicine, Iowa State University. mcarossino1@lsu.edu

Session: Virology, **2023-01-22, 6:00 - 8:00**

Objective: While it has been demonstrated that SARS-CoV-2 can infect numerous domestic and wild animals, there is a significant gap in our understanding of SARS-CoV-2 infections in wildlife species, particularly as it relates to (i) their permissiveness, (ii) their ability to become reservoirs further driving the emergence of novel variants, (iii) spillover/spillback potential to other animals and humans, and (iv) virulence of emerging variants from wildlife. Circulation of SARS-CoV-2 in wildlife is of significant public health concern, with a critical need to identify new reservoirs/susceptible species and to study the human-animal interface. Natural infection and transmission of SARS-CoV-2 among white-tailed deer (WTD), the predominant cervid species in North America, has caused a significant public health concern, particularly related to the possibility of spillback transmission to humans; the latter is now reported in Ontario, Canada. There is a significant gap in our knowledge regarding adaptive, deer-specific mutations and their role in viral dynamics, fitness, pathogenicity, and transmissibility in animal reservoirs and the animal-human interface.

Methods: We will 1) develop recombinant spike variants to investigate the kinetics of WTD-derived SARS-CoV-2 strains in domestic and wild ruminant cells and human airway epithelium to determine their spillback potential, 2) study the pathogenicity, fitness and spillover transmission of WTD-derived SARS-CoV-2 variants in WTD, and 3) study the pathogenicity, fitness and spillover transmission of WTD-derived SARS-CoV-2 variants in wild rodent species which could serve as bridging hosts facilitating spillback to other animals and humans in North America.

Results: NA

Conclusions: This work will significantly enhance our understanding of SARS-CoV-2 dynamics in wildlife species, the animal-human interface, and spillback/spillover transmission.

Financial Support: This project was supported by Agriculture and Food Research Initiative Competitive Grant no. 2023-70432-39465 from the USDA National Institute of Food and Agriculture

Notes:

P054 - Identification of avian influenza resistance host factors through CRISPR/Cas9 knockout screening in DF-1 cells

Evelyn A. Weaver[1], Tae H. Kim[1]

[1]Department of Animal Science, Pennsylvania State University. euw70@psu.edu

Session: Virology, **2023-01-22, 6:00 - 8:00**

Objective: The United States is the world's largest producer of poultry and the second-largest exporter of poultry meat. The recent high-pathogenic avian influenza outbreaks have devastated the poultry industry worldwide, including the ongoing outbreaks in the United States, with over 55 million birds affected across 47 states. Despite the threat to human health, substantial economic impacts, and an astronomical number of bird losses, there is a lack of strategies against the avian influenza virus beyond the passive biosecurity measures. This study aims to utilize whole genome knockout CRISPR screening and functional genomics to identify, annotate and characterize candidate gene(s) involved in avian influenza resistance in an in vitro avian cell culture model.

Methods: Initially, we will establish a Cas9-expressing DF-1 cell line. Subsequently, the cells will be transfected with a lentivirus guide RNA library encompassing whole genome knockouts, which will be cloned into the CROP-seq lentiviral vector. This process will yield a pool of DF-1 cells, each barcoded with unique gRNA sequences. These cells will then be exposed to either a low or high pathogen avian influenza strain for a period of 48 hours. Surviving cells will undergo genotyping to identify the gRNA barcodes, utilizing next-generation sequencing-based gRNA abundance enrichment. With this information, we will pinpoint host-resistant factors. To further investigate and understand the function of each gene, we will employ single-cell RNA-seq and established scCRISPR-seq data processes for comprehensive gene annotation.

Results: This study is ongoing and we are currently developing the DF-1 cell line expressing Cas9.

Conclusions: These experiments will provide foundational knowledge that will expand our knowledge of potential candidate gene(s) that may play a role in avian influenza resistance in the chicken. Identifying candidate genes and understanding their functions will help develop antiviral strategies and aid in designing resilient influenza virus genome-edited chickens.

Financial Support: This project was supported by Agriculture and Food Research Initiative Competitive Grant no. 2022-09630 from the USDA National Institute of Food and Agriculture.

Notes:

P055 - Transcriptomic insights into tissue-specific expression in the invasive Asian long-horned ticks

Mohamed A. M. Moustafa[1], Dana Price[1]

[1]Department of Entomology, Rutgers University. m.moustafa@rutgers.edu

Session: Parasitology, **2023-01-22, 6:00 - 8:00**

Objective: We aimed to investigate the transcriptomic landscape of the Asian long-horned tick, *Haemaphysalis longicornis*, an expanding and invasive ectoparasite and vector of *Theileria orientalis* Ikeda in the United States.

Methods: Employing high-throughput RNA-seq technology, we comprehensively explored gene expression profiles across a diverse array of tissues and leg types, including ovaries, midguts, salivary glands, forelegs, and hindlegs from invading North American ticks. Our sequencing efforts yielded an extensive dataset encompassing > 107M Illumina reads. We generated a comprehensive transcriptome, showing a rich repertoire of coding sequences (24,760 CDS) from the obtained sequences. Subsequent data processing involved advanced statistical analysis using DESeq2, complemented by insightful visualizations through Venn diagrams, MA plots, heatmaps, and gene ontology enrichment analysis. These methods collectively provided valuable insights into tissue-specific gene expression patterns.

Results: We identified substantial differential gene expression across tissues and leg types. Between ovaries, midgut, and salivary glands, a total of 6220 genes demonstrated significant expression differences at a false discovery rate of 0.05%. Notably, 2675 genes were overexpressed in ovaries, 2753 in the midgut, and 792 in salivary glands. In contrast, between forelegs and hindlegs, 177 genes exhibited significant expression variations. Specifically, 164 genes were upregulated in the forelegs and are thus likely involved in chemosensory processes via the Haller's organ.

Conclusions: Our comprehensive transcriptomic exploration, coupled with in-depth pathway analysis, shed light on the molecular functions of the prominently overexpressed genes within each organ. These findings provide novel insights into the physiology governing development, saliva production, digestion, and chemoreception in the invasive ALT ticks. These gene products will be used to further assign molecular targets for reverse-vaccine development targeting *H. longicornis*.

Notes:

P056 - Metagenomic analysis to study associations between gastrointestinal nematodes and the fecal microbiome of North American bison

Jeba Jesudoss Chelladurai[1], Theresa Quintana[1], Grace Schieferecke[1], Guwin Weerawarna Nilaweera[1]

[1]College of Veterinary Medicine, Kansas State University. jebaj@vet.k-state.edu

Session: Parasitology, **2023-01-22, 6:00 - 8:00**

Objective: The overall objective is to increase our understanding of the complex interactions between gastrointestinal nematode parasites and the microbiome of North American Bison. The specific objective of this project was to understand the diversity and composition of the fecal microbiome of bison infected with low or high levels of gastrointestinal nematode parasites using shotgun metagenomics conducted on the Illumina platform.

Methods: Utilizing a herd-stratified case-control experimental design, bison were categorized as either infected with high levels or low levels of strongyle parasites based on parasite egg burdens quantified (as eggs per gram (EPG)) with the mini-FLOTAC method. For shotgun metagenomics, a total of 18 samples from 9 mid-western herds were selected. Each sample represents a fecal pool from three individual bison, classified as high EPG or low EPG. Following DNA extraction and QC, Illumina libraries were prepared and sequencing was performed on a NextSeq 500 system to obtain ~15 million read pairs each. Raw data in FASTQ format was analysed using the DRAGEN metagenomics pipeline.

Results: The metagenomic analysis identified a diverse range of microorganisms in the bison microbiome, including bacteria, archaea, fungi, and viruses. The most abundant bacterial phyla were Proteobacteria, Actinobacteria, Firmicutes, Euryarchaeota and Bacteroidetes. The diversity of the bison microbiome was assessed using alpha diversity and beta diversity metrics. The results showed that the bison microbiome was diverse, both within and between samples. Additionally, principal components analysis of the dataset revealed clustering, which was dependent on the taxa used in the analysis. Some associations were observed between parasite burden and the composition of the microbiome.

Conclusions: The results of this study provide new insights into the composition and function of the bison microbiome, as well as its interactions with parasites. Further research is needed to better understand the specific mechanisms by which the microbiome interacts with parasites and to develop new strategies for parasite control based on this understanding.

Notes:

P057 - Immunological evaluation of recombinant RAP-1 and RRA proteins of *Babesia bovis*

Manuel J. Rojas[1], Reginaldo G. Bastos[2], Jinna Navas[1], Jacob M. Laughery[1], Paul A. Lacy[2], Carlos E. Suarez[2]

[1]Department of Veterinary Microbiology & Pathology, Washington State University, [2]Animal Disease Research Unit, USDA Agricultural Research Service. suarez@wsu.edu

Session: Parasitology, **2023-01-22, 6:00 - 8:00**

Objectives: Bovine babesiosis is an acute tick-borne disease of worldwide impact caused by *Babesia bovis*. that can be controlled using pharmacotherapy, ixodicides, and live attenuated vaccines. All these approaches have severe limitations and more sustainable and efficient vaccines are needed. Attempts to develop vaccines based on immunodominant parasite antigens have been so far unsuccessful. Previous numerous studies identified the *B. bovis* RAP-1 protein as a strong candidate for subunit vaccine, but vaccines based on full size recombinant RAP-1 or a truncated version including its sub-immunodominant NT portion (RAP-1NT) were also unsuccessful. We propose here that a vaccine based on RAP-1 should also include the RAP-1 Related Antigen (RRA) as a component. RRA can be considered as an immune-subdominant truncated version of RAP-1, lacking the highly antigenic repeats in the CT-terminus of the protein (RAP-1CT), that may be a functional replacement for RAP-1 in the face of strong anti-RAP-1 immune responses.

Methods: We generated recombinant RRA, RAP-1NT, and RAP-1CT proteins and compared the antigenicity of RRA, RAP-1NT and RAP-1 CT, the immunodominant component of RAP-1 using sera of a total of 9 cattle vaccinated with attenuated parasites and challenged with a virulent strain of *B. bovis* using ELISA and immunoblots.

Results: The rRAP-1NT and rRRA proteins where consistently poorly recognized by antibodies in vaccinated and protected animals, which, in contrast, strongly recognize the immunodominant RAP-1CT.

Conclusions: We confirmed that the NT portion of RAP-1 and the RRA protein are poorly immunogenic during infection. We plan next to use purified recombinant RRA and RAP-1NT in to test whether these two immune-subdominant antigens combined with an adjuvant known to have strong immune stimulatory effects, such as the Salmonella flagellin FliC, can elicit protective immune responses against *B. bovis* in a cattle vaccine trial.

Financial Support: USDA National Institute of Food and Agriculture (NIFA) (Award Number: 2020-67015-31809; Proposal Number: 2019-05375, Accession Number: 1022541) United States Department of Agriculture (ARS-USDA CRIS 2090-32000-040-00-D) International Development Research Center (IDRC) (Livestock Vaccine Innovation Fund (Grant 108525)

Notes:

P058 - Efficacy of buparvaquone against *Theileria orientalis* (US Ikeda genotype) infection in cattle

Reginaldo Bastos[1, 2], Amany Hasan[2, 3], Cynthia Onzere[2], David Herndon[1], Nicolas Villarino[4], Lindsay Fry[2]

[1]Animal Disease Research Unit, USDA Agricultural Research Service, [2]Department of Veterinary Microbiology & Pathology, Washington State University, [3]Department of Animal Medicine, University of Alexandria, Egypt, [4]Department of Veterinary Clinical Sciences, Washington State University. reginaldo.bastos@usda.gov

Session: Parasitology, **2023-01-22, 6:00 - 8:00**

Objective: *Theileria orientalis*, the causative agent of oriental theileriosis in cattle, is an apicomplexan hemoparasite with global distribution. Parasite genotype Ikeda is considered the most virulent, and it has been associated with outbreaks and devastating economic losses for the cattle industry in Japan, Australia, and New Zealand. The emerging of *T. orientalis* Ikeda in the US occurred in concert with the invasion of the country in 2017 by its major tick vector, the Asian longhorned tick *Haemaphysalis longicornis*. Infection of cattle herds with *T. orientalis* Ikeda has since been detected in several states in the US. Vaccines and therapeutics to control the parasite are unavailable and such strategies are much needed to assist US stakeholders in controlling the spread of the disease caused by the Ikeda genotype. Therefore, the objective of this study was to investigate the efficacy of buparvaquone against the *T. orientalis* US Ikeda genotype infection in cattle.

Methods: A group of 13 calves were inoculated with either *T. orientalis*-infected *H. longicornis* salivary glands (subcutaneous inoculation) or blood stabilate (intravenous inoculation). Infection evolved to persistence, which was characterized by detection of parasite DNA in the animal's peripheral blood by PCR whereas hematocrit and temperature were maintained at normal levels. Of the infected animals, seven calves were randomly selected and treated with the buparvaquone label dose of 2.5mg/Kg. Six animals remained untreated and served as controls. After treatment, parasite load in all animals was monitored by endpoint and quantitative PCR targeting the *T. orientalis* major piroplasm surface protein gene. Quantitative PCR results of parasite load before and after the buparvaquone treatment were compared by the Mann-Whitney test, and a P value <0.05 was considered statistically significant.

Results: Results demonstrated that buparvaquone efficiently controls parasite load as shown by the lack of detection of parasite DNA in peripheral blood starting at one week after treatment. Parasites remained undetectable in blood for 5 to 10 weeks after treatment; however, recrudescence was observed in all treated animals. Interestingly, despite the reappearance of parasite DNA in blood, parasite load was significantly lower ($P<0.001$) compared to pre-treatment levels. Control animals remained consistently PCR positive for 20 weeks after infection. Analysis of parasite resistance after the buparvaquone treatment and alternative doses and regimens for the drug are currently under investigation.

Conclusions: In conclusion, results demonstrate that buparvaquone is effective against *T. orientalis* US Ikeda genotype infection in cattle, showing a transient efficacy in reducing parasite load in peripheral blood. Considering these results and the current spread of *T. orientalis* in the US, future studies are needed to investigate alternative doses and regimens for buparvaquone and also novel therapeutics to control this economically important parasite of cattle.

Financial Support: The research was funded by the USDA-ARS CRIS 2090-32000-044-000-D.

Notes:

P059 - Efficacy of *Bacillus thuringiensis* crystal proteins against monogastric and ruminant gastrointestinal nematodes

Katherine H. Petersson[1], Elizabeth P. Kass[1], Kelly Flanagan[2], Duy Hoang[2], Nicholas Cazeault[2], Hanchen Li[2], Qian Ding[2], Florentina Rus[2], Ernesto Soto[2], Martin Nielsen[3], Gary Ostroff[2], Raffi Aroian[2]

[1]University of Rhode Island, [2]University of Massachusetts Chan Medical School, [3]Univeristy of Kentucky. kpetersson@uri.edu

Session: Parasitology, **2023-01-22, 6:00 - 8:00**

Objective: The objectives of this study were to analyze anthelmintic efficacy of *Bacillus thuringiensis (Bt)* crystal (Cry) proteins CryH18, CryH1 and CryH19 against monogastric and ruminant gastrointestinal nematodes.

Methods: BtCryH18 was evaluated: 1.1) *In vitro* against equine cyathostomins, ovine *Haemonchus contortus*, and swine *Ascaris suum.* Larval development inhibition assays were performed against cyathostomins and *H. contortus* at concentrations ranging from 1100 to 0.0011 ng/ml Bt CryH18. Larval motility of *A. suum* was determined at 40 µg/ml Bt CryH18; 1.2) *In vivo* against *Ancylostoma ceylancium* and *Heligmosomoides polygyrus* in rodents. Hamsters infected with *A. ceylanicum* and mice infected with *H. polygyrus* were stratified into treatment groups (n=5) and orally administered 20 mg/kg Bt Cry1H18 or 50 mg/kg Bt CryH18, respectively, on day (d) 0. 1.3) *In vivo* against *H. contortus* in ovine. Lambs (n=15) were experimentally infected with 10,000 *H. contortus* L3i and stratified sequentially by FEC into one of three treatment groups: CryH18-15 (15 mg/kg BW), CryH18-30 (30 mg/kg BW) or control. CryH18-30 and control treatment were orally administered on d0. CryH18-15 was administered on d4. FEC were monitored daily until d7 when lambs were euthanized, and total abomasal worm counts were determined. BtCryH1 was evaluated: 2.1) *In vivo* against *H. contortus* in ovine. Lambs (n=15) were experimentally infected with 10,000 *H. contortus* L3i and stratified sequentially by FEC into one of three treatment groups: CryH1-15 (15 mg/kg BW), CryH1-30 (30 mg/kg BW) or control. FEC were monitored daily until d7 when lambs were euthanized, and total abomasal worm counts were determined. BtCryH19 was preliminarily evaluated: 3.1) *in vivo* against Dorset lambs (n=2/group) infected with 10,000 HC infective larvae.

Results: BtCryH18 1.1). BtCryH18 demonstrated robust AH activity *in vitro* against cyathostomins, *H. contortus,* and *A. suum;* 1.2) BtCryH18 demonstrated significant reductions in both FEC and worm burdens in rodents experimentally infected with *A. ceylanicum* and *H. polygyrus*; 1.3) CryH18 decreased FEC by 85%, 97% and abomasal worm burdens by 69%, 93% in lambs administered 15 mg/kg BW and 30 mg/kg BW of CryH18, respectively. BtCryH1 2.1) There was no observed reduction in FEC for lambs administered 15 mg/kg BW and 30 mg/kg BW CryH1 IBaCC treatment groups, respectively therefore the sampling ceased after 4 days and animals were not euthanized. BtCryH19 3.1) Results are pending.

Conclusions: These studies provided evidence of varying anthelmintic efficacy of BtCry proteins against nematodes of monogastric and ruminant animals. Further studies to optimize and identify new novel anti-parasitic Cry proteins are warranted.

Financial Support: This project was supported by Agriculture and Food Research Initiative Competitive Grant no. 2021-67015-34574 from the USDA National Institute of Food and Agriculture.

Notes:

P060 - Evaluation of extracellular vesicles as anti-tick vaccines in white-tailed deer

Julia Gonzalez[1], Cristina Harvey[1], Carita de Souza Ribeiro-Silva[2], Brenda Galvan-Leal[1], Kelly Persinger[3], Tammi Johnson[3], Adela Oliva Chavez[1,4]

[1]Department of Entomology, Texas A&M University, [2]Departamento de Biociencias e Tecnologia, Universidade Federal de Goias, Brazil, [3]Texas A&M AgriLife Research, [4]Department of Entomology, University of Wisconsin-Madison. aolivachavez@tamu.edu

Session: Parasitology, **2023-01-22, 6:00 - 8:00**

Objective: Ticks and tick-borne pathogens significantly affect the public health of thousands of people in the US and impose an important economic threat to livestock worldwide. Current tick control measures are focused on the use of synthetic acaricides. Nevertheless, the emergence of acaricide resistance and the presence of wildlife as an alternative host transport mechanism to introduce tick populations into new areas has prevented the efficient management of ticks. Thus, non-chemical control measures that can reliably reduce ticks within wild reservoirs are needed. This project aims to evaluate the antigenicity of proteins packed in extracellular vesicles within tick salivary glands and midguts and examine the degree of tick control obtained by vaccination of white-tailed deer (*Odocoileus virginianus*) using these extracellular vesicles (EVs).

Methods: *Amblyomma americanum* female ticks were fed on 1-year-old white-tailed deer (WTD) for 5 days on three different infestations. Salivary glands and midguts were cultured *ex vivo* in vesicle free-tick media and extracellular vesicles were isolated by ultracentrifugation for the vaccination of three 2-year-old WTD. Each WTD was vaccinated with 400 µg of protein (200 µg from salivary glands and 200 µg from midguts) and received two boosters at 28 and 49 days, using Titer Max Gold as adjuvant. Two control deer were injected with Titer Max Gold and PBS only. Serum samples were obtained every seven days and reactivity was evaluated by western blot and ELISA. At 58 days post vaccination/boost, WTD were infested with 100 *A. americanum* nymphs, 50 females, and 50 males that were allowed to feed to repletion.

Results: The western blot analysis showed that all three vaccinated WTD seroconverted and recognized proteins in both vesicle populations and in the secreting organs. Multiple bands were recognized in salivary EVs (size range: 50 - 20 kDa) and midgut EVs (size range: 400 kDa - 20 kDa) by deer serum as early as 14 days post vaccination. Interestingly, protein bands that were visible in the salivary glands were not observed in the vesicles secreted by these organs, demonstrating the differences in proteomic profile. Although not statistically significant, there was a reduction in the number and engorgement weight of females recovered from two of the vaccinated animals. Further, early tick mortality during attachment was observed in one of the vaccinated animals.

Conclusions: Extracellular vesicle proteins are antigenic and led to the production of antibody responses after vaccination. Vaccination with tick vesicles resulted in moderate control, although not statistically significant. Our future studies will identify antigenic proteins recognized by antibodies within the serum of vaccinated animals.

Financial Support: These studies were supported by the United States Department of Agriculture (USDA) National Institute of Food and Agriculture (NIFA) project #TEX09134 to AOC..

Notes:

P061 - Transcriptome analysis of hepatic tissue during the development of liver abscess in Holstein Steers

N Beneduzi[1], O Benitez[2], D Paiva[1, 3], Y Borges[2], P. R. Broadway[4], T. G. Nagaraja[5], K Hales[6], C. S. Barboza[2], W. L. Crossland[6], M. A. Ballou[1], V. Machado[1]

[1]Department of Veterinary Science, Texas Tech University, [2] School of Veterinary Medicine, Texas Tech University, [4]USDA-ARS, [5]Department of Diagnostic Medicine/Pathobiology, Kansas State University, [6]Department of Animal and Food Sciences, Texas Tech University. natalia.beneduzi@ttu.edu

Session: Immunology, **2023-01-22, 6:00 - 8:00**

Objective: The objective of this study was to perform transcriptomic analysis of hepatic tissues after an experimental model for liver abscesses in ruminant calves based on an acidotic diet and oral pathogenic challenge.

Methods: Fifteen Holstein calves (initial BW: 90.8 ± 9.1) received a diet regimen that alternated cycles of a low-starch and high-starch acidotic diet for 20 days. Then, five calves were randomly assigned as negative controls (CON), and 10 calves received intraruminal inoculation of *Fusobacterium necrophorum* subsp. *necrophorum* (8.00×10^8 CFU) and *Salmonella enterica* serovar Lubbock (2.67×10^9 CFU). Hepatic tissue samples were collected one day prior to inoculation (-1), and at 7, and 14-days post-inoculation. All calves were euthanized on day 14 and presence of liver abscesses was evaluated via gross pathology. Liver samples were flash frozen and total RNA isolated via RNeasy Fibrous Tissue Mini Kit (Qiagen) for RNA sequencing analysis (Illumina NovaSeq 6000). DESeq2 R package was used for differential gene expression analysis (DEG) considered as $P \leq 0.05$ and log2FC ≥ 1. DEGs were evaluated for GO and KEGG enrichment analyses.

Results: Of the 10 calves inoculated, 3 developed liver abscesses (LA), while 7 did not (no-LA). A total of 968 DEG were identified among all experimental groups. Notably, on days -1 , 7 and 14, tissue from LA livers had greater expression of *BOLA-DQB* compared to the no-LA ($P < 0.01$). This gene is specifically associated with the MHC II region, which can be expressed by dendritic cells and macrophages and may play a role in the initial immune response. Genes associated with inflammation and tissue remodeling, such as *CHI3L1* ($P < 0.01$) and *DNTT* ($P < 0.001$) were downregulated in CON in comparison to LA group. Of note, the expression of genes like *SLC22A15* ($P < 0.01$), associated with the transport of toxins, and *SESN3* ($P < 0.01$), which is activated in response to cellular stress conditions such as inflammation, were upregulated in LA but not in CON. Interestingly, no significant gene expression differences were observed on day 14 between the CON and no-LA groups, unlike day 7.

Conclusions: These findings indicate that LA development leads to changes in hepatic tissue gene profile, particularly an upregulation of genes associated with inflammation and adaptive immune response. Our transcriptome data suggest that LA pathogenesis triggers a distinct gene expression response compared to infected animals that do not develop an abscess. These results offer valuable novel insights on key genes regulating LA pathogenesis in bovine.

Financial Support: The study was supported by the Foundation for Food & Agriculture Research (FFAR; Grant ID: ICASALAWG-0000000057). The content of this publication is solely the responsibility of the authors and does not necessarily represent the official views of FFAR.

Notes:

P062 - Characterization of bovine CD4 T cell memory responses following BCG vaccination

Haley Greiman[1, 2], Ellie Putz[2], Steven Olsen[2], Paola Boggiatto[2]

[1]Iowa State University, [2]National Animal Disease Center. hgreiman@iastate.edu

Session: Immunology, **2023-01-22, 6:00 - 8:00**

Objective: *Mycobacterium bovis*, the causative agent of bovine tuberculosis (bTB), is a zoonotic pathogen that contributes to economic losses in the cattle industry and poses a public health risk worldwide. Bacillus Calmette-Guerin, or BCG, is a live attenuated strain of *M. bovis* that is considered a potential vaccine candidate against bTB. However, BCG affords widely variable levels of protection against challenge and interferes with diagnostic tuberculin skin testing, and as such, it is not currently approved for use as a livestock or wildlife vaccine. Many efforts have been made to develop bTB vaccines that are reliable and do not interfere with diagnostic testing, but BCG continues to be the most effective option. Previous work has shown that a T helper 1 immune response is essential for protection against virulent *M. bovis* infection, and that $CD4^+$ central and effector memory T cells are induced following virulent challenge. Therefore, we sought to identify and characterize antigen specific $CD4^+$ central and effector memory T cell responses in BCG vaccinated cattle that may contribute to protection against *M. bovis*.

Methods: An *in vitro* recall response assay was conducted using peripheral blood mononuclear cells (PBMCs) isolated from BCG vaccinated cattle. Briefly, PBMCs were incubated for seven days with complete RPMI media, purified protein derivate of *M. bovis* (PPDb), or Concanavalin A. Proliferation, cell surface marker expression, and cytokine production of antigen stimulated $CD4^+$ T cells were measured concurrently using flow cytometry in order to characterize BCG-specific memory T cell subsets. Data were analyzed using a simple linear regression, and pairwise comparisons of Least Squares means were conducted to determine significant differences between stimulation conditions and cell types at each time point following vaccination.

Results: Significant numbers of PPDb-specific $CD4^+CD45RO^+CCR7^-CD62L^-$ T cells ($P \leq 0.05$), characterized as effector memory T cells, were observed up to 20 weeks following BCG vaccination. PPDb-specific $CD4^+CD45RO^+CCR7+CD62L^+$ T cells, characterized as central memory T cells, were also observed in the peripheral blood, though cell numbers were significant only at early time points following vaccination. Interestingly, a vast majority of both PPDb-specific central and effector memory T cells were capable of producing interferon gamma.

Conclusions: Collectively, the results presented indicate that vaccination of cattle with BCG induces significant development of PPDb-specific memory $CD4^+$ T cells with effector phenotypes consistent with a T helper 1 immune response. The exact role of the PPDb-specific, T helper 1 polarized, effector memory T cells in the protection against *M. bovis* requires further experimentation. However, the characterization of the kinetics of the bovine memory response induced by BCG vaccination provides insight to the development of improved vaccines for the control of bovine tuberculosis.

Financial Support: Funding provided by USDA ARS and Iowa State University.

Notes:

P063 - Transcriptome analysis of high-risk stocker cattle associates consistent inflammation-related pathways with BRD

Haleigh Prosser[1], Bradly Ramirez[1], Robert Valeris-Chacin[1], William Crosby[2], Paul S. Morley[1], Amelia Woolums[2], Brandi Karisch[3], Matthew Scott[1]

[1]Veterinary Education, Research, and Outreach Program, Texas A&M University and West Texas A&M University, [2]Department of Pathobiology and Population Medicine, Mississippi State University, [3]Department of Animal and Dairy Sciences, Mississippi State University. hmprosser1@tamu.edu

Session: Immunology, **2023-01-22, 6:00 - 8:00**

Objective: Bovine respiratory disease (BRD) is the costliest disease complex within the North American cattle industry. To counter this, cattle producers often administer tulathromycin to cattle as metaphylaxis to lower the risk of BRD morbidity. Utilizing RNA sequencing, we sought to evaluate the interaction of tulathromycin metaphylaxis and clinical BRD treatment outcome on host gene expression.

Methods: On day 0 of a 70-day study period, 84 commercial heifers (mean weight = 239 kg, s.d. = 16 kg) were randomly enrolled into two treatment groups: metaphylaxis with tulathromycin according to label instructions (META, n = 42) or negative control (CONT, n = 42). Scheduled jugular blood samples were collected on days 0, 7, 14, and 21, and additional jugular blood samples were collected at time of treatment from cattle diagnosed with BRD. For each scheduled timepoint, RNA from seven META and seven CONT animals who remained healthy throughout the study were sequenced (HEALTHY, sample n = 56), and RNA from samples collected at treatment were sequenced (TREAT n = 16, sample n = 32) via NovaSeq 6000 (150bp PE; ~40M reads/sample) and HISAT2/StringTie2 assembly. Differentially expressed genes (DEGs) were identified via edgeR glmLRT testing (FDR < 0.05); two analyses were retained for functional enrichment of DEGs via KOBAS-i (FDR < 0.05): an equal case-control analysis of 28 animals at days 0, 7, and 14, and a comparison between day 0 and time of treatment in cattle treated for BRD.

Results: There was no differential gene expression at day 0 between cattle which developed BRD or not, regardless of metaphylaxis administration. When evaluating cattle at days 7 and 14 in the case-control analysis, 1,410 and 136 DEGs were identified, respectively; 47 were shared, involved in increased neutrophil degranulation, decreased biosynthesis of specialized proresolving mediators (SPMs), and decreased regulation of the immune system in BRD cattle. When comparing cattle who would later develop BRD at day 0 to when they received BRD treatment, 89 and 471 DEGs were identified at the first treatment in cattle treated once or treated twice or more, respectively; these DEGs significantly enrich for the same aforementioned pathways. Further, 271 DEGs were identified at subsequent treatments in cattle who were treated twice or more times, enriching for the same aforementioned pathways.

Conclusions: Our results displayed a genomic pattern in cattle experiencing BRD, regardless of metaphylactic tulathromycin administration, hallmarked by decreased gene expression for SPM production and immune regulation and increased neutrophil degranulation. These findings indicate a consistent inflammatory response at time of treatment. Moreover, the at-treatment gene expression in cattle displaying more severe BRD (treated 2+) seems to closely resemble that of cattle that respond to first treatment, suggesting that inflammatory patterns are similar in many BRD cases, regardless of treatment frequency.

Financial Support: USDA NIFA AFRI Competitive Grant #2019-67017-29111 & 2020-67016-31469.

Notes:

P064 - The effect of pair housing in dairy calves on immune function and stress response to weaning and social mixing

K.N. Pachniak[1], L.S. Caixeta[1], M.C. Cheeran[1], S.M. Godden[1], N.R. Noyes[1], W.A. Knauer[1]

[1]Department of Veterinary Population Medicine, University of Minnesota. schr1123@umn.edu

Session: Immunology, **2023-01-22, 6:00 - 8:00**

Objective: A majority of dairy operations in the US house calves individually prior to weaning and social mixing. As a result of this strategy, dairy calves experience increased amounts of stress during the weaning and social mixing transition, impacting calves' immune function. This study assessed the impact of housing type prior to weaning on stress and immune response during weaning and post weaning social mixing.

Methods: Thirty Holstein heifer calves were enrolled in one of two treatment groups within 7 days after birth: pair (n=20; 10 pairs) or individually (n=10) housed groups. Calves in both treatment groups were managed in outdoor hutches following the same standard operating procedures. All calves were weaned from days 43-56 and spent an additional seven day adjustment period, post-weaning, in the hutches. Calves were socially mixed within their treatment group on day 63 of life. Whole blood was collected one week prior to the start of weaning, on days 43, 44, 46, 48, and 50 (relative to weaning), and again on days 63, 64, 66, 68, and 70 (relative to social mixing). The blood samples were processed and stored at -80 ℃ for future analysis.

Results: White blood cells will be harvested from the whole blood, and be used for the determination of gene expression through qPCR. The stress cortisol and cytokine concentrations will be measured using radioimmunoassay and ELISA assays, respectively. The statistical analysis will be completed using Microsoft Excel, GraphPad Prism 10, and R-Studio (Build 524). We expect to see significant differences between the individually and pair housed treatment groups in stress cortisol response, immune gene expression (IL1β, TNFα, MPO, SELL, TLR4), and cytokine response to LPS stimulation (IL1β, TNFα, IL6). The final analysis will be completed December 2024.

Conclusions: This experiment will be used to better understand the impact of housing management on immunological development and response in relation to the influx of stress during the weaning and post-weaning social mixing transition phases in dairy calves.

Financial Support: This work was supported by the USDA National Institute of Food and Agriculture through project number MIN-62-139, accession no.7002806, multistate project number NC1029.

Notes:

P065 - Epithelial-dendritic cell cross-talk during bovine cryptosporidiosis

Deborah Molehin[1], Brina S. Lopez[1]

[1]Midwestern University. blopez@midwestern.edu

Session: Immunology, **2023-01-22, 6:00 - 8:00**

Objective: Cryptosporidiosis caused by *Cryptosporidium parvum* (*C. parvum*) in cattle remains a disease of global health importance. Characterized by life-threatening diarrhea in neonatal calves, infants, and children, severe clinical disease is rare in mature cattle and adult human populations. Innate immunity, including intestinal epithelial cells (IEC) and dendritic cells (DC), is the first line of defense against *C. parvum* and is critical in determining the outcome of infection. Crosstalk between epithelial cells and DCs is crucial in directing protective immunity against *C. parvum* infections. In this study, we evaluated the role of crosstalk between IECs and DCs in the immune response against C. parvum and how these interactions develop as a function of age.

Methods: Enteroid-derived 2D monolayers were generated on transwells using intestinal crypts obtained from mature cattle. Monocyte-derived DCs (MoDCs) were generated from adult cattle or a 3-month-old calf and cultured in the compartment below enteroid-derived monolayers. The apical monolayer surface was stimulated with either Pam3CSK4 or *C. parvum* sporozoites. Cytokine secretion was quantified using a multiplex cytokine panel. Cell metabolism was analyzed using an Extracellular Flux Seahorse Analyzer. Data were analyzed using a repeated measures ANOVA.

Results: Mitochondrial function of MoDCs following IEC stimulation revealed increased extracellular acidification rate, basal respiration rate, and ATP production, indicating increased energy demands following stimulation of IEC compared with untreated controls. Furthermore, real-time PMA/I stimulation decreased maximum respiration rate and spare respiratory capacity for stimulated conditions, corresponding to mitochondrial collapse and consistent with MoDC maturation. Altered cytokine secretion in stimulated cultures compared to untreated controls was observed.

Conclusions: Using a culture system that mirrors *in vivo* infection, the impact of age on bovine IEC-DC crosstalk to C. parvum was assessed. These results will aid in identifying and developing rational and effective age-specific therapies.

Financial Support: This project was supported by Agriculture and Food Research Initiative Competitive Grant no. 2021-67016-34572; from the USDA National Institute of Food and Agriculture

Notes:

P066 - Programming the immune system for resilience: maternal and neonatal vitamin D supplementation

Corwin D. Nelson[1], Kieran Meade[2], John Driver[3], Jose Santos[1], K.C. Jeong[1], Haipeng Yu[1]

[1]University of Florida, [2]University College Dublin, [3]University of Missouri. cdnelson@ufl.edu

Session: Immunology, **2023-01-22, 6:00 - 8:00**

Objective: Determine the effects of maternal and neonatal vitamin D supplementation on development of immunity and health of calves.

Methods: Pregnant cows (n = 120 at 240 d gestation) will be randomly assigned to receive 0 or 1.5 ug/kg BW of calcidiol as a dietary supplement added. Calves born to those cows will be randomly assigned to receive 0 or 1.5 ug/kg BW calcidiol added to milk replacer resulting in four treatments for calves in factorial arrangement. Transfer of passive immunity, development of cell and antibody mediated immunity, and single cell RNA sequencing of bone marrow cells will be used to assess immune development along with assessment of health and growth measures. Data will be analyzed for main effects of maternal vitamin D and neonatal vitamin D, and the interaction of maternal and neonatal vitamin D treatments using mixed models.

Results: The experiment is ongoing but preliminary results show that maternal calcidiol increased ($P < 0.01$) serum 25-hydroxyvitamin D concentrations of calves, which were correlated with serum IgG1 concentrations and monocyte CD14 expression.

Conclusions: Maternal and neonatal calcidiol supplementation benetifs vitamin D status of calves. The impacts of calcidiol on programming of immunity and long-term health and performance will be assessed.

Financial Support: This project was supported by Agriculture and Food Research Initiative Competitive Grant no. 2023-67015-39734 from the USDA National Institute of Food and Agriculture.

Notes:

P067 - Effects of an innate immune stimulant on dairy calf respiratory health, *Salmonella* shedding, and nasopharyngeal cytokine gene expression

Grace M. Jakes[1], L.D. Sommer[2], J.N. Gotte[2], T.J. Applegate[2], S. Dow[2], S.M. Raabis[2]

[1]Dept. of Microbiology, Immunology, and Pathology, Colorado State University, [2]Dept. of Clinical Sciences, Colorado State University. grace.jakes@colostate.edu

Session: Immunology, **2023-01-22, 6:00 - 8:00**

Objective: Bovine respiratory disease (BRD) is a leading cause of morbidity and mortality in dairy calves. Alternative treatment strategies are needed to reduce antimicrobial use. Innate immune stimulation using toll-like receptor (TLR) agonists has emerged as a strategy to reduce BRD incidence, but the prophylactic effects of this treatment have not been evaluated in pre-weaned dairy calves. Calves that develop enteric disease may be at a higher risk for BRD; however, evidence of direct links between disease processes is limited. *Salmonella* is an important enteric pathogen in dairy calves, but the impact of *Salmonella* infection on the risk of BRD development has not been determined. The objectives of this study were to evaluate the effects of an innate immune stimulant on dairy calf respiratory health, *Salmonella* shedding, and cytokine gene expression.

Methods: 50 pre-weaned Holstein calves were assigned to one of two intranasal dosing groups administered at 1, 2, and 3 weeks of age. Control: 2 mL sucrose diluent (n=25); LTC: 0.1 mL liposome TLR complex (LTC) in 1.9 mL sucrose diluent (n=25). Clinical and ultrasound scores were collected twice per week using the University of Wisconsin-Madison calf health scoring system up to 10 weeks of age. Deep nasopharyngeal swabs (DNPS) were collected on each calf pretreatment, and at 2, 3, 4, and 8 weeks of age. Cytokine gene expression from DNPS was evaluated using RT-qPCR for the bovine cytokines IFN-γ and MCP-1. Fecal screening for *Salmonella* was performed using a standard enrichment protocol followed by PCR amplification for the *invA* gene (a highly conserved gene in *Salmonella* spp. crucial for colonization and commonly used for identification).

Results: There were no differences between treatment groups on the odds of developing clinical or subclinical respiratory disease based on respiratory score and lung ultrasound. Incidence of subclinical respiratory disease was higher than clinical disease presentation, but both of these rates were relatively low (20% and 32% respectively) in this population. *Salmonella* shedding was observed in 22% of calves, though a *Salmonella* positive status did not impact the odds of developing respiratory disease during the study. *Salmonella* shedding status did not differ by treatment.

Conclusions: These results suggest that innate immune stimulation may not improve respiratory disease risk in healthy pre-weaned calves, or that calves may have been overstimulated by the LTC treatment. *Salmonella* shedding was identified in pre-weaned dairy calves; however, links to BRD risk were not identified in this study.

Financial Support: The authors would like to acknowledge Colorado State University College of Veterinary Medicine and Biomedical Sciences (CSU CVMBS) College Research Council Interdisciplinary Pilot Grant for project funding and National Institutes of Health T32 Training Grant for graduate student support.

Notes:

P068 - Cellular and humoral immune responses to persistent colonization of *Brucella abortus* strain RB51 in cattle

Paola Boggiatto[1], Haley Greiman[1], Shollie Falkenberg[2], Ellie Putz[1], Kaitlyn Sarlo-Davila[1], Steven Olsen[1]

[1]National Animal Disease Center, USDA-ARS, College of Veterinary Medicine, Auburn University. paola.boggiatto@usda.gov

Session: Immunology, **2023-01-22, 6:00 - 8:00**

Objective: *Brucella abortus* strain RB51 (RB51) is the commercial vaccine used in the United States and many parts of the world against bovine brucellosis. RB51 was licensed for use in 1996, and it has been shown to be safe and efficacious in cattle. Additionally, it elicits humoral and cellular responses in calves and adult animals. In 2017, an epidemiological trace back investigation performed by the Centers for Disease Control and Prevention (CDC) identified human cases of brucellosis caused by infection with RB51. These infections resulted from the consumption of unpasteurized dairy products, which were traced back to otherwise healthy animals that were shedding RB51 in their milk. The goal of this study was to understand the host cellular and humoral immune response to RB51 in persistently colonized cattle.

Methods: Blood samples were collected at various time points from an RB51 shedder cow and conventional cattle vaccinated with RB51 to analyze the cellular and humoral immune responses to RB51. RB51-specific *in vitro* recall assays followed by flow cytometry analysis and enzyme-linked immunosorbent assays were used to characterize T cell and humoral responses, respectively. Mammary gland secretions were collected periodically to assess RB51 shedding.

Results: We demonstrate that in the presence of persistent RB51 colonization, there is a lack of peripheral anti-RB51 $CD4^+$ T cell responses with a concurrent high anti-RB51 IgG humoral response. This lack of response does not appear to be driven by an exhaustion T cell phenotype. Additionally, revaccination with RB51 does not elicit anamnestic responses in persistently colonized animals. Sustained shedding of RB51 remains present at high levels.

Conclusions: These data would suggest that RB51 persistence is cattle is driven by a lack of $CD4^+$ T cell responses. Persistently colonized animals are not immunologically ignorant to the antigen, as they exhibit very high antibody titers. The presence of anti-RB51 IgG suggests that T cell help was present at some point following initial vaccination. While we did not identify exhaustion as a mechanism for driving the lack of T cell responses, we cannot completely rule it out at this time. By understanding the mechanism(s) that result in RB51 persistence, we aim to develop intervention strategies to improve on the current vaccine and/or prevent vaccination of susceptible animals, which pose a risk to human health.

Notes:

P069 - Dose titration assessing the impact of an intramammary liposome-TLR agonist (LTC) on differential somatic cell count

Emily Leonard[1], Brian Crooker[2], Steve Dow[3], Luciano Caixeta[1]

[1]Department of Veterinary Population Medicine, University of Minnesota, [2]Department of Animal Science, University of Minnesota, [3]Department of Clinical Sciences, Colorado State University

Session: Immunology, **2023-01-22, 6:00 - 8:00**

Objective: Mastitis on dairy farms is commonly controlled using antibiotics, but concerns for antimicrobial stewardship are rising. Another approach to control mastitis is by utilizing immunotherapeutic agents to upregulate the innate immune system in the mammary gland. A liposome-Toll-like receptor (TLR) agonist (LTC) immune stimulant generates synergistic immune activation by combining TLR3 and TLR9 agonists with charged liposomes and a mucosal adhesive agent. Our objective with this pilot project is to determine the lowest effective dose of LTC that induces a mammary gland immune response without signs of clinical mastitis.

Methods: Eight mid-lactation (90 ± 3 DIM) dairy cows, without clinical mastitis and a SCC < 200,000 cells/mL, were assigned to two 4x4 Latin Squares with experimental periods of 7d separated by a 28d interval. Intramammary administration of treatments were randomly assigned to cows and consisted of LTC (0.125mL [HI], 0.06mL [MED], 0.03mL [LOW], and 0mL [CON]) diluted to a 10mL volume using LTC diluent (Tris buffered, 10% sucrose, pH 7.2). All treatments were administered to the right rear quarter. On the first day of each experiment period, prior to treatment infusion, a milk sample from each quarter was collected. Additional samples were collected every 12h during the following 7d. The effect of LTC on the total leukocyte count (TLC), neutrophils (NEU), lymphocytes (LYM), and macrophages (MAC) during the study period was determined using a linear mixed-effects model accounting for repeated measures.

Results: There is no evidence that HI, MED, or LOW treatments differed from CON for counts of TLC (P=0.28), NEU (P=0.19), LYM (P=0.48), or MAC (P= 0.36). The MED treatment reveals a peak in TLC, NEU, LYM, and MAC counts between 72h and 84h post-treatment.

Conclusions: The MED treatment is the most promising effective dose of LTC, but further analyses are needed. We will explore this dose further by measuring cytokine gene expression using targeted RT-PCR to assess the innate immune response in the mammary gland.

Financial Support: This work is funded by the U.S. Department of Agriculture, National Institute for Food and Agriculture (2021-67015-34558).

Notes:

P070 - Cytochrome P450 pathway dynamics during experimental bovine coliform mastitis

J.D. Wilson[1], T. Nyholm[2], D. Zimmerman[2], J. Lippolis[2], V. Mavangira[1]

[1]Veterinary Microbiology and Preventative Medicine, Iowa State University, [2]USDA-ARS. joew@iastate.edu

Session: Immunology, **2023-01-22, 6:00 - 8:00.**

Objective: Systemic bovine mastitis caused by *Escherichia coli* (*E. coli*) is characterized by altered production of lipid inflammatory mediators including those derived from the cytochrome P450 (CYP450) pathway. We previously documented altered CYP450 pathway metabolites and an increased activity in their degradative pathway known as soluble epoxide (sEH) in cows with natural coliform mastitis suggesting that manipulating this pathway might mitigate disease severity. We assessed the temporal dynamics of sEH during coliform mastitis to determine its validity as a therapeutic target using a controlled experimental mastitis challenge. We hypothesized that CYP450 and sEH activity alterations are influenced by stage of clinical disease and differ by mammary quarter infection status.

Methods: Experimental mastitis was induced in six lactating Holstein dairy cows through intramammary infusion (IMI) of the right front quarter with 400 CFU of *E. coli* (P4 strain) suspended in phosphate buffered saline (PBS). For vehicle control, an equivalent PBS volume was infused into the left rear quarter. Clinical assessments including appetite and rectal temperature monitoring, and sampling (quarter milk and blood) were performed before IMI (t=0), 6, 12, 24, and 48 hours. Milk, plasma, and PBMCs were assessed for oxygenated lipid metabolites (oxylipids) to determine CYP450 and sEH activity. The sEH activity was also determined in isolated milk somatic cells, PBMCs, and tissue samples via ex-vivo enzyme activity assay. All variables were analyzed for paired means (infected vs. control quarters) and for temporal changes with repeated measures with significance set at α=0.05.

Results: Four of 6 cows became hyperthermic (rectal temperature≥103.9°F) and had decreased in feed intake 12 hrs. post-IMI. Increased production of CYP450-derived oxylipids was detected at 6 and 12 hrs. and increased downstream products up to 12hrs in milk regardless of quarter. The sEH index indicated increased activity in the mammary gland at 6 hrs. ($P<0.01$) and a numerical increase by 12 hrs. ($P=0.056$) compared to 6 hrs. in the infected quarter. The degree of CYP450 and sEH metabolite alterations were greater in the infected than control quarter. No differences were detected for oxylipid metabolites in plasma or PBMCs. Further, direct sEH ex-vivo activity was increased at 6 hrs. compared to any other time points with no differences between quarters. Direct sEH activity did not differ in PBMCs over time.

Conclusions: The CYP450 and sEH pathways in cows with experimental coliform mastitis increased only in the mammary gland shortly after infection and appeared to precede systemic clinical signs. The impact of CYP450 and sEH activity during bovine coliform mastitis should be further explored to justify interventions targeting these pathways.

Financial Support: This project was supported by Agriculture and Food Research Initiative Competitive Grant no. 2020-67015-38956 from the USDA National Institute of Food and Agriculture.

Notes:

P071 - Upregulation of antileukoproteinase in neonatal foals and postpartum mares

Camille M. Holmes[1], Bettina Wagner[1]

[1]Cornell University. cmh335@cornell.edu

Session: Immunology, **2023-01-22, 6:00 - 8:00**

Objective: Antileukoproteinase (SLPI) is a pleiotropic molecule secreted widely throughout the body, involved in the immune response as an upholder of homeostatic conditions. Recently developed monoclonal antibodies (mAbs) from our group have facilitated the characterization of SLPI in equine. It is produced by a broad cell range including neutrophils, monocytes, and epithelial cells, and is detectable in the serum, saliva, colostrum, and mucosal secretions of horses when they are healthy, with increased secretion during periods of disease. High SLPI values in the colostrum prompted us to measure maternal and neonatal SLPI concentration from the final weeks of pregnancy through weaning, aimed at better understanding dynamics in parturition and early life.

Methods: Serum samples were collected by jugular venipuncture, aliquoted, and stored at -20°C until analysis. Samples were taken biweekly from late gestation mares, up until parturition. Following parturition, a day 0 serum sample was taken from both the mare and foal, and a day 0 colostrum sample was also collected. Frequent sampling during the first month of life included days 2, 6, 10, and 14, with an additional milk sample taken on day 10. Samples were then collected monthly until the foals reached 7 to 8 months of age, at which they were weaned. Serum and milk samples were measured by fluorescent bead-based assay, and a recombinant SLPI of known concentration was used to generate a standard curve and quantify samples.

Results: Mares and foals showed a peak in secretion on day 0 ranging from 383.0 to 1258 ng/mL in foals and 482.5 to 21,300 ng/mL in mares. Mares showed significantly higher secretion on day 0 postpartum and day 2 postpartum compared to normal SLPI serum values in adult horses ($p<0.0001$). Then, serum SLPI concentrations decreased and reached the normal adult range by day 6 postpartum. Moreover, the increase of maternal SLPI could be quantified prepartum in a separate cohort of mares, with SLPI serum values significantly higher ($p=0.0039$) within the two weeks prepartum, compared to paired samples taken one week postpartum. Meanwhile, foals showed prolonged high secretion until 90 days of age. Foal serum SLPI concentrations were significantly higher than those in their dams on days 6 ($p<0.0001$), 14 ($p<0.0001$), 30 ($p<0.0001$), 60 ($p=0.0059$), and 90 ($p=0.0007$) of age. After 4 months of age, foal SLPI decreased to normal adult levels. Comparison of colostrum SLPI concentrations to those in foal serum taken on day 2 of age did not correlate.

Conclusions: Together this suggests that SLPI plays an important role in mares during foaling and in the early life of foals. However, the lack of correlation between colostrum and foal serum SLPI indicates that maternal transfer is not the dominant or sole source of systemic SLPI concentrations in neonatal and young foals. Work is ongoing to compare neonatal SLPI values in healthy versus septic foals.

Financial Support: Harry M. Zweig Memorial Fund for Equine Research

Notes:

P072 - Swine immune reagent development and characterization for monitoring pig immune status and biomedical research

Gourapura J Renukaradhya[1], Crystal Loving[2], Joanna LaBresh[3], Raksha Suresh[1], Milan Royer[2], Kristen E Walker[2], Jovan Aquino[2], Joan K Lunney[2]

[1]The Ohio State University, [2]USDA-ARS, [3]Kingfisher Biotech, Inc. gourapura.1@osu.edu

Session: Immunology, **2023-01-22, 6:00 - 8:00**

Objective: The USDA Swine Immune Toolkit Initiative has been involved in generating priority immune reagents for understanding swine immunity, facilitating biomedical research, and pipelining resultant tools to marketing.

Methods: With the help of commercial partners, we expressed targeted swine immune molecules as soluble proteins using a yeast expression system and then produced panels of monoclonal antibodies (mAbs) against each target. Once developed each set of mAbs was compared for reactivity in assays of quantitation of target protein expression.

Results: We successfully generated panels of mAbs reactive to porcine IL-6, IL-13, IL-28B, CXCL10, and BAFF. We determined mAb reactivity to orthologous proteins for most panels of mAbs. A sensitive sandwich ELISA is now available for IL-13 and CXCL10; other targets are being screened for best mAb pairs. Reactivity tests for intracellular staining of porcine immune cells using flow cytometry assay for labeled porcine α-CXCL10 was successful and is underway for α-IL-6, α-IL-13 and a-IL-28 mAbs using different cell stimulation conditions. Immunohistochemistry analyses for binding of α-CXCL10 mAb on formalin fixed pig lymph nodes and spleen tissues was confirmed successfully. Analysis of a large panel of commercial α-human cluster of differentiation (CD) antigen mAbs for cross-reactivity with porcine cells is ongoing. For each target, our goal is to provide the veterinary community with new commercial reagents and standardized assay techniques for their research efforts. Finally, efforts are proceeding to characterize the swine leukocyte antigen (SLA) tetramers for antigen presentation studies.

Conclusions:
Tools and reagents generated by this project will undoubtedly advance our understanding of swine immune responses to disease and vaccines and enhance the use of swine for biomedical research efforts.

Financial Support: Supported by USDA-NIFA AFRI grant # 2019-67015-29815 and USDA ARS project 8042-32000-117.

Notes:

P073 - Characterizing the changes in the antigen-specific immunoglobulin glycosylation in gilts throughout pregnancy

Diane Mutumwinka[1], Poja Choudhary[1], Heather Wilson[1], George Mutwiri[1]

[1]University of Saskatchewan. lkr324@usask.ca

Session: Immunology, **2023-01-22, 6:00 - 8:00**

Objective: Understanding glycan modification of immunoglobulins (Igs) in pregnancy is critical as a recent publication in mice indicated that the deacetylation of sialic acid on maternal serum and colostrum IgGs can alter the cell-mediated immune function of B cells in neonatal pups. It was elucidated that the expression of sialic acid acetyl esterase (SIAE), that removes acetyl groups from sialic acids on Igs, was induced in later, but not early, pregnancy. The maternal IgGs with deacetylated sialic acids may then bind to the B cells in the pups and possibly impact the function of these cells. It is currently unknown whether Igs in other species undergo similar changes in glycosylation patterning during pregnancy. Because pigs do not receive maternal antibodies *in utero*, we can use pigs as a model to clarify the role of glycosylation of antibodies in neonatal immunity. Our objective is to characterize the changes in glycosylation patterning in maternal serum Igs during pregnancy and elucidate their impact on the immune responses of the dam and the neonates.

Methods: To establish that the SIAE enzyme is present in serum, we performed Western blot analysis on total proteins from serum obtained at day 0, day 30 and day 100 gestation from gilts immunized with a porcine epidemic diarrhea virus spike protein (PEDVS) vaccine and piglets (negative control). To determine if the gene coding for SIAE enzyme changes in expression over pregnancy, we obtained peripheral blood mononuclear cells (PBMCs) at the indicated time points above. Finally, to quantify global changes in glycosylation patterning in PEDVS-specific serum IgG, we used Protein-A agarose columns plus PEDVS antigen to immunoprecipitate the IgGs from gilts prior to pregnancy and at day 100 gestation. These PEDV-specific antibodies were then sent for lectin High Performance Liquid Chromatography (HPLC) analysis.

Results: Western blot analysis indicated that SIAE enzyme was present in serum in early and late pregnancy at comparable levels. PCR analysis is on-going, but we validated the specificity of the SIAE primers. Preliminary HPLC analysis indicated that the acetylation of the sialic acid on the IgGs did not appear to change over time. However, we observed a modest increase in the presence of N-glycans on IgGs from the day 100 pregnancy samples relative to the day 0 samples.

Conclusions: Western blot analysis and the HPLC data suggest that there are no changes in the level of the SIAE protein or the acetylation of sialic acids on serum maternal IgGs over pregnancy. However, these changes and the modest change in the N-glycosylation patterning of the PEDVS-IgG should be confirmed with more biological replicates. The enzyme responsible for the increase in N-glycosylation of the maternal IgGs should be investigated further at the gene, protein, and functional levels. This work will provide critical insights into the glycosylation patterning of IgG isotypes in pregnant mammals (using pigs as the example) in serum and the impact on neonatal cells.

Financial Support: Financial support was provided by (NSERC) Discovery Grant to George Mutwiri. VIDO also receives operational funding from the Government of Saskatchewan through Innovation Saskatchewan and the Ministry of Agriculture and Canada Foundation for Innovation through Major Science Initiatives.

Notes:

P074 - The WC1 multigenic array in the immune response to zoonotic pathogens in agricultural species

Lauren Le Page[1], Oriana Attridge[1], Cynthia L. Baldwin[1], Janice C. Telfer[1]

[1]University of Massachusetts Amherst. telfer@umass.edu

Session: Immunology, **2023-01-22, 6:00 - 8:00**

Objective: Gamma delta T cells are a crucial component of the immune response to a number of increasingly relevant and largely zoonotic pathogens to which efficacious vaccination is lacking. In ruminants and swine, gamma delta T cells represent a major population of peripheral blood and epithelial tissue-resident lymphocytes. Upon activation, gamma delta T cells elicit a variety of effector functions and play an indispensable role of orchestrating the downstream immune response. These characteristics make gamma delta T cells a promising candidate for recruitment by vaccination. WC1 is expressed as a multigenic array on gamma delta T cells in ruminants. In cattle there are 13 unique WC1 genes (WC1-1 to WC1-13), each comprised of 6-11 SRCR domains that selectively bind unprocessed antigen in a manner that resembles a pattern recognition receptor (PRRs). WC1 functions as a hybrid PRR and co-receptor for the gamma delta TCR. We hypothesized that a swine WC1 multigenic array has co-evolved with pathogens and thus sought to characterize its diversity and ligand-binding potential.

Methods: We used 5' and 3' RACE and RT-PCR to isolate full-length cDNA clones, and the MAKER annotation pipeline to annotate *Sscrofa11.1.* Binding pull-down assays were carried out with recombinant WC1 SRCR protein and fixed bacteria.

Results: We isolated cDNA and genomic evidence for a porcine WC1 multigenic array consisting of 9 genes (WC1-1 to WC1-9), each encoding 6 SRCR domains with unique pathogen binding potential. We annotated *Sscrofa11.1* for sequence derived from full-length cDNA transcripts representing the 9 porcine WC1 genes. We mapped 7 of the 9 genes, leaving two (WC1-4 and WC1-8) unplaced in the current assembly. We defined three subpopulations of porcine gamma delta T cells based on expression of WC1 and CD2, and characterized WC1 antibody reactivity. Finally, we confirmed that porcine WC1 SRCR domains are capable of directly binding whole fixed bacteria including *Leptospira spp* and *Mycobacterium bovis*.

Conclusions: Porcine WC1 exists as a multigenic array, is expressed on gamma delta T cells and binds to pathogens.

Financial Support: This project was supported by Agriculture and Food Research Initiative Competitive Grants no. 2015-06970 and 2021-06958 from the USDA National Institute of Food and Agriculture.

Notes:

P075 - Tissue localization and acute peripheral transcriptomic response following Salmonella enterica Typhimurium infection of pigs

Crystal L Loving[1], Jayne E Wiarda[1, 2], Kristen A Byrne[1], Christopher L Anderson[1]

[1]USDA-Agricultural Research Service, [2]Oak Ridge Institute for Science and Education. crystal.loving@usda.gov

Session: Immunology, **2023-01-22, 6:00 - 8:00**

Objective: Most foodborne isolates of *Salmonella* cause an acute, self-limiting, transient disease in swine, though it can be recovered from lymphoid tissues for weeks following infection. A clear understanding on the immune response to acute and persistent *Salmonella* infection in pigs is a necessary first step to developing targeted intervention strategies for preharvest reduction. The main objectives were to understand the acute and transitioning peripheral immune response of pigs to *Salmonella* inoculation and evaluate early *Salmonella* localization to immune cells in tissues.

Methods: Groups of pigs (n=5 per timepoint) were oronasally inoculated with *Salmonella enterica* serovar Typhimurium (ST) or mock-inoculated and blood collected at 2 and 8 days post-inoculation (dpi). Necropsies were performed on day 2 and 8 dpi to collect tonsil, cecum, and cecal contents. Whole blood RNA was extracted and sequenced. PBMC were isolated, cryopreserved, thawed, and subjected to partitioning and labeling (10X Genomics), and sequencing. ST enumeration was performed on cecal contents and tonsils. ST abundance and localization in tonsil and cecum was assessed using immunohistochemistry (IHC), including association with myeloid cells.

Results: ST was recovered from tonsil at 2 and 8 dpi, but only on 2 dpi from cecal contents. However, IHC revealed little ST in tonsil cells, suggesting extracellular ST detection using culture. ST-positive cells were detected in cecum. In whole blood, more than 400 genes were increased in expression over controls on 2dpi, but by 8dpi no significant differences were noted between mock and ST groups. Genes associated with macrophage activation and myeloid leukocyte activation were increased (GO terms). Single-cell RNA-sequencing analysis revealed specific cell types responding to ST infection, mostly resolved by 8 dpi.

Conclusions: ST inoculation caused a transient, but robust peripheral immune activation. Deciphering the immune response, particularly at the single-cell level reveaed responses of specific cell types. IHC paired with culture methods suggested differences between detection of extracellular and intracellular ST, particularly in tissues relevant for persistence.

Financial Support: Research was funded by USDA-ARS CRIS project #5030-3200-225-00D and an appointment to the Agricultural Research Service (ARS) Research Participation Program administered by the Oak Ridge Institute for Science and Education (ORISE) DE-SC0014664.

Notes:

P076 - Evaluating immunological reagents and expanding molecular assays across ruminant species

Jamison R. Slate[1, 2, 3], Paola Boggiatto[1, 2], Mitch Palmer[1, 2]

[1]Iowa State University, [2]USDA-Agricultural Research Service, [3]Oak Ridge Institute for Science and Education. jrslate@iastate.edu

Session: Immunology, **2023-01-22, 6:00 - 8:00**

Objective: The World Health Organization estimates that over 60% of emerging infectious diseases reported globally are zoonotic in origin, thus leaders in the One Health initiative have come together to generate the One Health Zoonotic Disease Prioritization Process: a process to prioritize zoonotic diseases of greatest concern and develop action plans to address potential spillover events (or disease transmission from one species to another). As seen from the coronavirus disease 2019 (COVID-19) pandemic, spillover events can have devastating impacts on the human health sector, but they are also concerning for the animal health and agricultural sectors as pathogens adapt to new hosts. Therefore, it is imperative to study disease susceptibility, progression, and dissemination in a variety of livestock, wildlife, and companion animal species. However, the lack of reagents and assays to study diseases of concern across species has become a limiting factor for researchers. Subsequently, the objective of this study was to determine if reagents and assays developed for cattle (*Bos taurus*), a well-characterized agricultural ruminant, could be used in other less studied ruminants like bison (*Bison bison*), white-tailed deer (*Odocoileus virginianus*), and elk (*Cervus canadensis*).

Methods: Blood from ruminant species was collected via the jugular vein in ACD tubes, and PBMCs were isolated using Histopaque (-1077 for cattle, deer, & bison; -1083 for elk) density centrifugation. PBMCs were seeded into flat bottom 96-well culture plates (1 x 10^6 cells/well) and 48-well culture plates (2 x 10^6 cells/well) for supernatant or lysate collection, respectively. PBMCs were rested in 20% cRPMI media overnight (37°C, 5% CO_2). Cells were stimulated (No stimulation, Concanavalin A (ConA) [5 µg/mL], PMA/Ionomycin [1x], LPS [0.5 µg/mL], and Resiquimod [1 µg/mL]) for 6 hours for cell lysates or 48 hours for supernatant collections. Supernatants were used to test the cross-reactivity of Bovine IFNγ and IL-17A ELISAs; cell lysates were used to isolate RNA (via TRIzol extraction) and prepare complementary DNA (cDNA) for SYBR Green RT-qPCR analysis. Primers for each species were designed using the Primer3 web tool and included the following list of genes: *ACTB, GAPDH, IFNG, IL17A, NFKB1, STAT1, STAT3, IRF3, NLRP3, TNF,* and *IL12B*.

Results: Using BLASTP, all species had high predicted sequence homology compared to *Bos taurus* IFNγ (bison -99%, deer -96%, & elk -95%) and IL-17A (bison -99%, deer -99%, & elk -99%), and ELISA cytokine quantification reflected a range of antibody cross-reactivity. RT-qPCR results showed changes in gene expression based on stimulation conditions (ConA: mitogenic stimulation; PMA/Ionomycin: pancellular activation; LPS: TLR4 signaling, emulating bacterial stimulation; Resiquimod: TLR7/8 signaling, emulating viral stimulation), as well as similarities and differences across species.

Conclusions: Studying zoonotic pathogens and spillover events can be difficult due to limited reagents; however, we propose using a One Health approach to implement reagents and assays across similar species to help overcome some of these research hurdles. Herein, we show reagents and assays for agricultural ruminants (cattle) could be used to characterize immune responses in wild ruminant species of concern (bison, deer, and elk) *in vitro*.

Financial Support: This research was supported by funding from the USDA.

Notes:

P077 - Humoral responses of elk to experimental challenge

Paola Boggiatto[1], Ellie Putz[1], Steven Olsen[1]

[1]USDA-Agricultural Research Service. steven.olsen@usda.gov

Session: Immunology, **2023-01-22, 6:00 - 8:00**

Objective: Characterize humoral responses of naive and vaccinated elk to experimental challenge with virulent *B. abortus* strain 2308.

Methods: Elk heifers, approximately 8-10 months of age, were vaccinated with saline (control) or 2 x 10^{10} CFU of *Brucella abortus* strain RB51 (RB51) vaccine. Immunologic responses after inoculation demonstrated significantly greater (P<0.05) antibody responses to RB51 antigens in vaccinates as compared to controls. Pregnant elk (n=8/trt) were experimentally challenged at the beginning of the third trimester with 10^7 CFU of *B. abortus* strain 2308 (2308) administered on the conjunctival surface. Sera, conjunctival fluid, and fecal samples were obtained at regular intervals after experimental challenge and evaluated for IgG or IgA antibody responses to 2308 or RB51 an fluorescence polarization (FPA), standard tube agglutination (STAT), and ELISA assays. Controls and vaccinates were necropsied within 1-3 weeks of parturition (2 controls, 3 vaccinates), or at 14 to 17 wks after parturition (6 controls, 5 vaccinates).

Results: No difference (P>0.05) in abortion or infection after experimental challenge was observed between treatments. Both treatments demonstrated seroconversion to 2308 on standard tube agglutination (STAT), fluorescence polarization assay (FPA) and an IgG ELISA assay but vaccinates demonstrated significant reductions in responses on the FPA and ELISA assays at later sampling times after experimental challenge. Elevations in IgG and IgA responses to 2308 were detected in conjunctival fluid but not in feces. Conjunctival fluid demonstrated an amnestic IgG response, but not IgA, to RB51 antigens which was not observed in sera.

Conclusions: Our data suggests that vaccinated elk may demonstrate more rapid declines in IgG humoral responses after experimental infection and amnestic IgG responses to the vaccine strain in conjunctival fluid. Although STAT demonstrates a minimal decline over time in both treatments, we observed reduced FPA responses in vaccinates in later sampling times. This observation has regulatory implications as elk titers declined despite persistence of *Brucella* infection. The observations related to humoral responses in conjunctival fluids raises intriguing questions on mucosal immunity in elk.

Notes:

P078 - Protective effects of Green tea extract against oxidative-stress mediated airway inflammation and mucus hypersecretion in asthmatic mice model

Jeong-Won Kim[1], Jin-Hwa Kim[1], Ji-Soo Jeong[1], Chang-Yeop Kim[1], Seo-Ryeong Lee[1], Eun-Hye Chung[1], Eui-Ju Hong[1], Tae-Won Kim[1], Je-Won Ko[1]

[1]College of Veterinary Medicine, Chungnam National University. rheoda@cnu.ac.kr

Session: Immunology, **2023-01-22, 6:00 - 8:00**

Objective: We investigated whether green tea extract (GTE) prevents asthma pathogenesis in an ovalbumin (OVA)-induced mouse model of asthma.

Methods: There were five groups with eight females per group were used: normal control (PBS sensitization/challenge), OVA (OVA sensitization/challenge), DEX (perorally treated with 3 mg/kg/day dexamethasone and OVA sensitization/challenge), GTE100, and GTE400 (perorally treated with 100 or 400 mg/kg/day GTE, respectively, and OVA sensitization/challenge).

Results: The GTE100 and GTE400 groups showed a decrease in airway hyperresponsiveness ($p < 0.01$) and the number of inflammatory cells ($p < 0.01$) in the bronchoalveolar lavage fluid (BALF) compared to the OVA group. GTE treatment also reduced interleukin (IL)-13 (GTE100, $p < 0.05$; GTE400, $p < 0.01$), IL-5 ($p < 0.01$), and IL-4 (GTE100, $p < 0.05$; GTE400, $p < 0.01$) levels in the BALF and OVA-specific immunoglobulin E ($p < 0.01$) levels in the serum compared to those in the OVA group. GTE treatment decreased OVA-induced mucus secretion ($p < 0.01$) and airway inflammation (GTE100, $p < 0.05$; GTE400, $p < 0.01$). In addition, GTE suppressed the phosphorylation of mitogen-activated protein kinases; jun N-terminal kinase (GTE100, $p < 0.05$; GTE400, $p < 0.01$), extracellular signal-regulated kinase ($p < 0.01$), and p38 (GTE100, $p < 0.05$; GTE400, $p < 0.01$), which generally occurs after exposure to OVA. GTE administration also reduced matrix metalloproteinase-9 (GTE100, $p < 0.05$; GTE400, $p < 0.01$) activity and protein levels.

Conclusions: GTE effectively inhibited asthmatic respiratory inflammation and mucus hyperproduction induced by OVA inhalation. These results suggest that GTE has the potential to be used for the treatment of asthma.

Financial Support: This work was supported by the National Research Foundation of Korea (NRF) grand funded by the Korea government (MSIT) (RS-2023-00211435314820926400001)

Notes:

P079 - Anti-IL-10 does not affect broiler systemic IL-10 concentrations in models with or without Salmonella Typhimurium

K. Fries-Craft[1], S. Schmitz-Esser[1], E.A. Bobeck[1]

[1]Department of Animal Science, Iowa State University. kfcraft@iastate.edu

Session: Immunology, **2023-01-22, 6:00 - 8:00**

Objective: During infection, *Eimeria spp.* induce host production of IL-10 to evade immune responses, resulting in coccidiosis and establishing the ideal environment for necrotic enteritis due to secondary infection with *Clostridium perfringens* (CP). Dietary anti-IL-10 may be able to protect bird health during disease; however, the specific effects on host immunity and necrotic enteritis development are unknown. Additionally, necrotic enteritis challenge models may rely on early inoculation with other bacteria, like *Salmonella* Typhimurium (ST) to improve repeatability. The study objective was to evaluate intestinal lesion score, fecal oocyst shedding, and systemic IL-10 in birds fed anti-IL-10 during coccidiosis and necrotic enteritis challenges using models ± ST.

Methods: Three replicate studies were conducted for 25d using Ross 308 broilers obtained at hatch (20 birds/ raised wire cage) and randomly assigned to diets ± 0.03% anti-IL-10. On d0, birds in replicates 1 and 2 were inoculated with $1*10^8$ colony forming units (CFU) of ST while birds in replicate 3 did not receive ST at placement. In all replicates, blood was collected from 6 birds/ treatment on d14 and half the remainder were orally inoculated with $1.5*10^4$ sporulated oocysts of wild-type *Eimeria maxima* (EM) M6. On d18 and 19, half the *Eimeria*-challenged birds were inoculated with $1*10^8$ CFU of CP. Additional blood samples were collected at d21 and d25, corresponding to 7 and 11d post-inoculation (pi) with EM and 3 and 7dpi with secondary CP. Plasma was collected from all blood samples to determine systemic IL-10 by ELISA. Jejunal lesion scores were recorded from 6 birds/ treatment on d21 (7dpi EM, 3dpi CP) and excreta from each cage was collected on d22 to evaluate fecal oocyst shedding. Plasma IL-10 and oocyst shedding data were analyzed using a mixed model with diet and challenge effects (SAS 9.4) and lesion scores were analyzed by ordinal logistic regression within each replicate ($P \leq 0.05$, trends at $0.05 \leq P \leq 0.10$).

Results: In replicates 1 and 2, early ST inoculation did not affect baseline plasma IL-10. Dietary anti-IL-10 did not affect systemic IL-10 at any timepoint in any of the three replicate studies, nor were any challenge effects observed. In all three replicates, feeding anti-IL-10 did not affect the likelihood of assigning a lower jejunal lesion score during challenge with primary EM or secondary CP. No differences in fecal oocyst shedding were observed in replicate 1; however, anti-IL-10 tended to reduce oocyst shedding 2.2-4.9-fold during EM challenge compared to control in replicates 2 and 3 ($P=0.07$). No anti-IL-10 effects on fecal oocyst shedding were observed in birds challenged with EM+CP.

Conclusions: Plasma IL-10 results indicate that dietary anti-IL-10 does not have an effect on systemic IL-10 concentrations and its potential effects are restricted to the intestinal compartment. Regardless of the model, EM±CP does not affect systemic IL-10 and pathogen effects are likely limited to intestinal compartments. While anti-IL-10 may reduce oocyst shedding, more research is needed to evaluate the effects of anti-IL-10 on intestinal responses to EM±CP. Jejunal cytokine gene expression evaluation is ongoing to better characterize local responses anti-IL-10.

Financial Support: The authors thank USDA-NIFA for financial support of this research (grant 2021-67015-34533)

Notes:

P080 - Deletion of a single gene abrogates the ability of mycobacterial pathogens to establish a persistent infection

Asmaa Mahmoud[1, 2], Gaber Abdellrazeq[1, 3], Victoria Hulubei[1], William Davis[1]

[1]Department of Veterinary Microbiology and Pathology, Washington State University, [2]General Organization for Veterinary Services, Ministry of Agriculture, Egypt, [3]Department of Microbiology, Alexandria University. asmaa.mahmoud@wsu.edu

Session: Vaccinology, **2023-01-22, 6:00 - 8:00**

Objective: Mycobacterial pathogens cause serious diseases in humans and animals. Bacillus Calmette Guérin (BCG) is the only available vaccine, and it doesn't provide full protection. Efforts continue to develop an effective vaccine that elicits sterile immunity. Studies of the gene *rel,* regulator of the stringent response, in a mouse model with *Mycobacterium tuberculosis* and a bovine model with *Mycobacterium avium* subsp. *paratuberculosis* have demonstrated products encoded by genes regulated by the gene *rel,* regulator of the stringent response, interfere with immune clearance. When *rel* is deleted, an immune response is elicited by mutants that clears infection. The objective of the present study was to determine how and when gene products regulated by *rel* interfere with the immune response to mycobacteria. Comparative studies were conducted with cattle vaccinated with BCG and a BCG *rel* deletion mutant.

Methods: Ten steers one-year-old were grouped into three groups; one group of four steers was vaccinated with BCG, the second group of four steers was vaccinated with BCG*rel,* and the third group of two steers was used as unvaccinated controls. An ex vivo tissue culture platform was used to detect the immune responses; (A) a flow cytometric assay was used to compare the *in vivo* 1) the proliferative recall response, 2) expression of cytokines that regulate the immune response, 3) content of molecules involved in intracellular killing of bacteria. (B) a qPCR assay was used to detect the functional activity of CD8 cytotoxic T cells elicited by vaccination with BCG or with BCG*rel*. A full factorial generalized linear mixed model was used to analyze the data obtained in each dataset using the SAS software procedure PROC GLIMMIX.

Results: The in vivo recall response was detected 14 days post-vaccination. Comparison between BCG and BCG*rel* vaccinated steers revealed deletion of *rel* did not interfere with the proliferative T cell recall response, expression of cytokines IFN-γ, TNFα, IL-17, IL-22, or intracellular killing of bacteria mediated by perforin, granzyme B, and granulysin. However, a difference was observed in the functional activity of CD8 T cells from steers vaccinated with BCG*rel* in comparison with CD8 T cells from steers vaccinated with BCG. Vaccination with BCG*rel* elicited a vigorous CD8 CTL response. Extensive intracellular killing of bacteria in infected target cells was observed with CTL from steers vaccinated with BCG*rel*. Minimal intracellular killing was observed with CTL from steers vaccinated with BCG.

Conclusions: The data indicate that deletion of *rel* may improve the efficacy of BCG as a vaccine. Deletion of *rel* disrupts the ability of mutants to establish an infection and development of an immune response that clears the infection. Identification of Further studies are needed to identify the gene products that interfere with development of CTL activity should provide the information needed to develop a vaccine that elicits sterile immunity.

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

Notes:

P081 - Sheep-associated malignant catarrhal fever vaccine candidate: safety and immunogenicity in cattle

Daniela D. More[1], Katherine N. Baker[1], Maria Herndon[2], Naomi Taus[1], Cristina W. Cunha[1]

[1]USDA, [2]Washington State University. daniela.d.more@wsu.edu

Session: Vaccinology, **2023-01-22, 6:00 - 8:00**

Objective: Sheep-associated malignant catarrhal fever (SA-MCF) is a viral often-fatal disease caused by ovine herpesvirus 2 (OvHV-2) and transmitted by sheep through nasal virus shedding to non-adapted species such as cattle, bison, and deer. There is no vaccine available to control the disease, which negatively impacts the livestock industry in the United States. The objective of this study was to test the safety and immunogenicity of a viral-vectored vaccine targeting ovine herpesvirus 2 (OvHV-2) glycoprotein B (gB).

Methods: The vaccine candidate is a recombinant alcelaphine herpesvirus-1 (AlHV-1) that lacks its latency-associated and gB coding genes but expresses the OvHV-2 gB homologous gene. Calves (n=4 per group) were immunized with the viral vaccine ($8x10^{4.5}$ $TCID_{50}$) plus 20% adjuvant (Emulsigen®) or received only mock immunization containing culture medium plus adjuvant. Three immunizations were delivered intramuscularly at two-week intervals. Animals were monitored daily for clinical signs to evaluate vaccine safety. Blood samples and nasal swabs were collected at several time points until 84 days post-prime immunization (DPI) to address immune responses induced by vaccination. Specific humoral anti-OvHV-2 gB responses were quantified both in plasma and nasal secretions by ELISA and tested by *in vitro* virus neutralization assay; cellular immunity was assessed by CBC analysis of whole blood and leucocyte phenotyping by flow cytometry; and cytokine and chemokine profiling using bead-based 15-multiplex assay (Milliplex®). AlHV-1 quantitative PCR was performed to monitor potential vaccine virus infection throughout the study.

Results: The AlHV-1-OvHV-2-gB vaccine candidate was safe since no clinical signs were observed in any of the immunized animals. Also, AlHV-1 DNA was not detected in peripheral blood leucocytes or nasal secretions of vaccinated animals at any timepoint tested, suggesting that the viral vector was not able to establish a latent infection and be shed. Regarding humoral responses, vaccination induced significant levels of anti-OvHV-2 gB IgG in vaccinated animals when compared to mock-immunized calves after 27 DPI, lasting until the end of the study. Mucosal antibodies, however, were shown to be transient, with significant difference in total IgG content only at 27 DPI, and similar levels between groups at the end of the experiment. Neutralizing antibodies against the vaccine virus were abundantly present in serum and nasal secretions of all vaccinated animals at 41 DPI. We observed 100% inhibition of viral plaque formation in all samples from vaccinated animals, whereas mock immunized animals showed only 9% inhibition on average. For mucosal neutralization, the average inhibition of plaque formation was 49% for vaccinated and 24% for mock-immunized animals. Regarding cellular immunity, following each immunization we observed modulation of B, T ($CD3^+$), T $CD8^+$ cells, and both T GD^+ and GD^- subpopulations and increased production of cytokines and chemokines, including IFN-γ and IP-10 in vaccinated animals compared to controls. Overall, these results indicate that vaccination induced broad systemic and mucosal immune responses.

Conclusions: The AlHV1-OvHV-2-gB vaccine is safe and immunogenic for cattle and represents a promising candidate to be tested in vaccination-challenge trials to evaluate efficacy against sheep-associated MCF.

Financial Support: This work is supported by USDA-ARS, CWU grant N. 2090-32000-045-00D. Dr. More was supported by an ARS Research Participation Program administered by ORISE, contract N. DE-SC0014664.

Notes:

P082 - Broadly protective bovine parainfluenza 3 virus and bovine viral diarrhea virus vaccine

Tae Kim[1], Huldah Sang[1], Waithaka Mwangi[1]

[1]Kansas State University. wmwangi@vet.k-state.edu

Session: Vaccinology, **2023-01-22, 6:00 - 8:00**

Objective: Develop a contemporary live vaccine capable of inducing broad protection against multiple bovine parainfluenza 3 virus (BPI3V) as well as bovine viral diarrhea virus (BVDV) strains.

Methods: Pilot studies have demonstrated that a prototype vaccine containing mosaic antigens designed using conserved determinants identified in a few BVDV-1a, b, and BVDV-2 genomes, conferred broad protection against BVDV-1 & 2 strains. To improve protective efficacy and broaden coverage, we have designed novel envelop (E2) and non-structural (NS2-5) mosaic antigens, designated E2-NS2-5, using data from >200 genome sequences. We have also designed novel mosaic BPI3V antigens, designated F2 and HN2 (F2-HN2), respectively, using data from all sequenced genomes. In addition, we have developed a BPI3Vc vector backbone that contains defined mutations shown to attenuate BPI3Va and used it to generate attenuated BPI3Vc-F2-HN2 and BPI3Vc-E2-NS2-5 recombinant viruses. We hypothesize that immunization of calves with the recombinant BPI3Vc expressing the novel F2-HN2 or the E2-NS2-5 mosaic antigens, will confer broad protection against diverse BPI3V and BVDV strains. The hypothesis will be tested through completion of the following specific aims: 1) Test whether mucosal or parenteral immunization of calves with the recombinant BPI3Vc-F2-HN2 virus will safely confer broad protection against challenge with wildtype BPI3Va-c strains. A commercial BPI3Va vaccine will serve as a positive control and sham treated calves will serve as negative controls. *In vivo* attenuation, safety, immunogenicity, and protective efficacy of the recombinant BPI3Vc-F2-HN2 virus against representative BPI3Va-c, strains will be evaluated in calves following intranasal or subcutaneous immunization. Immune sera will be tested, *in vitro*, for cross-neutralization of disparate BPI3V strains; and 2) Determine whether mucosal or parenteral immunization of calves with the recombinant BPI3Vc-E2-NS2-5 virus, will confer protection against representative BVDV-1a, b, and BVDV-2 strains. Calves will be immunized as above and then challenged with representative BVDV-1a, b, or BVDV-2 strains to evaluate safety, immunogenicity, and protective efficacy. A commercial BVDV vaccine will serve as a positive control and sham treated calves will serve as negative controls. Immune sera will be tested, *in vitro*, for neutralization of diverse BVDV strains. At the termination of the animal studies, tissue samples will be obtained to analyze and score pathological lesions. The significance of the differences in immune readouts, viremia, clinical scores, and pathological lesions between the treatment and controls will be analyzed and compared.

Results: This is a new project and completion of the proposed studies is expected to result in: 1) Confirmation of recombinant BPI3Vc virus attenuation *in vivo*; 2) Determination of whether the BPI3Vc-F2-HN2 prototype vaccine can confer broad protection against diverse BPI3V strains; and 3) Determination of whether the BPI3Vc-E2-NS2-5 prototype vaccine can confer broad protection against diverse BVDV strains.

Conclusions: Successful development of a broadly protective vaccine for control of diverse BPI3V and BVDV strains is expected to improve management of bovine respiratory disease complex to increase cattle productivity and profitability.

Financial Support: This project was supported by Agriculture and Food Research Initiative Competitive Grant no. 2023-67015-39736 from the USDA National Institute of Food and Agriculture

Notes:

P083 - Progress on the development of an "intelligently-designed" vaccine to prevent diseases due to *Histophilus somni*

Thomas J. Inzana[1], Dianjun Cao[1], Amelia Woolums[2], Merrilee Thoresen[2], Kelsey M. Harvey[2]

[1]Long Island University, [2]Mississippi State University. thomas.inzana@liu.edu

Session: Vaccinology, **2023-01-22, 6:00 - 8:00**

Objective: Our hypothesis is that current vaccines to prevent bovine diseases due to *Histophilus somni*, including bovine respiratory disease (BRD), are inadequate because antigens expressed by bacteria in the host differ from culture-grown cells used for vaccine manufacture. The natural growth state of *H. somni* is a biofilm, and during chronic infection (e.g. BRD, myocarditis, etc.), a biofilm is prevalent, and half of the bacterial genome is differentially expressed when the bacteria form a biofilm, compared to planktonic growth. In addition, iron binding proteins are expressed by bacteria in the host that are not expressed in rich culture medium.

Methods: An *lpx*L1 mutant of *H. somni* is being generated by allelic exchange mutagenesis to avoid vaccine endotoxicity. *H. somni* will be grown planktonically under iron-restricted conditions to enhance expression of outer membrane iron binding proteins. Outer membrane vesicles containing the iron-restricted proteins will be combined with the extracellular polymeric matrix of the *H. somni* biofilm, which contains many proteins and a polysaccharide not present in planktonic cultures. This vaccine will be combined with a novel adjuvant that strongly induces a TH1/Th2 response. The vaccine-adjuvant mixture will be tested for immunogenicity and protective efficacy in comparison to a commercial vaccine in a bovine model for BRD, and then tested for efficacy and safety in a commercial cattle herd.

Results: This project was just initiated in May 2023, but a candidate *lpx*L1 mutant may have already been generated by allelic exchange with a chloramphenicol resistance gene.

Conclusions: This project has just begun. However, if successful, this work may lead to a new generation of vaccines with improved efficacy for opportunistic pathogens responsible for chronic infections.

Financial Support: This work is supported by USDA-NIFA grant 2023-67015-39655 to TJI.

Notes:

P084 - Development of a bovine IL-18 expression vector for *Histophilus somni*

D. Cao[1], X. Zhu[2], T.J. Inzana[1]

[1]Department of Veterinary Biomedical Sciences, Long Island University, [2]Virginia-Maryland College of Veterinary Medicine, University of Maryland. dianjun.cao@liu.edu

Session: Vaccinology, **2023-01-22, 6:00 - 8:00**

Objective: A more effective vaccine against diseases caused by *Histophilus somni (H. somni)* is needed because *H. somni* can survive within macrophages and neutrophils, but current bacterin vaccines may only induce a humoral antibody response. Growing evidence indicates that a robust cellular immune response is essential to clear infected cells and is likely required to provide optimum protection against *H. somni* infection. Interleukin-18 (IL-18) is a multifunctional pro-inflammatory cytokine that regulates both innate and acquired immune responses. Il-18 modulates the immune system by coordinating the production of interferon-gamma and activating natural killer cells and Th1 cells. Therefore, inclusion of IL-18 may boost the efficacy of *H. somni* vaccines. Recognizing the significance of IL-18 in immune orchestration, we have undertaken the development of expression vectors for bovine IL-18 (bIL-18) in both *H. somni* and bovine cells. However, progress has been hindered by the lack of a suitable expression vector to express bIL-18 in *H. somni*. The aim of this study was to identify an effective promoter for driving the expression of bIL-18 in *H. somni*, with the ultimate goal of developing a more efficacious vaccine.

Methods: A 582-bp DNA fragment encoding mature bovine IL-18, positioned downstream of a 146-bp *sodC* promoter from *Actinobacillus pleuropneumoniae*, was synthetized as a gBlock fragment, denoted as *sodC*-bIL-18. To construct a plasmid for bIL-18 expression in *H. somni*, the *sodC*-bIL-18 gBlock fragment was digested with SpeI and ligated into plasmid pNS3K at the XbaI site, resulting in plasmid pNS3K-*sodC*-bIL-18. A mammalian expression plasmid, pN1-bIL-18, was created by replacing an 854-base pair DNA fragment that contained cloning sites and enhanced green fluorescent protein (EGFP) with the NheI/NotI fragment in pNS3K-*sodC*-bIL-18 that contained the mature bIL-18 gene. To express bIL-18 in *H. somni*, pNS3K-*sodC*-bIL18 was electroporated into *H. somni* strains 2336 and 129pt. After 4 hours culture at 30°C, the cells were harvested and analyzed for IL-18 expression using Western blotting. For bIL-18 expression in host cells, 2.5 µg of pN1-bIL-18 plasmid was electroporated into BT cells, electroporated cells were cultured, and tested for IL-18.

Results: The sodC promoter of *A. pleuropneumoniae* was determined to be an effective promoter for driving the expression of foreign proteins in *H. somni*. The 3012 bp recombinant plasmid pNS3K-sodC-bIL-18, encoding for 193 amino acids of mature bIL-18, was electroporated into *H. somni* strains 2336 and 129pt. Western blotting with rabbit anti-bovine IL-18 antibody confirmed that an approximately 22.3 kDa peptide (similar to the predicted size of mature bIL-18) from cell lysates reacted with bIL-18 specific antibody. In addition, pN1-bIL-18 plasmid-transfected bovine turbinate cells produced a similar size bIL-18 protein in Western blots.

Conclusions: The results indicated that the constructed bIL-18 expression plasmids successfully expressed bIL-18 in both *H. somni* and bovine cells. These constructs may provide a valuable tool for enhancing the cellular immune response to *H. somni* vaccines. Further animal studies will be required to evaluate the adjuvant potential of bIL-18 within the context of an *H. somni* vaccine.

Notes:

P085 - Bovine papular stomatitis virus as a vaccine vector for cattle

Gustavo Delhon[1], Sushil Khatiwada[2], David Doub[2], Seth Harris[1], Sabal Chaulagain[2], Mostafa El-Gaffary[2], Daniel L. Rock[2]

[1]School of Veterinary Medicine & Biomedical Sciences, University of Nebraska, [2]Department of Pathobiology, University of Illinois. ddoub@illinois.edu

Session: Vaccinology, **2023-01-22, 6:00 - 8:00**

Objective: Virus vectored vaccines are not available commercially for cattle even though compelling potential applications exist. Bovine papular stomatitis virus (BPSV), a highly prevalent parapoxvirus in cattle, causes largely asymptomatic infections or mild self-limited oral lesions in cattle. The ability of BPSV to accommodate large amounts of foreign DNA, induce low level of anti-BPSV immunity, and circulate and likely persist in cattle populations, make BPSV an attractive viral vector candidate for use in cattle. This study, using vaccination/challenge experiments, demonstrates the protective efficacy of a BPSV vector expressing protective antigens from Bovine herpesvirus 1 (BoHV-1) in cattle.

Methods: Recombinant BPSV vectors were constructed expressing either BoHV-1 glycoprotein gD ($BPSV^{gD}$), or gD and glycoprotein gB ($BPSV^{gD/gB}$). BPSV serologically-positive calves were inoculated with $BPSV^{gD}$ or $BPSV^{gD/gB}$ intramuscularly and intranasally, and boosted prior to intranasal high dose challenge with the BoHV-1 Cooper strain ($2x10^{8.5}$ $TCID_{50}$/animal) at 70 days post immunization.

Results: Following immunization**,** no BPSV lesions were observed at inoculation sites, indicating BPSV vectors are attenuated in the natural host. Infectious virus was not recovered from nasal and oral fluids following virus inoculation. However, specific BPSV DNA sequences were detected by PCR in nasal fluids from calves between day 7 and 21 post-infection indicating low levels of virus replication occurred following inoculation. $BPSV^{gD}$ or $BPSV^{gD/gB}$ induced BoHV-1 neutralizing antibodies in calves with titers ranging from 1:8 to 1:64 at the time of challenge infection and provided protection following a high dose BoHV-1 challenge at day 70 post infection. Control calves (N=2), presented ocular and nasal discharges and labored breathing, and one of them coughing. In the immunized group, the two calves with the highest neutralizing antibody titers exhibited no clinical signs other than short duration low grade fever, while one calf showed additional clinical signs for only a single day. BoHV-1 DNA in nasal swabs declined gradually post-challenge in both groups with overall amounts trending lower in vaccinated animals

Conclusions: The results described here indicate that immunization of calves with BPSV vectors containing heterologous BoHV-1 proteins gB and gD induced neutralizing antibodies and provided protection from BoHV-1 challenge infection. Notably and of likely consequence for potential future applications, the BPSV vector was effective in inducing immune responses in BPSV serologically-positive animals. This result is consistent with the known high reinfection potential of BPSV and its high prevalence and transmissibility in bovine populations; thus, prior BPSV infection does not pose a significant obstacle for a BPSV-based vector. Based on results shown here, BPSV shows promise as a vector for use in cattle especially where a multivalent vaccine is required, such as with Bovine respiratory disease.

Financial Support: This research was supported by the U.S. Department of Agriculture, National Institute of Food and Agriculture, award number 2019-67015-29966

Notes:

P086 - First trials and development of effective caseous lymphadenitis inactivated vaccine in South Korea

Gyeong-Seo Park[1], Byoung-Joo Seo[1], So-Min Lee[1], Min-A Jeong[1], Dong-Geun Cho[2], Won-Il Cho[2], Chong-Han Kim[1]

[1]Woogene B&G, [2]Ohsan Black Goat Farm. gyeoungseopark@gmail.com

Session: Vaccinology, **2023-01-22, 6:00 - 8:00**

Objective: Caseous Lymphadenitis (CLA) is a chronic and subclinical bacterial disease of goats and sheep caused by *Corynebacterium pseudotuberculosis* infection. Until 2014, there were no reports of CLA outbreaks in South Korea. However, in recent years, the prevalence of *C. pseudotuberculosis* has been steadily increasing. Vaccination was the primary option to prevent and control CLA, but the CLA vaccine has not been commercialized in South Korea. Accordingly, we became the first in South Korea to develop the inactivated CLA vaccine by recently obtaining field isolates and evaluating its safety and efficacy in *in-vivo*.

Methods: To assess the safety of the CLA vaccine, mice and guinea pigs were subcutaneous (S.C) inoculated with inactivated *C. pseudotuberculosis*. After confirming safety in laboratory animals, clinical trials were conducted at Korea Native Black Goat (KNBG). Twenty-five, 3-month-old female KNBG were randomly housed and divided into 5 groups; Group 1 (Vaccine group 1, 10^7 CFU/ml), Group 2 (Vaccine group 2, 10^6 CFU/ml), Group 3 (Vaccine group 3, 10^5 CFU/ml), Group 4 (Positive control), and Group 5 (Negative control). The vaccine groups were intramuscularly (I.M) inoculated with an inactivated vaccine with different contents of antigens at 0 weeks post-vaccination (WPV) and with a booster vaccination at 4 WPV. At 8 WPV, the vaccine groups and positive control groups were intramuscularly challenged with 10^6 CFU/ml of field strain *C. pseudotuberculosis*. All groups were monitored for the development of clinical signs such as abnormal behavior, and dyspnea. Blood was collected from all groups of KNBGs on designated days. The body weight of all KNBGs was measured at 0-, 8-, and 12 weeks post-vaccination (WPV), and the average weekly weight gain (AWWG) were measured at 8, and 12 WPV. The serum from all groups was tested for anti-CLA-specific IgG using a commercially available ELISA kit. All KNBGs were humanly euthanized at 20 WPV for pathological evaluations. Two-way ANOVA and nonparametric one-way ANOVA were used to analyze.

Results: The field isolate was identified as *C. pseudotuberculosis* and used as a master cell bank for CLA vaccine development. In the vaccine groups, clinical symptoms such as high fever, dyspnea, abnormal behavior, and sudden deaths were not observed until the end of the experiment. In the vaccine group, CLA-specific IgG was detected at a significantly high level and maintained until the end of the experiment. Necropsy was performed to identify gross lesions and bacterial growth and obtain pathological tissue. Bacterial infection was confirmed only at the site of the challenge and the prefemoral. In the pathological evaluations, the inflammation was predominantly observed in the prefemoral lymph nodes in group 3 and group 4.

Conclusions: The CLA vaccine demonstrated safety and efficacy against *C. pseudotuberculosis* infection. Furthermore, considering that the genetic diversity of *C. pseudotuberculosis*, which has become widespread in South Korea, is less than 0.5%, our vaccine is expected to effectively prevent a wide range of strains. In summary, our CLA vaccine has the potential to prevent CLA and foster the growth of South Korea's domestic KNBG industry.

Financial Support: This work was funded by the Ministry of SMEs and Startups (MSS, Republic of Korea)[S3063938].

Notes:

P087 - Inactivated whole cell antigen vaccine protects dogs from Rocky Mountain Spotted Fever independent of an adjuvant used

Perle Latre de Late[1], Swetha Madesh[1], Jonathan Ferm[1], Dominica Genda[1], Ian Stoll[1], Jodi McGill[2], Kim D. Yong[3], Abha Sahni[4], Sanjeev Sahni[4], Roman R. Ganta[1]

[1]Department of Veterinary Pathobiology, University of Missouri, [2]Department of Veterinary Microbiology and Preventive Medicine, Iowa State University, [3]Veterinary Diagnostic Laboratory, University of Missouri, [4]Department of Pathology, University of Texas Medical Branch. plh4n@umsystem.edu

Session: Vaccinology, **2023-01-22, 6:00 - 8:00**

Objective: Rocky Mountain Spotted Fever (RMSF) is a potentially fatal tick-borne disease in dogs and people, and it is spread across North, Central and South Americas. It is caused by *Rickettsia rickettsii*, an obligate, intracellular bacterial pathogen that is transmitted by several species of hard ticks including *Dermacentor* species, *Rhipicephalus sanguineus*, and *Amblyomma americanum*. Clinical signs of the disease are persistent fever, headache, nausea, vomiting, muscle pain, lack of appetite, edema, and petechial rashes. If untreated, the disease can quickly progress into the life-threatening illness in people and dogs, with fatality rates ranging from 30 to 80%. Doxycycline is the only treatment against RMSF. In recent years, the severe form of the disease is frequently reported in parts of the USA and Mexico. There is no vaccine to prevent RMSF in either dogs or people. We previously demonstrated that the whole cell inactivated antigen vaccine (WCA) offers complete protection against virulent *R. rickettsii* infection in dogs when using Montanide gel as the adjuvant. In the current study, we investigated three adjuvants (Montanide, Quil A and aluminum hydroxide) to optimize safety and immunogenicity of WCA using the canine model for RMSF.

Methods: Vaccines prepared with heat inactivation method and with the three different adjuvants were used. Three vaccination groups of dogs were included in this study (n=5 for each group). Adjuvant only preparations were administered to control groups (n=6; 2 animals per adjuvant preparation). A priming vaccination was performed on day 0, followed by a booster vaccination on day 30 and I.V. infection challenge with 10^5 *R. rickettsii* on day 50. Infection progression was monitored for 30 days.

Results: Independent of the adjuvant used, all dogs vaccinated with WCA were protected from RMSF, whereas nonvaccinated *R. rickettsii*-infected dogs developed the disease. All dogs receiving WCA developed vaccine-specific IgG responses following the primary vaccination, which increased following the booster vaccination. Vaccine with Quil A induced a higher IgG response compared to Montanide and aluminum hydroxide. Vaccine-specific IgG2 response is more prominent than the IgG1 response during vaccination. Montanide and aluminum hydroxide seem to induce a balanced IGg1 and IgG2 response. Although weak compared to vaccinated animals, the IgG2 response is also observed following infection in unvaccinated animals, suggesting that the IgG response alone is not sufficient in preventing the disease progression. In vaccinated animals, the pathogen remains undetectable in the blood and various tissues; lung, liver spleen, Kidney, bone marrow, lymph node, cerebellum, thymus, and heart, whereas several tissues tested positive for the pathogen DNA in non-vaccinated animals. Vaccinated animals had the limited induction of pro-inflammatory cytokines like IFN-gamma, IL-6, and TNF-alpha, while IL-8 (a chemoattractant of neutrophils and inducer of phagocytosis) remained high.

Conclusion: This study provides the first evidence of the protective ability of WCA against RMSF in dogs tested using three different adjuvants. Our vaccine assessment experiments using the three different adjuvants will allow us to gain insights regarding the host adaptive immune response critical for protection, in addition to advancing the progress towards the vaccine development.

Financial Support: This work was supported by the grant number R01AI152417 from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, USA.

Notes:

P088 - Generation of a targeted disruption mutant in *Anaplasma phagocytophilum* and its application as a vaccine candidate

Jonathan Ferm1, Huitao Liu1, Dominica Genda1, Roman Ganta1

[1]University of Missouri Columbia. jdf7hw@umsystem.edu

Session: Vaccinology, **2023-01-22, 6:00 - 8:00**

Objective: *Anaplasma phagocytophilum* is an obligate intracellular bacterium responsible for causing granulocytic anaplasmosis in a wide range of species globally. Transmitted by *Ixodes* species ticks, it is known to cause infections in people, dogs, horses, sheep, and cattle. Currently, there are no vaccines to prevent *A. phagocytophilum* infections or the disease resulting from it in any vertebrates, while treatment options are limited to tetracycline derivatives. Recently, our team developed and successfully applied targeted mutagenesis methods to two closely related tick-borne bacteria; *Ehrlichia chaffeensis* and *Anaplasma marginale*. We also discovered that the phage head-to-tail connecter protein (*phtcp*) gene is essential for the bacterial *in vivo* growth and persistence. Functional disruption mutations in the *phtcp* genes of *A. marginale* and *E. chaffeensis* caused reduced infection and rapid clearance in cattle and dogs, respectively. Furthermore, prior infection with the *phtcp* mutants provided protective immunity against wild type infections. In the current study, we extended targeted mutagenesis to disrupt the *phtcp* gene in *A. phagocytophilum*. We then evaluated the mutant as a potential live vaccine to prevent the disease in the canine host.

Methods: Hematological parameters, bacterial persistence in blood, and IgG response were assessed in both vaccinated and non-vaccinated animals prior to and following infection challenge with the wild type *A. phagocytophilum.*

Results: Wild type bacterial DNA was not detected in any vaccinated animals following infection challenge, while identified several times in non-vaccinated dogs, although animals in both groups induced pathogen-specific IgG. Vaccinated animals had normal hematological values throughout the study period, while non-vaccinated dogs had increased neutrophils and monocytes following the infection challenge.

Conclusion: This study further demonstrates that the *phtcp* gene homologs are essential for the intracellular survival of Anaplasmataceae pathogens and that the gene function disruption mutant organisms are ideal vaccine candidates to control infections in natural incidental hosts.

Financial Support: This work was supported by the PHS grants AI070908 and AI152418 from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, USA. Support also from USDA APHIS NSTP Fellowship Program.

Notes:

P089 - Genetically modified live vaccine to prevent monocytotropic ehrlichiosis in dogs resulting from *Ehrlichia canis*

Dominica D. Genda[1], Arathy Nair[2], Jonathan D. Ferm[1], Huitao Liu[1], Roman R. Ganta[1]

[1]University of Missouri, [2]Kansas State University. dgm5m@missouri.edu

Session: Vaccinology, **2023-01-22, 6:00 - 8:00**

Objective: Canine monocytotropic ehrlichiosis (CME) is a multisystemic tick-borne disease distributed worldwide and characterized by acute, subclinical, and chronic forms. Obligate intracellular rickettsial bacterium, *Ehrlichia canis* is one of the etiological agents responsible for causing the disease. It is transmitted by *Rhipicephalus sanguineus* (brown dog tick) in the USA. High fever, lethargy, lack of appetite, and depression have been reported during the acute form of the disease. Currently, there are no available vaccines to prevent the disease and treatment options are limited to tetracycline derivatives. We previously genetically mutated the phage head to tail connector protein (*phtcp*) gene in the related species *Ehrlichia chaffeensis* and *Anaplasma marginale.* Infection with the mutated bacteria showed that they have attenuated growth in their natural hosts and induce sufficient immune protection to prevent disease resulting from I.V. and tick transmission wild type infections. In the current study, we deleted the *phtcp* gene homolog from *E. canis* and the mutant was tested as a modified live vaccine candidate to see if it would be able to prevent the wild-type *E. canis* infection in dogs.

Methods: The vaccination group of five dogs were infected with mutated bacteria while the control group of 4 dogs were left uninfected, 29 days later both groups were challenged with wildtype infection. Throughout the experiment all animals were assessed for changes in clinical signs and blood samples were collected throughout the study from both groups. Blood samples were analyzed by PCR, ELISA, culture recovery, and complete blood cell count assessment. *Rhipicephalus sanguineus* ticks were allowed to feed for one week after wild type challenge to acquire infection and were tested by PCR for xenodiagnosis.

Results: Our data demonstrated that the vaccine protected dogs against wild-type I.V. infection. All animals had IgG responses after mutant and or wildtype infection. All non-vaccinated animals had steady infection after challenge while vaccinated animals tested negative for wild type infection throughout the course of the study.

Conclusions: This is the first study describing the targeted mutagenesis deletion of a gene from *E. canis* and subsequent use in developing a live vaccine.

Financial Support: This work was supported by the PHS grants AI070908 and AI152418 from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, USA.

Notes:

P090 - Development of mucosally administered multivalent vaccine to prevent poultry infection by multiple *Eimeria* species

Shifeng Wang[1], Roy Curtiss[1], Soo-Young Wanda[1], Mark Jenkins[2]

[1]College of Veterinary Medicine, University of Florida, [2]Animal Parasitic Disease Laboratory, USDA-Agricultural Research Service. rcurtiss@ufl.edu

Session: Vaccinology, **2023-01-22, 6:00 - 8:00**

Objective: To design, construct and evaluate a *Salmonella*-vectored vaccine to prevent coccidiosis caused by *Eimeria*species in poultry.

Methods: Standard methods for genetic modification of *Salmonella* and molecular biology tools to codon optimize/harmonize sequences encoding protective *Eimeria* antigens to assemble into bi-functional plasmid vectors.

Results: We use significantly improved genetically designed *Salmonella* vaccine vectors to synthesize and deliver multiple *Eimeria maxima* and *E. tenella* antigens to induce protective immunity against coccidiosis in broilers. Our *Salmonella* vaccine vectors reprogrammed for sequential physiological functions are to the best of our knowledge the most extensively genetically modified living organism yet created. We designed these vaccine vector strains to eliminate means by which they (i) inhibit induction of immune responses and (ii) formation of biofilms that enhance persistence, and with improved means for (iii) regulated delayed attenuation, (iv) regulated delayed synthesis of protective antigens and (v) regulated delayed lysis in vivo. The timing of events has been programmed to maximize the ability of vaccines to colonize internal lymphoid tissues in chickens to produce and deliver protective antigens prior to lysis, which precludes persistence in vivo and viability if shed. The regulated delayed attenuation attributes were designed to enhance induction of immunity to diverse *Salmonella* serotypes and to other *Enterobacteriaceae*. The Protective Immunity Enhanced *Salmonella* Vaccine (PIESV) constructs have been designed for use with newly constructed bi-functional plasmid vectors that specify synthesis and delivery by secretion protective *Eimeria* antigens encoded for maximal synthesis by *Salmonella* and also then serve as DNA vaccines encoding *Eimeria* antigens to be synthesized in chicken host cells to enable their synthesis and post-translational modification. Constructs currently being made will be evaluated for inducing protective immunity to challenge with *E. maxima* and *E. tenella* oocysts to eliminate disease and ensure efficient feed conversion and maximal growth attributes. They will also be evaluated to induce protective immunity to multiple *Salmonella* serotypes often colonizing chickens and passed through the food chain to humans. An added hope is that vaccine use will ultimately decrease drug use in the poultry industry to reduce pressures for selective enrichment of drug-resistant microbes.

Conclusions: Since the project has just been initiated, success to date has been limited to selection and modification of the PIESV vector strain and bi-functional plasmid vector to use and the selection of antigen-encoding genes with designing the modified optimized sequences for commercial synthesis to enable insertion into the plasmid vector.

Financial Support: This project is supported by Agriculture and Food Research Initiative Competitive Grant no. 2022-08214 from the USDA National Institute of Food and Agriculture.

Notes:

P091 - Enhancing the production of type I interferons to create rationally-defined Marek's disease vaccines

John Dunn[1], Maria Bahamon Naranjo[2], Sonsiray Alvarez Narvaez[1], Steven Conrad[1], Jef Boeke[2]

[1]USDA-Agricultural Research Service, [2]New York University Langone. john.dunn@usda.gov

Session: Vaccinology, **2023-01-22, 6:00 - 8:00**

Objective: Marek's disease (MD) is an oncogenic disease of poultry caused by Marek's disease virus (MDV), and commonly controlled by vaccination with a live attenuated virus strain. Vaccine breaks have been common in the past, making the need for novel vaccine development. Viruses encode gene products which inhibit secretion of the type I interferons (IFN-Is). Our goal is to ablate MDV genes which frustrate the production of IFN-Is during infection to create vaccine strains with improved protection. Our collaboration with the Boeke laboratory (NYU-Langone) will give us access to rapidly-assembled MDV viral genomes. This proposal will hopefully result in new and more protective vaccine strains for MDV.

Methods: The current phase of the project is generation of the entire MDV genome cloned into yeast assemblon vector for rapid manipulation (Boeke lab), and the identification of MDV genes that suppress IFN-I production (Dunn lab). We selected 5 MDV genes (US3, UL18, UL48, UL46 and UL9) that have been suspected to modulate the IFN-beta production for cloning into five different recombinant lentiviruses. These MDV-lentiviruses will be used for gene delivery and subsequent expression into HD11 chicken macrophage-like cells. Simultaneously, we have developed a model of IFN-I production in HD11 using double-stranded DNA (dsDNA). We will use this model to assess the ability of integrated MDV genes to reduce production of IFN-I in the macrophage-like cells.

Results: The Boeke lab now has the entire genome cloned on two yeast assemblon vectors. Transfection of the DNAs into chicken cells to confirm infectious virus is underway. Using this vector, we have knocked out 3 of the 5 genes of interest with URA3. The Dunn lab has successfully integrated US3 in the HD11 genome to determine if this gene has an inhibitory effect in IFN-I production. In parallel we are repeating the process with the other four target MDV genes.

Conclusions: We plan to have candidate viruses ready to evaluate during the next performance period and anticipate that this project will result in several new and highly effective vaccine candidates for MDV.

Financial Support: This project was supported by Agriculture and Food Research Initiative Competitive Grant no. 2020-67015-31470 from the USDA National Institute of Food and Agriculture.

Notes:

P092 - Generation of a Newcastle disease vaccine that protects against infectious laryngotracheitis and Marek's disease

Stephen Spatz[1], Sherif Ibrahim[1], Lei He[1], Qingzhong Yu[1]

[1]USDA-Agricultural Research Service. Stephen.spatz@usda.gov

Session: Vaccinology, **2023-01-22, 6:00 - 8:00**

Objective: A structural-based vaccine design will be used to create bivalent vaccines against infectious laryngotracheitis virus (ILTV) and Marek's disease virus (MDV) using an NDV-based vector (rLS-Ch-IL4R) that has been proven safe for in *ovo* administration. The two antigens targeted are glycoprotein B (gB) of MDV1 and glycoprotein D (gD) of ILTV. Various gene constructs will be inserted into the NDV genome, including native membrane-bound, chimeric membrane-bound (the ectodomain of gB fused to the membrane anchor of NDVs F protein), and a secreted version containing a trimer clamp. The recombinants NDV/MDVgB and NDV/ILTgD will be tested for safety, genetic stability, and protective efficacy in challenge models. Once the best gene variants for gB (MDV) and gD (ILTV) are identified, a trivalent vaccine capable of protecting against all three diseases will be created.

Methods: Transformation-associated cloning was used to assemble PCR fragments representing the NDV genome in yeast. An independent transcription unit (ITU) was engineered downstream of the inverted IL4 gene. The open reading frame of glycoprotein D of ILTV was amplified using PCR and cloned into this ITU using in-fusion cloning. HEp2 cells were transfected with the rLS-Ch-IL4R plasmid and accessory plasmids encoding vital NDV proteins, and infectious virus was recovered. Viral stocks were generated in embryonated eggs. Another construct was generated in which an ITU was placed upstream of the inverted IL4 gene. To do this, a synthetic ITU (633 nucleotides) was inserted into the *SnaB*I and *Rsr*II sites of rLS-Ch-IL4R. PCR with flanking primers and restriction enzyme digestions confirmed that the rLS-Ch-IL4R infectious clone contained the new ITU.

Results: Two constructs were created that contained new independent transcription units (ITUs) both upstream and downstream of the inverted interleukin 4 gene. One construct, which was made in yeast, generated infectious virus. The other construct is still awaiting rescue.

Conclusions: We plan to have candidate viruses containing variants of gB (Marek’s disease virus) and gD (infectious laryngotracheitis virus) ready for evaluation during the next performance period and anticipate that this project will result in several new and highly effective vaccine candidates for MDV and ILTV.

Notes:

P093 - Evaluation of return on investment following the use of different avian coccidiosis vaccines based on their attenuation

Santiago De Castro Verges[1], Martina Dardi[1], Marina Solé[1], Shyong Wey Ong[1], Kesavan Sivagiganesan[1]

[1]Hipra Animal Health. santiago.castro@hipra.com

Session: Vaccinology, **2023-01-22, 6:00 - 8:00**

Objective: Avian coccidiosis is one of the most widespread diseases around the world, and its ability to survive chemical disinfectants hampers poultry production at all levels. Vaccination has become an efficient tool during the last 20 years, and especially in breeders it is now common practice. Avian coccidiosis vaccines mainly differ with regard to how the vaccinal strains are attenuated and how this process is carried out. Currently there are 3 types of avian coccidiosis vaccines: non-attenuated vaccinal strains (NAT), attenuated by embryo passage vaccinal strains (AEP), and attenuated by precociousness vaccinal strains (APP). With the clear objective of evaluating which type of vaccinal strain is better for broiler breeders, a field comparison between a NAT vaccine and a APP vaccine was carried out, where different performance parameters were monetized in order to calculate the final outcome of the different vaccination schemes.

Methods: The field evaluation was performed in 4 different broiler breeder flocks, in the West Malaysia region, analysing performance parameters such as egg laying, hatchability and mortality throughout the life cycle of the birds, including the rearing and laying phases, monetizing these data and evaluating the final return on investment. Two flocks were vaccinated with an attenuated by precociousness coccidiosis vaccine (APP), and the other 2 flocks were vaccinated with a non-attenuated coccidiosis vaccine (NAT). The 4 flocks belonged to the same genetics line, the same hatchery, had the same nutrition programme, were reared in the same density, and the only independent parameter was the avian coccidiosis vaccine applied at hatchery. The statistical analysis was done using the R software v3.1. A p-value < 0.05 was chosen as the limit for statistical significance. A T-test or a linear regression model including week as covariate were performed to compare performances between vaccines.

Results: Total female mortality rates were statistically significantly lower with the APP vaccines (8.16% APP vs 8.69% NAT $p>0.0001$). Secondly, despite the fact that egg production rates were higher in the APP group, no statistical difference was observed to the NAT group ($p=0.956$). Regarding mean hatchability rates, significant differences were found between the two groups (89.29% APP vs 87.8% NAT $p>0.007$). Comparing these data and monetizing them with the cost of the vaccines, day-old chicks and feed, the difference for every 1000 birds vaccinated within an APP coccidiosis vaccine compared to a NAT vaccine was 1,337.44$ in favour of the APP vaccine.

Conclusions: The outcomes of this study show that the application of the APP coccidiosis vaccine result on a larger return on investment when compared to the NAT vaccine. Safety and duration of immunity may explain the differences in terms of performance in a long-term evaluation. Other authors in the past have reported the effects of coccidiosis on testes development. Nevertheless, further studies are necessary to confirm these outcomes.

Notes:

P094 - Development of an intranasal highly pathogenic avian influenza nanovaccine: Kazakhstan's new project with international collaboration

Kairat Tabynov[1], Talgat Karibayev[2], Maksat Berdikulov[2], Leila Yelchibayeva[1], Kuantay Zharmambet[1], Aidana Kuanyshbek[3], Nikolai Petrovsky[4], Gourapura J. Renukaradhya [5], Kaissar Tabynov[1]

[1]International Center for Vaccinology, Kazakh National Agrarian Research University (KazNARU),, [2]National Reference Center for Veterinary, Kazakhstan, [3]Almaty Branch of National Reference Center for Veterinary, Kazakhstan, [4]Vaxine Pty Ltd, Australia, [5]Ohio Agricultural Research and Development Center, The Ohio State University. ktabynov@gmail.com

Session: Vaccinology, **2023-01-22, 6:00 - 8:00**

Objective: Development of a novel, safe, and effective intranasal (needle-free) nanovaccine against HPAI, where chitosan nanoparticles conjugated with mannose are used as a mucosal delivery system for a recombinant vaccine virus antigen obtained by reverse genetics.

Methods: Immunology, virology and genetic engineering methods will be used in the research.

Results: The obtained nanovaccine formulation will be evaluated for safety, immunogenicity and protective efficacy including protection against virus transmission in chickens under different immunization regimens. Moreover, the protective potential of intranasal nanovaccine in the cross-immunization regimen of birds with injected inactivated vaccine will be evaluated. Within the framework of the project, investigations of commercial influenza vaccines of subtype H5 available in the territories of Kazakhstan and the Eurasian Economic Union on the effectiveness against epizootic viruses of HPAI subtype H5N8 clade 2.3.4.4b, are also planned by request of the poultry industry. The project also plans to conduct a pilot vaccine development stage for further commercialization. This work will determine the duration of immunity in birds inoculated with the developed vaccine against HPAI, as well as the shelf life of the preparation under conditions of routine storage. In addition, draft regulatory documents will be prepared. The results of this work will bring the development to NASA's Technology Readiness Level 6 (TRL-6) according to the BIRAC scale for the biotechnology field (veterinary medicine, vaccines), and thereby increase the chances of attracting investment for commercialization of the vaccine.

Conclusions: This project represents a significant stride in combatting HPAI through the development of an intranasal nanovaccine. The international collaboration, leveraging cutting-edge technology, holds promise in avian influenza vaccination strategies and safeguarding global food security.

Financial Support: This research has been funded by the Science Committee of the Ministry of Science and Higher Education of the Republic of Kazakhstan (Grant No. AP19675939)

Notes:

P095 - Evaluation of inactivated vaccine formulated with new polymer adjuvant Montanide™ GEL P PR against salmonellosis in layers

Marie-Eve Koziol[1], Sarah Kharief[1], Nicolas Versillé[1], Juliette Ben Arous[1]

[1]Seppic. marie-Eve.koziol@airliquide.com

Session: Vaccinology, **2023-01-22, 6:00 - 8:00**

Objective: Injectable vaccines dedicated to avian species are usually formulated with oil-based adjuvants allowing to obtain emulsions, especially water-in-oil ones. Oily adjuvants are very effective immunologically speaking, nevertheless, they could be less tolerated by sensitive species. MONTANIDE™ GEL P PR (GEL P), an aqueous adjuvant based on polymeric technology is developed to meet the growing need for better innocuity in the avian market. Salmonellosis is an avian disease caused by infection with a Gram negative bacteria, *Salmonella enteritidis*. Salmonellosis vaccines are usually inactivated vaccines that may lead to severe reactions at the injection site. The target of the following experiment is to evaluate the safety, efficacy and protection of an inactivated vaccine adjuvanted with GEL P.

Methods: In this trial, in order to evaluate the safety of the vaccine, 32-day-old SPF egg laying chickens were injected subcutaneously (SC) in the neck or intramuscularly (IM) in the chest, with 1 ml of the inactivated vaccine based on GEL P or standard water-in-oil. Similarly, the efficacy and protection were evaluated by injecting (SC or IM) 0.5 ml of the inactivated vaccine based on GEL P or standard water-in-oil adjuvant. Blood samples were collected at D7, 14, 21 and 28 after injection. The antibody titers were determined by ELISA.

Results: The collected data demonstrates that the GEL P group is safer, whereas oily adjuvant exhibits higher reactogenic properties especially with IM route that leads to the highest score. In terms of efficacy, both water-in-oil and GEL P vaccines induced a good immune response since the antibody levels are well above the acceptable protective threshold two weeks after vaccination whatever the mode of injection.

Conclusions: These results show that MONTANIDE™ GEL P PR is balanced in terms of safety profile, and protection against potential reactogenic bacterial diseases and suitable candidate adjuvant for the formulation of inactivated poultry vaccines either in IM or SC application.

Notes:

P096 - *Haemonchus contortus* vaccine using novel controlled release delivery in sheep

Darby Harris[1], Doug Jones[2], Carly Kanpie[3], Paola Boggiatto[3], Wenbin Tuo[4], Steven Olsen

[1]College of Veterinary Medicine, Mississippi State University, [2]College of Veterinary Medicine, Iowa State University, [3]Infectious Bacterial Disease, USDA-Agriculture Research Service, [4]Betlsville Area Research Center, USDA-Agriculture Research Service. steven.olsen@usda.gov

Session: Vaccinology, **2023-01-22, 6:00 - 8:00**

Objective: *Haemonchus contortus*, commonly known as the barber pole worm, is a parasitic nematode that resides in the abomasum of small ruminants. These blood-feeding strongyles can be found globally and cause animal suffering and significant economic losses for producers. Given the widespread increase of anthelmintic resistance in *H. contortus* and other gastrointestinal nematodes, new solutions must be generated. Vaccination against these nematodes has been shown to be effective, but the current commercial vaccine requires serial injections to provide sufficient immunity. The goal of the study is to determine if delivering antigenic targets for *Haemonchus contortus* in a novel Vaccine Platform for Extended Antigen Release (VPEAR) implant device will induce a strong humoral immune response which could be used as a means of reducing worm burden and disease caused by the parasitic nematodes in sheep.

Methods: Four different groups of proteins or peptides are compared against a negative control antigen. Forty sheep had a VPEAR implant device surgically placed subcutaneously on the right fore flank a few centimeters caudal to the elbow joint. The immune response is being measured using antibodies detected by indirect ELISA. After measuring the immune response for 8 weeks, all groups will be challenged with oral gavage of live *H. contortus* larvae.

Results: Although this research is still ongoing, reduced rejection of the implants as compared to previous studies indicate acceptance of the implants, possibly due to improvements in the technique. Preliminary data shows that a humoral immune response to *Haemonchus contortus* antigens is being generated. Difference in serum antibody titers to whole worm antigen and proteins used in VPEAR were different between groups.

Conclusion: VPEAR is proving to be a viable platform for use in livestock. Although this research is still ongoing, the preliminary data shows that a humoral immune response is being generated against *Haemonchus contortus* antigens.

Financial Support: Student Support: USDA Agricultural Research Service Boehringer Ingelheim Veterinary Scholars Program

Notes:

P097 - Immunogenicity and protection of a novel glycoengineered vessicle vaccine against *Glaesserella parasuis*

Jinjing Liu[1], Isabel A. Carvalho[1], Yung-Fu Chang[1]

[1]Cornell University. jl3538@cornell.edu

Session: Vaccinology, **2023-01-22, 6:00 - 8:00**

Objective: *Glaesserella parasuis* is a common bacterium that causes significant economic losses in pigs. While traditional treatment of this infectious disease relied on antibiotics, the rising concern of antimicrobial resistance has led to the development of vaccines for prevention. However, more than 15 serovars and non typable strains have been identified, and current commercial *G.parasuis* vaccines offer limited heterologous protection, highlighting the need for a more effective cross-protective vaccine against heterologous strains. Encouragingly, *G. parasuis* capsular polysaccharide (CPS) displayed cross-protection in mice, indicating its potential as a vaccine candidate. In this study, we aim to generate glycoconjugate outer membrane vesicle vaccines and examine their ability against heterologous *G.parasuis* strains and their potential as a cross-protective vaccine.

Methods: In this study we focused on *G.parasuis* serovar 4, 5 and 14 to test the antigenicity and cross-protection. To overcome the limitation of CPS as T-cell independent and improve vaccine efficacy, we applied protein glycan coupling technology (PGCT) to bioconjugate *G.parasuis* CPS with either outer membrane vesicles (OMVs) or carrier protein. Glycoconjugate OMV (glycOMV) was confirmed by TEM and dot blotting. Dendritic cells (DC) were treated with GlycOMV to test the CPS antigenicity. For animal experiments six-week-old BALB/c mice were vaccinated three times at three weeks interval, and blood was collected before each vaccination. Mice were challenged with $2x10^9$ CFU bacteria two weeks after the third vaccination. Clinical signs and survival rates were recoded after the challenge. ELISA was applied to monitor the antibody titer change, and flow cytometry was used to detect the immune response after vaccination.

Results: GlycOMVs were found to be engulfed by DCs, leading to an increase in their CD86 and CD40 expression. These findings indicated the ability of glycOMVs to promote the antigen presenting process. Serotype-specific antibodies were detected after the second vaccination and further increased after the third one. Moreover, antibodies generated by serovar 5 could also react with serovar 4 or 14 strains. In our animal experiment, we observed that more than 80% of mice vaccinated with glycOMV were survived after challenge. In contrast, no mice survived in mice immunized with empty OMV and only 20% survived in the negative control group. The significantly higher survival rate in glycOMV demonstrated the protective immune response elicited by CPS against lethal *G.parasuis* infection.

Conclusions: By conjugating the polysaccharide from *G.parasuis* with OMVs, we have successfully generated vaccines that can efficiently deliver glycotopes to the immune system, and effectively elicit antigen-specific antibody responses in mice. These results highlight the potential of using CPS as a promising vaccine candidate against *G. parasuis* infection. Overall, the development and use of effective vaccines are crucial in reducing the need for antibiotics and combating antimicrobial resistance. Our study highlights the potential of a cross-protective vaccine against *G. parasuis*, which could have significant implications for the livestock industry and public health. We expect the vaccine to provide a promising avenue for controlling this bacterium and reducing economic losses in swine industry.

Notes:

P098 - Novel pseudorabies virus (PRV) vectored subunit vaccine against African swine fever

Selvaraj Pavulraj[1], Caleb S Kleinpeter[1], Shafiqul I Chowdhury[1]

[1]Department of Pathobiological Sciences, Louisiana State University. chowdh@lsu.edu

Session: Vaccinology, **2023-01-22, 6:00 - 8:00**

Objective: This is the first year of this study funded by a USDA grant. African swine fever (ASF) is a highly contagious and fatal disease of domestic and wild pigs caused by the ASF virus (ASFV). The virus infection causes disease conditions ranging from chronic or persistent infection to acute hemorrhagic fever. The acute form of the disease is exceptionally fatal with 100% mortality in pigs and characterized by hemorrhage, lymphopenia, and disseminated intravascular coagulation. This devastating pig disease has spread over three continents including Africa, Asia, and Europe. In 2021, the disease appeared in America after a 40-year absence—it was introduced to the Dominican Republic and Haiti. ASF has never been isolated in the United States (US); however, there is a significant risk of ASF incursion into the US. Therefore, ASF poses a substantial threat to the US swine industry. There are no commercial vaccines available to prevent and control ASF. Experimentally, live attenuated or gene-deleted ASFV vaccines have shown protective efficacy. However, the safety of these vaccines is a concern since there is the risk of reversion to virulence or recombination of vaccine viruses with field viruses. In the current study, our primary goal is to construct and test the protective efficacy of the novel PRV quadruple mutant virus (PRVqmv) vectored subunit ASFV vaccines for pigs (A cocktail of ten chimeric ASFV proteins). Our objective in this project is to i) Construct and *in vitro* characterize three different variant versions of PRVqmv Sub-ASFV vaccines (combination of chimeric ASFV proteins with or without M448R/MGF505-7R and CP204L proteins that are consistent targets of cytotoxic T cells and enhanced ASFV infectivity *in vitro*, respectively). ii) Evaluate the safety, stability, intranasal replication, and protective immunogenicity of three versions of PRVqmv Sub-ASFV vaccines in pigs. iii) Determine the protective efficacy of the PRVqmv Sub-ASFV vaccine against virulent ASFV-G challenges in pigs.

Methods: We have constructed a codon-optimized (porcine) ASFV chimeric gene expression cassettes i.e., i) ASFV MGF505-5R - P2A - B646L- F2A - F317L- GMCSF; ii) ASFV B602L - P2A - E183L - T2A - E199L - F2A - EP153R; iii) ASFV M448R - P2A - MGF505-7R - E2A - CP204L and incorporated them into PRV gG-deletion, gE-US9 deletion and TK-deletion plasmid vectors respectively. The chimeric polyprotein design included different self-cleavable peptide 2A (P2A, E2A, F2A, and T2A) to generate the individual proteins in the transfected/infected cells. Currently, sequential co-transfection of PRV quadruple gene mutant (PRVqmv) full-length genomic DNA with the resulting ASFV chimeric genes insertion vector DNA by homologous recombination is in progress. In the future, a putative PRVqmv expressing chimeric ASFV genes will be isolated, plaque purified, and characterized for the individual chimeric ASFV protein expression *in vitro*.

Results: We successfully designed and synthesized all three ASFV chimeric gene expression cassettes. Further validated the expression of all chimeric ASFV proteins upon transfection into the 293T cells using ASFV protein-specific and/or tag-specific antibodies.

Conclusions: This is the first year of the project. We expect to generate and *in vitro* characterize the three versions of the PRVqmv Sub-ASFV vaccine virus.

Notes:

P099 - Safe and broadly cross-protective live attenuated influenza virus vaccines for use in swine

Daniela Rajao[1], C. Joaquin Caceres[1], Brianna Cowan[1], Tavis Anderson[2], Amy Vincent Baker[2], Daniel Perez[1]

[1]University of Georgia, [2]National Animal Disease Center, USDA-Agricultural Research Service. daniela.rajao@uga.edu

Session: Vaccinology, **2023-01-22, 6:00 - 8:00**

Objective: Influenza A virus (FLUAV) causes significant economic losses to the global swine industry and vaccination remains the most effective method of defense against influenza in pigs. However, currently available swine influenza vaccines result in limited protection against all the antigenically distinct FLUAV that currently co-circulate in pigs. The major goal of this project is to develop live attenuated influenza virus (LAIV) vaccine strategies that are not only safe and effective, but also greatly impaired in their capacity to reassort with field strains.

Methods: We previously developed a stable and efficacious LAIV strategy for FLUAV carrying temperature-sensitive (ts) mutations in the PB2 and PB1 open reading frame (ORF), in addition to a C-terminal HA epitope tag in the PB1 ORF (termed Flu-att). To further improve the immune stimulation of the Flu-att, we have modified the hemagglutinin (HA) gene segment of swine-adapted H1N1 (A/CA/04/2009, CA/09) and H3N2 (A/turkey/Ohio/313053/2004, OH/04) viruses to express the porcine IgA-inducing protein (IGIP; Flu-IGIP). We have also modified the neuraminidase (NA) gene segments to express an interleukin immune stimulator. To improve safety and reduce reassortment potential, unique molecular markers were incorporated into the sequence of specific internal gene segments (PB2, PA, NP, and NS) of both Flu-att strains.

Results: Plasmids were constructed containing the modifications described above. Following transformation, recombinant plasmid stocks were amplified and confirmed. Construction of internal gene plasmids containing unique molecular markers is ongoing. Virus rescue with individual genes in the Flu-att backbone was successful for IGIP-H1, IGIP-H3, and N2-IL. Virus rescue with different combinations of gene segments is ongoing. Sequences will be confirmed, and viral stocks prepared. Once all viruses are confirmed, LAIV vaccine strains will be tested in vitro for growth kinetics and stability, as well as the reassortment potential using co-infection assays. Strains confirmed to be stable will be tested in pigs for their safety, transmission, and immune responses. The best vaccine candidate will then be selected to be tested in pigs for efficacy against infection with heterologous viruses.

Conclusions: These studies will generate and demonstrate that modified LAIVs are safe and effective in protecting pigs against influenza.

Financial Support: This project was supported by Agriculture and Food Research Initiative Competitive Grant no. 2022-67015-37205 /project accession no. 1028058 from the USDA National Institute of Food and Agriculture.

Notes:

P100 - Intradermal vaccination with a STING-targeting nanoparticle adjuvant enhances immunity against swine influenza

Juan F. Hernandez-Franco[1], Gourapura J. Renukaradhya[2], Harm HogenEsch[1, 3]

[1]Department of Comparative Pathobiology, Purdue University., [2]Department of Animal Sciences, The Ohio State University, [3]Purdue Institute of Inflammation, Immunology and Infectious Diseases. jfhernan@purdue.edu

Session: Vaccinology, **2023-01-22, 6:00 - 8:00**

Objective: The development of cross-protective vaccines targeting the potential pandemic-causing swine influenza A virus (swIAV) remains a global health challenge. Commercially available swIAV vaccines administered via intramuscular (IM) injection provide limited cross-protection, contributing to poor protection against circulating virus variants. Intradermal (ID) vaccination may induce a more robust immune response as it targets a tissue that contains diverse populations of antigen-presenting cells. The goal of this research was to determine whether ID vaccination utilizing a novel adjuvant combination of a stimulator of interferon genes (STING) agonist and nanoparticles (NanoST) elicit a superior cross-reactive antibody response than immunization via IM or intranasal (IN) routes.

Methods: We compared the efficacy of a commercial IM-injected multivalent swIAV vaccine and a monovalent whole-inactivated H1N2 swIAV (WIV) vaccine administered via IM injection, needle-free ID injection, or IN spray. The NanoST adjuvant, a combination adjuvant system consisting of a cationic phytoglycogen-based nanoparticle combined with the STING agonist ADU-S100, was formulated with the ID, IN, and IM-administered monovalent WIV vaccines. Pigs received two vaccinations three weeks apart, followed by a heterologous H1N1 challenge two weeks after the second vaccination. Serum, nasal swab, lung lysate, and BAL fluid were tested for H1N1-OH7, H1N2-OH10, and H3N2-OH4-specific IgG and IgA. The avidity of the antibodies was determined. Hemagglutination inhibition (HI) titers were evaluated using serum and BAL fluid. Serum virus neutralization (VN) titers were accessed. H1N2 and H1N1-specific antibody-secreting cells (ASCs) in the bone marrow of vaccinated pigs were enumerated by the ELISpot assay.

Results: The WIV/NanoST vaccine administered intradermally induced a significantly greater cross-reactive nasal ($p < 0.05$) and serum ($p < 0.05$ to $p < 0.001$) antibody response compared to IN and IM immunization with WIV/NanoST or the commercial vaccine. The avidity of the antibodies in pigs vaccinated intradermally was significantly higher in comparison to pigs vaccinated with the IN ($p < 0.001$), IM ($p < 0.05$), and commercial ($p < 0.01$) vaccines. Bone marrow from ID and IM-immunized pigs had increased numbers of IgG ($p < 0.01$ and $p < 0.05$, respectively) and IgA ($p < 0.01$ and $p < 0.05$, respectively) virus-specific ASCs. Pigs that received the commercial vaccine or WIV/NanoST via ID administration had significant HI ($p < 0.001$ and $p < 0.0001$, respectively) and VN ($p < 0.001$ and $p < 0.05$, respectively) titers.

Conclusions: Intradermal vaccination with the STING-targeted NanoST adjuvant system enhanced the efficacy of the immune response generated by a monovalent WIV vaccine. This investigation provides a strong rationale for the development of NanoST as a safe and versatile adjuvant platform to develop next-generation swIAV vaccines.

Financial Support: This work was supported by USDA-NIFA Grant 2019-67015-29814 and USDA Hatch formula funds of project IND020164H.

Notes:

P101 - A minimally replicative PRRSV vaccine targeting improved safety and efficacy

Wenjuan Fang[1], Sheela Ramamoorthy[1]

[1]North Dakota State University. sheela.ramamoorthy@ndsu.edu

Session: Vaccinology, **2023-01-22, 6:00 - 8:00**

Objective: Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) causes severe respiratory illness in young piglets and early abortion in pregnant sows. It continues to remain the leading cause of economic losses to the pork industry world-wide. An effective PRRSV vaccine is yet to be developed, likely due to the high genetic and antigenic variation exhibited by PRRSV. Generally, attenuated vaccines are more effective than inactivated vaccines against PRRSV. However, they are associated with serious safety concerns due to reversion to virulence or recombination with field strains.

Methods: To improve the safety and efficacy of PRRSV modified live vaccines (MLVs), selected coding regions of the M and N genes of a PRRSV infectious clone were mutated such that the propensity to accumulate premature stop codons during viral replication was increased without altering the amino acid composition of the genes. PRRSV VR 2385 M and N genes encoding the desired mutations were commercially synthesized. They were shuttled into a DNA launched infectious clone by restriction digestion.

Results: Recombinant PPRSV with mutated M&N genes or a mutated N gene alone was successfully rescued by transfection and passage in M145 cells. The rescued mutated viral strains were serially passaged in M145 cells. The mutations were stable over 7 serial passages and viral titers of over $1x10^6$ $TCID_{50}$ were obtained.

Conclusions: The PRRSV modified live vaccine (MLV) constructs will be tested in 2-3 old PRRSV negative weanling pigs to determine if the suicidal PRRSV MLV is cleared from the host shortly after vaccination and assess whether minimal replication of the MLV in the host will elicit protection which is superior to currently available commercial MLVs. As vaccine safety is paramount, development of this novel approach to improve vaccine safety can have far reaching application to other viruses with high mutation rates and well characterized reverse genetics systems.

Notes:

P102 - Validation of a live-virus PRRSV vaccine candidate for efficient attenuation and better protection

Laura C. Miller[1], Sarah J. Anderson[2], Alexandra Buckley[2], Erin Schirtzinger[1], Kaitlyn M. Sarlo Davila[2], Damarius Fleming[2], Albert Van Geelen[3], Kelly Lager[2], Jiuyi Li[4], Yongming Sang[4]

[1]Kansas State University, [2]USDA-Agricultural Research Service, [3]USDA-Animal and Plant Health Inspection Service, [4]Tennessee State University. lauracmiller@vet.k-state.edu

Session: Vaccinology, **2023-01-22, 6:00 - 8:00**

Objective: Porcine reproductive and respiratory syndrome (PRRS) is a complex and costly disease in the swine industry, due in part to the high degree of genetic variation among PRRS virus (PRRSV) field isolates. Although there are PRRSV vaccines currently available, they can have varying degrees of cross-protection depending on genetic similarity**.** We have identified several host interferons that have superior antiviral properties potentiating immune responses in pigs. Preliminary evaluation of a cohort of optimized antiviral IFNs including each of IFN-α, IFN-β (IFNmix) and IFN-ω subtypes was demonstrated *in vitro* through reverse genetic incorporation into the Type 2 PRRSV p129 expression vector. We performed *in vivo* studies in pigs to compare our novel vaccine candidates to commercially available MLV vaccines using a contemporary challenge virus (NADC-34). Here, we present the clinical data.

Methods: The *in vivo* studies were conducted in commercial pigs (n = 10/group): sham vaccine + sham challenge, sham vaccine + challenge, MLV-commercial + challenge, MLV-PRRSVp129-IFN-ω + challenge, MLV-PRRSVp129-IFNmix + challenge. Pigs in all treatment groups were monitored for clinical signs, weighed, and temperature recorded throughout the study. Serum was collected to evaluate viral load with real-time-RT-PCR and the immune response with a commercial PRRSV ELISA, and whole blood was used to evaluate gene expression.

Results: This pilot study demonstrated that antiviral IFN vaccine prototype efficacy was comparable to commercially available PRRSV MLV vaccine. In this study, pigs administered the novel vaccines had similar ELISA titers prior to challenge and reduction in viral load in the serum after challenge to those given the commercial MLV. In addition, the MLV-PRRSVp129-IFNmix numerically reduced temperature and viral load greater than MLV-PRRSVp129-IFN-ω.

Conclusions: A DNA-launched reverse genetics system for PRRSV and co-expression of immunomodulatory peptides designed to directly reverse PRRSV suppression on the pig's IFN signaling and associated immune response has the potential to enhance vaccine efficacy against heterologous PRRSV strains compared to currently available vaccines.

Financial Support: This research was supported by the intramural research program of the USDA-NIFA-AFRI FASE-EPSCoR. The findings and conclusions in this preliminary presentation have not been formally disseminated and should not be construed to represent any agency determination or policy.

Notes:

P103 - T cell epitope content comparison of swine H1 influenza A virus hemagglutinin to identify the best vaccine match

Andres H. Gutierrez[1], William D. Martin[1], Anne S. De Groot[1]

[1]EpiVax, Inc.. annied@epivax.com

Session: Vaccinology, **2023-01-22, 6:00 - 8:00**

Objective: T cell Epitope Content Comparison (EpiCC) is an algorithm that compares the putative T cell epitope content shared between commercial vaccines and field isolates to identify the best vaccine match based on T cell epitope relatedness and coverage. The vaccine that covers more of the T cell epitope content of an isolate may confer broader cross-reactive cell-mediated immune response. Based on EpiCC and leveraging an analysis of a large population of field strains, we developed a web application for porcine circovirus type 2 (PCV2). Similar web applications can be developed for other swine pathogens. To illustrate the application of EpiCC, we compared the putative T cell epitope content of hemagglutinin (HA) from circulating H1 swine influenza A virus (sIAV) strains and randomly selected strains from different phylogenetic clades as potential vaccines to identify the best match. For each clade, we also identified the best vaccine candidate.

Methods: 930 HA sequences of H1 sIAV circulating in America from 2019 to 2023 were downloaded from GISAID. This set comprised strains from alpha (86), beta (5) beta-like (4), gamma (340), delta1 (16), delta2 (278), npdm (187), and unclassified (14) clades. Putative SLA class I and II T cell epitope content in the input sequences were identified using PigMatrix. EpiCC assessed the relatedness of T cell epitopes contained in HA protein sequences between field isolates and 3 randomly selected vaccine strains from the most frequent clades. EpiCC generated a score for each vaccine-isolate comparison and an assessment of T cell epitope coverage.

Results: Vaccine-isolate comparisons tended to be clade-specific. T cell epitope coverages of the randomly selected gamma, delta2, and npdm vaccines were on average, 89.24%, 84.54%, and 89.37%, respectively for strains of the same clade, and between 31.78% to 67.56% for strains from different clades. EpiCC results showed that certain vaccines were better matches for frequently isolated circulating strains. These results could be applied to identify the best vaccine match for a specific farm or in an outbreak. Moreover, strains with the highest overall EpiCC scores for each clade were identified. These strains had higher EpiCC scores than randomly selected vaccines.

Conclusions: EpiCC scores were clade-specific. We were able to identify vaccine candidates with better T cell epitope coverage than randomly selected vaccines. EpiCC can be applied to commercial and autologous vaccines to assess T cell epitope coverage against diverse circulating viruses. EpiCC may complement current methods for selecting the best-matched vaccine. Clinical data may help to refine EpiCC predictions and understand the relationship between shared epitope content and clinical outcomes.

Notes:

P104 - Vet-ting for victory: exploring bison producers' access to veterinary services in Ontario, Canada

Natassia H Lambrou[1], Kelsey L Spence[1], Charlotte B Winder[1]

[1]Department of Population Medicine, University of Guelph, Canada. nlambrou@uoguelph.ca

Session: Epidemiology, **2023-01-22, 6:00 - 8:00**

Objective: The primary objective of this study was to explore the current access to veterinary services among bison producers in Ontario, Canada. Our secondary objective was to identify any perceived barriers to accessing veterinary services and determine how this may impact bison health and management practices across the industry.

Methods: Bison producers in Ontario were recruited through email to participate in virtual focus groups of 3-5 producers using Zoom. The audio from the focus group was transcribed verbatim using the online transcription platform Otter.ai, and further verified by the research team for accuracy and completeness. The transcripts were then imported into NVivo and analyzed using qualitative content analysis to identify themes across the data.

Results: One focus group with 3 producers has been conducted to date, with several additional focus groups scheduled. Preliminary analysis suggests two prominent barriers to accessing veterinary services raised by participating bison producers: a lack of new large animal veterinarians entering the field and increased costs associated with veterinary visits. As recruitment continues, the data from the focus groups will continue to be analyzed to determine how these barriers may impact bison health and management.

Conclusions: This research possesses the potential for far-reaching national implications and holds significant promise for informing future research within the farmed bison sector. The findings of this study will lay a strong foundation that policymakers and industry stakeholders can use to create more innovative initiatives aimed at improving bison producers' access to veterinary services. This, in turn, will play a crucial role in encouraging the sustained growth and development of the farmed bison industry in Ontario.

Notes:

P105 - Evaluating estimated carbon emissions and feedlot production data to address beef sustainability

Deanna M. Gennett[1], Taylor B. McAtee[1], David G. Renter[1]

[1]Department of Diagnostic Medicine and Pathobiology, Kansas State University. gennett@vet.k-state.edu

Session: Epidemiology, **2023-01-22, 6:00 - 8:00**

Objective: With climate change and agricultural sustainability considered major societal issues, the goal of this study was to provide insight on how feedlot cattle, directly and indirectly, affect greenhouse gas emissions, and to demonstrate relationships between feedlot cattle health, performance, and greenhouse gas emissions. The specific objective of this study was to determine how various feedlot metrics, including for cattle health and performance, are associated with estimates of greenhouse gas emissions.

Methods: Operational animal health and feedlot performance data were obtained from multiple commercial feedlots via formal data sharing agreements. These data were linked by unique lot (cohort) identifiers with outputs from a proprietary life cycle assessment system that estimates carbon equivalents from predicted greenhouse gas production. The primary dataset included over 10,000 lots (cohorts) of cattle containing a range of 10-358 animals per lot. Descriptive graphs and figures were created from the cattle health and performance metrics, and the corresponding lot-level emissions estimates for data analysis, visualization, and pattern identification.

Results: Using the estimated emissions, the cow-calf, or pre-feedlot, phase of production was associated with approximately 80% of a cattle's total lifetime emissions while the feedlot sector was associated with the remaining 20%. Our data indicated that the production and delivery of feed (49%) comprises the largest source of greenhouse gas production during a cattle's life cycle with the remaining coming from enteric fermentation (27%), manure (21%), and fuel (3%). Within their life cycle, steers produced 4.47% more emissions than heifers on a per animal basis, while heifers produced 7.39% more emissions on a per kilogram of live cattle weight basis (as a measure of beef production). Lifetime emissions per kilogram of cattle weight were reduced when days on feed in the feedlot and mortality were minimal and weight gain relative to feed intake was high.

Conclusions: Based on this study and other ongoing work, we can conclude that cattle health and performance in the feedlot have an impact on the quantity of greenhouse gas emissions that are generated during beef production. In other words, healthy and efficient cattle in the feedlot are associated with less greenhouse gas emissions and are overall more sustainable from an emissions perspective. Recognizing how cattle production systems impact carbon emissions is a first step towards improving beef sustainability and reducing the associated carbon footprint.

Financial Support: This study was funded by the College of Veterinary Medicine's Center for Outcomes Research and Epidemiology, and the Global Food Systems program, Kansas State University.

Notes:

P106 - 2023 update on the impact of management decisions on BRD morbidity, mortality, and performance in beef calves

Sarah Capik[1], Robert Larson[2], Brad White[2], David Amrine[2], Brandi Karisch[3], Kelsey Harvey[3], Jane Parish[3], Amelia Woolums[4], Hudson McAllister[5], Vinicius Gouvea[6], Matthew Scott[5]

[1]Tumbleweed Veterinary Services, [2]Department of Clinical Sciences, Kansas State University, [3]Department of Animal and Dairy Sciences, Mississippi State University, [4]Department of Pathobiology and Population Medicine, Mississippi State University, [5]VERO Program, Texas A&M University, [6]Dept. of Animal Sciences, Texas A&M University. mascott@cvm.tamu.edu

Session: Epidemiology, **2023-01-22, 6:00 - 8:00**

Objective: 1) Examine the effect of vaccination twice during preweaning on inflammatory mediators, preweaning performance, and BRD morbidity and mortality during backgrounding; 2) quantify the impact of marketing decisions on inflammatory mediators, BRD morbidity, mortality, and performance in weaned beef calves sent directly to backgrounding or sent via an auction market and order buyer; and 3) evaluate associations between pen- and yard-level management factors and health outcomes during the feedlot phase of production.

Methods: Objectives 1 and 2: In a 3-year randomized control trial with a split-plot design, 84 male calves per year will be vaccinated at ~90 and ~180 days of age with a 5-way modified-live respiratory vaccine or not during preweaning then marketed directly to a backgrounding facility or marketed via an auction market prior to transport to a backgrounding facility for 45 days. Blood was collected for analysis of inflammatory mediators (TNF-a, IL-1b, and haptoglobin via commercial ELISAs), and performance, BRD morbidity and mortality were recorded throughout. Objective 3: An existing relational feedlot database was used to explore the impact of pen- and yard-level factors (stocking density, shared resources between pens, animal flow) on BRD morbidity and mortality using generalized linear mixed effect models ($p<0.05$).

Results: Objectives 1 and 2: Year 1: During the cow-calf phase, no BRD occurred; average daily gain (ADG) was 0.94 kg ± 0.47. During backgrounding, 39.5% morbidity, 18.8% retreatment, and 1.2% mortality rates were recorded; ADG was 1.11 kg ± 0.45. Year 2: During the cow-calf phase, no BRD occurred; ADG was 0.91 kg ± 0.17. During backgrounding, 10.7% morbidity, 0.0% retreatment, and 0.0% mortality rates were recorded; ADG was 1.24 kg ± 0.31. ELISA for TNF-a and IL-1b for years 1 and 2 are complete. Full statistical analysis will be performed following the collection of Year 3 data. Objective 3: Results have been fully published as of 2022.

Conclusions: Objective 3 of this study is complete. Live animal data collection for Objectives 1 and 2 was postponed due to logistical constraints, with the expectation of final data collection occurring in 2024. Final statistical analyses will occur in spring 2025.

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Notes:

P107 - Optimization of targeted enriched metagenomics protocol to obtain strain-level data for *Mycoplasma bovis* in respiratory samples from cattle

Sal Lamsal[1], Christopher Panaretos[2], Ethan Dudley[1], Lee Pinnell[1], Matthew Scott[1], John Richeson[3], Hatem Kittana[1,4], Paul S. Morley[1], Robert Valeris-Chacin[1]

[1]Veterinary Education, Research, and Outreach, Texas A&M University, [2]Dept of Veterinary Pathobiology, Texas A&M University, [3]Dept of Agricultural Sciences, West Texas A&M University, [4]Texas Veterinary Medicine Diagnostic Lab. sal@tamu.edu

Session: Epidemiology, **2023-01-22, 6:00 - 8:00**

Objectives: Bovine respiratory diseases (BRD) is a major health challenge to the beef cattle industry. Mycoplasma bovis has been linked to chronic cases of BRD. However, advances in molecular techniques may provide further insights into *M. bovis* ecology, removing the bias associated with culture. Therefore, the objective of this study was to develop and optimize a targeted enriched metagenomics protocol for *M. bovis* (using RNA baits to enrich *M. bovis* DNA) in samples from the respiratory tract of beef cattle. Specifically, we evaluated different molarity strengths of the baits because lower bait molarity may improve specific binding in conditions of low target abundance and drastically reduce the cost of the method.

Methods: Thirty Nasopharyngeal swabs collected from West Texas A&M University feedlot were extracted using PowerSoil Pro (Qiagen) kit and were confirmed to be positive for *Mycoplasma bovis* using digital qPCR system. These samples were used to optimize a targeted enriched metagenomics (TE) protocol. The bait set (n=29,296 baits) used for the TE protocol were designed using syotti and produced by Agilent. This bait set was used in four different molarities; quarter, half, three quarters, and full (the one recommended by the manufacturer). All four molarities were tested in all samples. Two rounds of TE (double capture) and a pre-library preparation enrichment were used, both of which conditions have been shown to greatly increase the enrichment in *Mannheimia haemolytica* in our lab. DNA libraries will be submitted to Texas A&M Institute for Genome Sciences and Society (TIGGS) for sequencing in an Illumina platform (NovaSeq). Reads obtained from sequencing will be aligned to the kraken2 database to calculate the number and percentage of reads on target i.e., classified as M. *bovis.* The number and percentage of on target reads will be compared between molarity strengths to determine the optimal concentration of baits.

Results: We observed a linear decrease in the concentration of the final DNA libraries after the TE. Additionally, all DNA libraries met the minimum quality control for sequencing regardless of the bait molarities evaluated.

Conclusions: The lowest molarity evaluated (quarter) yielded good DNA libraries, suggesting that it can be used in the study of the strain-level dynamics of *M. bovis* in cattle with BRD at a much lower cost.

Financial Support: Texas AgriLife Research

Notes:

P108 - Effect of yeast supplementation on pro-inflammatory cytokines in steers inoculated with digital dermatitis

Marlee Henige[1], Kelly Anklam[1], Sair Ordaz Puga[2], Matthew Aviles[1], Summer Henschel[1], Julia Buettner[1], Ilkyu Yoon[3], George Dawson[3], Jeffrey Wheeler[3], Doerte Doepfer[1]

[1]School of Veterinary Medicine, University of Wisconsin, [2]College of Veterinary Medicine, University of Minnesota, , [3]Diamond V. henige@wisc.edu

Session: Epidemiology, **2023-01-22, 6:00 - 8:00**

Objective: Digital Dermatitis (DD) is currently the leading cause of lameness within the cattle industry causing major economic losses and decreased animal welfare. Due to concerns of antimicrobial resistance, there is growing interest in finding alternative methods for treatment and prevention of such bacterial diseases. Recent studies have demonstrated the positive effect *Saccharomyces cerevisiae* fermentation products (SCFP) have on the innate immune system of multiple species including cattle. Such reported benefits include increased interleukin levels, which play key roles in immune and inflammatory processes. The objective of this study is to evaluate the effect that SCFP based supplementation has on pro-inflammatory cytokines (IL-1beta and IL-6) in lightweight Holstein Friesian steers that have been experimentally inoculated with DD.

Methods: Cattle (n=120) enrolled in this study were randomly assigned to either a SCFP based supplementation or control supplementation group. A subset of cattle (n=25 per group) were randomly selected and inoculated with DD. The experimentally infected cattle were reintroduced to the healthy cattle 2-4 weeks post inoculation via pens of small groups to evaluate DD transmission rates. Whole blood samples were taken at different timepoints throughout the study for IL-1beta and IL-6 testing (stimulants include: Mock, PAM, Poly, and LPS5). Blood samples were collected upon arrival (week 1, n=43), prior to inoculation (week 7, n=40), 3 weeks post inoculation (week 10, n=49), and 6 weeks post inoculation (week 13, n=36).

Results: When IL-1beta levels of samples exposed to a stimulant were compared to the Mock value, there were significant findings. SCFP supplementation in cattle with M1 and M4 lesions had increased interleukin levels when exposed to PAM. Similarly, SCFP supplemented cattle with M4 lesion also had increased interleukin levels when exposed to LPS5, but the same cattle with M4.1 lesions had decreased interleukin levels. When looking at the comparison of the data for IL-6 levels revealed that SCFP supplemented cattle had increased interleukin levels the more days post M2 lesion when exposed to PAM. When exposed to LPS5, SCFP supplemented cattle generally had decreased interleukin levels, but when they were faced with M4.1 lesions the interleukin levels decreased. Further analysis revealed that IL-1beta levels in SCFP supplemented cattle prior to DD exposure were significantly decreased when exposed to LPS5 (95% CI [0.62, 0.88]) with decreased trends for PAM and Poly. Post DD exposure, SCFP supplemented cattle IL-1beta levels were significantly elevated for PAM (95% CI [1.03, 2.06]) and LPS5 (95% CI [1.12, 1.67]). Upon further analysis of IL-6 levels, SCFP supplemented cattle prior to DD exposure were significantly elevated for Poly (95% CI [1.28, 2.43]) and LPS5 (95% CI [1.29, 2.30]) with increased trends for PAM. Post exposure, SCFP supplemented cattle IL-6 levels were initially elevated for LPS5 and then decreased with time, whereas PAM caused the opposite effect.

Conclusions: These results suggest that SCFP based supplementation has a significant effect on IL-1beta and IL-6 levels in dairy steers and provides increased immune support. Future studies are warranted to further explore the effects that SCFP has on cattle's innate immune system.

Notes:

P109 - Factors influencing Ontario dairy producers' management and care of down dairy cattle.

J.E. Brindle[1], D.L. Renaud[1], T. Duffield[1], D. Haley[1], C.B. Winder[1]

[1]University of Guelph. brindlej@uoguelph.ca

Session: Epidemiology, **2023-01-22, 6:00 - 8:00**

Objective: The objective of this cross-sectional study was to describe how Ontario dairy producers manage down cows, and identify factors associated these practices.

Methods: An online survey was distributed to all dairy producers in Ontario, Canada (n = 3,367) and was available from November 2020 to March 2021. All dairy producers were identified through the provincial dairy organization and contacted via email and social media. The complete survey was comprised of 134 questions, 31 of which were related to down cow management. Descriptive statistics were evaluated, and two logistic regression models were generated using STATA17, exploring factors associated with 1) relocating down cows with hip lifters and 2) assisting cows to stand within an hour after going down.

Results: A total of 226 farmers responded, which was a response rate of 7.4%. Participants were predominantly male (68%), farm owners (78%) and between 30 and 39 years old (29%). The first model found that smaller farms (< 57 cows) were less likely to move down cows with hip lifters, compared with larger farms (> 129 cows) (odds ratio [OR] = 0.33; P = 0.04; 95% CI 0.11-0.97). In addition, farms that used hip lifters to lift cows had a higher odds of moving down cows with hip lifters (OR = 12.96; P < 0.001; 95% CI 2.92-57.49). The second model identified that farms that used hip lifters to move cows had a higher odds of assisting a cow to stand within an hour following recumbency (OR = 13.04; P < 0.001; 95% CI 3.23-52.65). Additionally, producers who waited longer to relocate a down cow (< 1 h vs. > 1 h) had a lower odds of assisting the cow to stand within one hour of finding them down (OR = 0.03; P < 0.001; 95% CI 0.006-0.129).

Conclusions: Ensuring the well-being of down dairy cows remains a significant concern for the industry. There was considerable diversity in producers' approaches to managing down cows. Implementing standardized practices could aid producers in applying evidence-backed management techniques on their farms, enhancing animal welfare.

Financial Support: This work is supported by funding from the Ontario Agri-Food Innovation Alliance, the Saputo Dairy Care Program, and Dairy Farmers of Ontario.

Notes:

P110 - Surveillance of Bovine Leukemia Virus prevalence in southeast Florida dairy herds

Monalisa Lima[1], Jessica Prim[1], Kristen Schmidt [1], Kristi Jones[1], Klibs Galvao[1], Ricardo Chebel[1], Fiona Maunsell[1], Joao Bittar[1]

[1]University of Florida. msousadiaslima@ufl.edu

Session: Epidemiology, **2023-01-22, 6:00 - 8:00**

Objective: This study aims to determine the prevalence of bovine leukemia virus (BLV) infection, a contagious disease of cattle, in dairy herds in southeast Florida.

Methods: For this study, samples were collected continuously over a six-month period from a total of 13 herds, with each herd contributing 40 cows, resulting in a total sample size of 520 (n = 520). Each participating farm received a kit containing all the materials needed to collect blood samples. Ten cows from each lactation, from the first to those in the fourth or more lactations, were randomly selected for testing. Blood samples were obtained from tail vessels using evacuated tubes without anticoagulants. These samples were allowed to clot for one hour and then centrifuged at 3,000 g for 10 minutes to separate the serum. Two 1 mL aliquots were frozen at −20°C for future testing. One of these aliquots was sent to the Iowa Veterinary Diagnostic Laboratory at Iowa State University for BLV testing using a commercial ELISA kit (Bovine Leukemia Virus Antibody Test Kit, ELISA, VMRD Inc, Pullman, Wash). The ELISA test demonstrates high sensitivity and specificity, exceeding 95%, especially for animals infected for at least 55 days (Nagy et al., 2003). Dry cows or cows with 21 days of lactation were excluded from the study as false negatives are more likely in this specific group. This approach has been shown to result in a 99% correlation with true prevalence based on whole herd sampling (Erskine et al., 2012). The prevalence of BLV was compared between lactations using the Chi-square method from the stats package of R studio (Version 2023.09.1). *P*-values were adjusted for multiple tests using Bonferroni corrections.

Results: Based on the results of the descriptive analysis, the average BLV prevalence was calculated at 66.5% (range = 36.2% - 83.1%). Once evaluating parity, the prevalence of BLV was lesser in first lactation (36.2%; confidence interval [CI] = 27.9 - 45.0%) than in second lactation (62.3%; CI = 53.4 - 70.7%; $P < 0.01$), third lactation (84.6%; CI = 77.2 - 90.3%; $P < 0.01$), and fourth or grater lactation (83.1%; CI = 75.5 - 89.1%; $P < 0.01$). The prevalence of BLV was lesser in the second lactation than in the third lactation ($P < 0.01$) and fourth or greater lactation ($P < 0.01$). There was no difference in BLV prevalence was found between the third and fourth lactations ($P = 0.86$).

Conclusions: These results present the different prevalence rates of BLV at different stages of lactation in the studied population, highlighting the increased prevalence of the disease directly correlated with the number of lactations. In addition, BLV prevention and control strategies must target not only the northern states but also the southeast of the country. Therefore, a clear understanding of the relationship between BLV prevalence and lactation stages enables more effective decision-making in herd management, animal selection, and disease control, leading to increased efficiency in livestock farming operations.

Financial Support; We would like to acknowledge research support from the Southeast Dairy Producers Checkoff for their financial support in carrying out this project.

Notes:

P111 - Temporal identification of bovine leukemia virus in dairy youngstock and impact on milking herd prevalence

Madison E. Sokacz[1], Kelly R. B. Sporer[2], Paul Bartlett[3], Casey Droscha[2], Bo Norby[3], Tasia M. Taxis[1]

[1]Department of Animal Science, Michigan State University, [2]CentralStar Cooperative Inc., [3]Department of Large Animal Clinical Sciences, Michigan State University. sokaczma@msu.edu

Session: Epidemiology, **2023-01-22, 6:00 - 8:00**

Objective: Bovine Leukemia Virus (BLV) is a delta-retro virus responsible for the chronic infection of bovine leukosis. The most noted negative impacts on animals infected with BLV are decreased profitability, longevity, and welfare. The identification of these negative impacts are the result of decades of BLV research focused on lactating cows, as the lactating cows are the division of a dairy operation that generates revenue. While some research has addressed early life BLV infections, to our knowledge, no studies have conducted a comprehensive longitudinal study assessing time points potentially associated with higher probability of infections in youngstock. Youngstock, in this case, refers to animals raised on a farm from birth until their first calf. To bridge the knowledge gap, this study aimed to investigate the time when youngstock exhibit the greatest probability of BLV infection.

Methods: A cohort of youngstock (n=254) from five Midwest commercial dairy herds underwent longitudinal evaluation for BLV infection. Blood samples were collected at three time points: neonate, heifer prior to breeding, and bred heifer. BLV infection was identified using a BLV qPCR. Following completion of sample collection, odds ratio analysis was conducted to assess relative infection probabilities at different time points.

Results: Analysis revealed an increase in youngstock BLV prevalence, particularly in the first year. Neonate BLV prevalence was 1.2%, rising to 11.9% in heifers prior to breeding and 13.9% in bred heifers. Time points overall significantly impacted prevalence (p=0.0001). Furthermore, odds ratio analysis showed significantly higher infection probabilities based on time points. Specifically, heifers prior to breeding compared to neonates had a greater probability of BLV infection (OR=10.9, 95% CI, [3.26, 36.34]), and in bred heifers compared to neonates (OR=13.5, 95% CI, [4.09, 44.77]). While there was a rise in prevalence from heifers prior to breeding to bred heifers, the odds ratio analysis showed this was not significant (OR=1.2, 95% CI, [0.72, 2.13]).

Conclusions: As the probability of BLV infection becomes increasingly significant as youngstock age, it is evident that youngstock are subjected to transmission risk following the neonatal age. This leads us to believe there are early life events that may be BLV transmission risks; potentially including dam status, vaccinations, the use of heifer growers, dehorning, and housing choices.

Financial Support: This project was supported by Agriculture and Food Research Initiative Competitive Grant no. 2020-67015-21562 from the USDA National Institute of Food and Agriculture.

Notes:

P112 - Brucellosis surveillance in Georgia in 2020-2022 years

Natia Kartskhia[1], Tengiz Chaligava[1], Vladimer Baratashvili[1], Marina Donduashvili[2]

[1]National Food Agency, [2]State Laboratory of Agriculture. n.kartskhia@gmail.com

Session: Epidemiology, **2023-01-22, 6:00 - 8:00**

Objective: Brucellosis is widespread in Georgia with a substantial number of small, large ruminants and human infections. For improving human and livestock health, livestock productivity, and international trade, it is important to control and eradicate brucellosis in the country. Georgia is a traditional agricultural country with a human population of about 3.7 million. About 50% of the population lives in rural areas and 90% of farms are backyard farms. During 2014 - 2017 years under the active surveillance program 538 721 large ruminants were tested for the presence of brucellosis, out of them 10 199 animals (1.89%) were positive animals in the herd level. The aim of the brucellosis passive surveillance campaign in 2020 - 2022 years was to identify brucellosis positive large and small ruminants in the villages, based on animals' clinical signs/human cases and conduct slaughtering of animals to decrease prevalence in herd level. Electronic integrated disease surveillance system was used for information exchange between human and animal health sectors (National food agency, national centre for disease control and prevention, state laboratory of agriculture).

Methods: Since 2018 animal testing continued under passive surveillance, animals with clinical signs of brucellosis or suspected to be a source of human infection were included in passive surveillance. Large and small ruminants samples were collected in accordance with biosecurity regulations. All sampled animals were tagged with unique ID number and tag number was labeled on the sample tube. Epidemiological information were recorded into the electronic integrated disease surveillance system. Samples were submitted to the state laboratory of agriculture by the state veterinarians. Rose bengal test were used as a screening test and positive samples were confirmed by fluorescence polarization assay (FPA) or enzyme-linked immunoassay (ELISA).

Results: In 2020 - 2022 years notification for brucellosis suspected cases/or human cases has been registered in nine regions. In total 176 villages, 768 holding and 4970 animals (4519 large ruminants and 451 small ruminants) were tested in 40 municipalities under the brucellosis passive surveillance campaign. Out of total tested villages, 368 positive animal were identified. Appeared prevalence was 7.4% on individual level. Based on data analyses it was determined that during the Brucellosis passive surveillance campaign, prevalence within a herd varied from 1.3 to 84.6 percentage. However data analyses shows that mode of prevalence is 9%, average 1.3%, minimum 2.2%, maximum 84.6%, median 16%.

Conclusions: Data from brucellosis passive surveillance campaign 2020 - 2022 years shows that brucellosis cases still occurs in Georgia with 7,4 % of prevalence on individual level. Previews years data analysis shows necessity of analysis from previous years shows the need for continued brucellosis surveillance. It is also necessary to continue vaccination campaign of female large and small ruminants with RB-51 and REV-1 vaccine.

Notes:

P113 - Estimation of basic reproduction number (R0) and analysis of spatio-temporal clustering of bovine brucellosis in Costa Rica

Ronald Castillo[1], Demelash Areda[2]

[1]World Organisation for Animal Health, France, [2]Ottawa University. demelash.biffa@gmail.com

Session: Epidemiology, **2023-01-22, 6:00 - 8:00**

Objective: Brucellosis is among the most economically significant diseases that impact the livestock industry, especially in the developing world. This zoonotic disease can be transmitted to humans through direct or indirect contact with infected animals or their byproducts. In Costa Rica, brucellosis is an endemic disease that affects a variety of domestic animals as well as human populations. In Costa Rica, the epidemiological data on brucellosis is insufficient. The objective of this study was to model the transmission dynamics and discern spatiotemporal clustering of brucellosis in Costa Rica.

Methods: We utilized R statistical computing for mathematical modeling and spatiotemporal cluster analysis of 4643 confirmed cases of brucellosis in 1141 outbreaks. The data was sourced from the SIVE application (System Integrated Epidemiological Surveillance), developed by SENASA (Costa Rican National Veterinary Services). Spatiotemporal analysis was conducted using the discrete Poisson model of the SaTScan® temporal space analysis tool. Additionally, we employed the R package for the Estimation of R_0 and Real-Time Reproduction Number.

Results: The results of the study showed that the basic reproductive number (R_0) of brucellosis in Costa Rica was as follows : 0.94 (95% CI=0.87-1.03) using the exponential growth method, 1.04 (95% CI=0.79-1.34) with the Maximum Likelihood method, and 1.01 (95% CI=1.015-1.016) using attack rate method. The number of spatial clusters of brucellosis varied significantly among the studied regions, with a higher occurrence in the Huetár Caribbean Region (6 clusters) and the lowest in the Brunca and Chorotega Regions.

Conclusions: This study confirms that brucellosis is unlikely to spread haphazardly among animal herds in Costa Rica. Consequently, implementing measures focused on specific geographic areas could significantly aid in controlling and preventing the disease and its associated impacts. As a follow-up to this study, we are currently working on developing mathematical models to provide information for decision-makers. This effort aims to generate valuable insights that will contribute significantly to the allocation of resources for the prevention and control of brucellosis in Costa Rica.

Notes:

P114 - Mathematical modelling assessment of *Salmonella* Dublin transmission and control in Northeastern US heifer raisers

Sebastián Llanos-Soto[1], Martin Wiedmann[2], Aaron Adalja[3], Christopher J. Henry[1], Elisha Frye[1], Paolo Moroni[1], Renata Ivanek[1]

[1]Department of Population Medicine and Diagnostic Sciences, Cornell University, [2]Department of Food Science, Cornell University, [3]School of Hotel Administration, Cornell University. sgl67@cornell.edu

Session: Epidemiology, **2023-01-22, 6:00 - 8:00**

Objective: Design a stochastic modeling framework of *Salmonella* Dublin transmission dynamics in a off-site heifer-raising operation to determine the efficacy of vaccination and improved cleaning in preventing and controlling *S.* Dublin.

Methods: We developed a modified Susceptible-Infected-Recovered-Susceptible model of the spread of *S.* Dublin in an off-site heifer-raising operation in the Northeastern United States. The model incorporated stochasticity via Monte Carlo simulations. To account for cattle susceptibility and the consequences of *S.* Dublin infection at different ages, we modeled animal progression through 3 successive age groups: weaned calves, growing heifers, and pregnant heifers. Vaccine efficacy was represented by a proportional reduction in the probability of death among young heifers. Improved cleaning was represented as an increase in the barn floor's scraping frequency from once (1x) to 3x, 5x, and 7x per week using a skid steer and 2x, 4x, 6x, and 8x per day using an alley scraper. The validated model was used to predict epidemiological and economic outcomes in a herd of 240 heifers over a 2-year simulation period in scenarios without (baseline) and with vaccination and improved cleaning. Epidemiological outcomes included the probability of an outbreak, infection-induced deaths, abortions, and carrier status of heifers departing to a dairy farm. An economic assessment was conducted to understand the influence that vaccination and cleaning improvements could have on the operating income of the heifer-raising operation. A Partial Rank Correlation Coefficient analysis determined the impact of model parameters on the outcomes of interest.

Results: Values for all epidemiological outcomes were increasingly reduced as cleaning became more frequent, with the outbreak probability decreasing from 92% (458/500 iterations) at baseline to 76%-59% (379 to 294/500 iterations) when alley scraping between 2x to 8x per day. At this cleaning frequency, deaths were reduced by 64% (from a median of 14 (interquartile range (IQR)=9-20) to 5 (IQR=0-12)), while carrier departures and abortions were reduced by 50% (from a median of 10 (IQR=7-12) to 5 (IQR=0-9)) and 60% (from a median of 10 (IQR=5-17) to 4 (IQR=0-11)), respectively. The performance of vaccination did not improve when combined with enhanced cleaning. The most influential parameters were the duration of the immune period and the *S.* Dublin shedding rate. Findings from the scenario analysis revealed that carriers act as important disseminators of *S.* Dublin to naïve herds, but their influence in *S.* Dublin transmission wanes after an endemic infection state is reached in the herd. The economic assessment indicated that the operating income of a heifer-raising operation could be substantially improved (from 170 USD per 100 head to a maximum of 230 USD per 100 head) through frequent cleaning (2x to 8x per day) with an alley scraper if the cost of each additional scraping per 100 head per year does not surpass 50 USD.

Conclusions: Increased scraping frequency can reduce the probability of *S.* Dublin outbreaks in a heifer-raiser operation and mitigate the consequences of infection in heifers. Preventing carrier development in heifer-raising operations is critical to limit *S.* Dublin dissemination to dairy farms.

Financial Support: National Institute of Food and Agriculture, USDA Hatch Funds (1014331 and 7000433), USDA Multistate Research Funds (1016738), and Cornell Institute of Digital Agriculture Research Innovation Fund.

Notes:

P115 - Screening for lumpy skin disease (LSD) in high-risk areas of Armenia

Khachik Sargsyan[1], Satenik Kharatyan[1], Varduhi Hakobyan[1], Hasmik Elbakyan[1], Tigran Markosyan[1], Tigranuhi Vardanyan[1]

[1]Scientific Center for Risk Assessment and Analysis in the Food Safety Area, Ministry of Economy, Armenia. tigranuhi.vardanyann@gmail.com

Session: Epidemiology, **2023-01-22, 6:00 - 8:00**

Objective: Lumpy skin disease (LSD) is an infectious disease in cattle caused by a virus of the family *Poxviridae*. LSD is primarily a vector-borne disease and is spread by blood-sucking vectors like mosquitoes, ticks, and houseflies. LSD emerged in Armenia in late 2015, primarily impacting cattle near the Iranian border. Vaccination is the only means of preventing the spread of the infection in endemic and newly affected regions. After the outbreak in 2015, Armenia implemented a vaccination strategy that utilized a heterologous, dry culture, sheep pox virus-based vaccine against LSD in cattle. Implementation of systematic mass vaccination assisted in disease stabilization. However, continued disease monitoring is important, as circulating strains undergo mutations, and globalization can render preventive measures and vaccines ineffective. Currently, our vaccination campaign is executed from April to June, thus we conducted an active surveillance study organized between 15 November and 15 December 2022 when the animals return from the pastures to understand the risk of transmission of LSD and evaluate if the stability of the epidemic situation is in question.

Methods: Based on epidemiological data from the Ministry of Economy and the Food Safety Inspection Body and interviews with individuals in at-risk communities, 15 cattle that lived near pastured puddles and lakes in the Syunik Region were identified for testing. Samples were collected from cattle that had been previously vaccinated with the sheep pox vaccine. Blood was collected from the cattle via the jugular vein into purple-topped vacutainers for viral testing, that were delivered to the laboratory in cold boxes within 24 hours. The blood samples were tested for LSD virus (LSDV) using a real-time polymerase chain reaction (RT-PCR) screening test from the Federal Centre for Animal Health (FGBI ARRIAH, Vladimir, Russia). Cycle threshold (Ct) values below 35 were considered positive.

Results: During the investigation, no animals were identified with typical clinical signs of LSD. Based on the RT-PCR results, none of the 15 samples tested positive for the presence of LSDV.

Conclusions: Prior to 2015, LSD had not been registered in Armenia. The disease was quickly controlled, and Armenia began a vaccination campaign in susceptible cattle in high-risk areas and performed passive surveys. Approximately 40% of cattle are vaccinated in Armenia annually. PCR testing of blood determined that LSDV was not detected in any of the animals' blood samples, which confirmed that the animals were free of LSDV. Our limitations include that this was a low sample size, and more work is needed.We suggest that the sustained annual vaccination of cattle is necessary to protect animals, especially those located on the border of Turkey and Iran, where LSDV is currently registered. Continued active surveillance is also important to monitor the disease. The integration of these strategies stands as a formidable barrier against the diffusion of this exceedingly infectious transboundary disease.

Notes:

P116 - Spatiotemporal dynamics of *Bacillus anthracis* infection transmission in northern Vietnam, 2022-2023

Van Khang Pham[1], C. Metrailer Morgan[2], Tan Luong Minh[2], Minh Hoa Luong[1], Thanh Hai Pham[1], Bich Ngoc Do[1], Jiranantasak Treenate[2], Thi Lan Huong Vu[3], Thanh Long Pham[3], Thi Mai Hung Tran[1], Nhu Duong Tran[1], Thuy Oanh Kim[4], H. Norris Michael[2], Quang Thai Pham[1], Thi Thu Ha Hoang[1], K. Blackburn Jason[2]

[1]National Institute of Hygiene and Epidemiology, [2]University of Florida, [3]Department of Animal Health, [4]USAID. pqt@nihe.org.vn

Session: Epidemiology, **2023-01-22, 6:00 - 8:00**

Objective: *Bacillus anthracis* is a zoonotic pathogen causing anthrax outbreaks in northern Vietnam since 2015. An April 2022 outbreak in Son La occurred after the butchering of a water buffalo, followed by infections in Ha Giang, Lai Chau, and Dien Bien provinces. This study aims to analyze the transmission dynamics and origins of *B. anthracis* strains in human patients and their source animals within the endemic region.

Methods: 83 diagnostic samples from 2022-2023 were collected from humans, buffalo, and soil in outbreaks of four provinces. Human samples were inoculated on trypticase soy agar with 5% horse blood. The soil sample was suspended in 1× phosphate buffer saline (PBS) and incubated at 65ºC for one hour in a shaking water bath at 200 rpm; then, the suspension was plated onto Polymyxin-Lysozyme-EDTA-Thallous-acetate agar and incubated at 37ºC for 24h. The buffalo hide was washed with 1× PBS, then cut into small pieces and suspended in 300 µl of NaCl 0.9%. The resulting suspension was homogenized using a pellet pestle motor, and 50-100 µl was plated onto HBA. The typical *B. anthracis* colonies from those samples were stuck for isolated colonies on HBA. 31 specimens were confirmed as *B. anthracis* by extracting DNA and using a previously established qPCR assay that targeted the chromosomal Ba-1 marker and the plasmid virulence markers. The *B. anthracis* isolates were whole genome sequenced using the Illumina Miseq platform. A twenty-five-marker multi-locus variable number tandem repeat analysis (MLVA-25) was conducted to investigate the relationships between human, soil, and cattle strains.

Results: Epidemiological and genetic analyses of 23 anthrax strains spanning 2022 to 2023 across four provinces. A high genetic homogeneity among 15 *B. anthracis* strains in Dien Bien (8), Lai Chau (5), and Ha Giang (2), all of which belonged to the canonical single nucleotide polymorphism analysis (canSNP) lineage of A.Br.011/009, predominantly consisting of trans-Eurasian (TEA) group strains, including the closely related Carbosap strain. In Son La, eight strains recovered from an outbreak in which early epidemiological investigations suggested a single animal was responsible for all exposures. Five *B. anthracis* isolates were recovered from human clinical cases: one from the buffalo hide, another from associated maggots, and one from soil at the carcass site. After being classified under the A.Br.001/002 lineage based on canSNP, those strains were not previously identified in Vietnam but have been reported in neighboring countries such as China, India, Indonesia, Thailand, and Australia. Surprisingly, four distinct MLVA-25 genotypes were identified among the eight isolates, suggesting an unusual level of genetic diversity given the limited geography and timing of cases, which contrasts with previous literature using MLVA-25.

Conclusions: The coupled spatial and phylogenetic data strongly indicate that this outbreak likely originated from multiple and potentially undetected animal sources. Considering these findings, future outbreak response efforts should prioritize intensive surveillance for additional animal cases and employ molecular epidemiological traceback methods to identify the sources of the pathogen, thus facilitating more effective prevention and control strategies.

Financial Support: This work is funded by the US Defense Threat Reduction Agency (DTRA) Grant #HDTRA1-20-1-0003 to Jason K. Blackburn.

Notes:

P117 - Cancer prevalence and associated risk factors in Maricopa County, Arizona: A retrospective study

Chauncey Hart[1], Demelash Areda[1]

[1]Ottawa University. demelash.biffa@gmail.com

Session: Epidemiology, **2023-01-22, 6:00 - 8:00**

Objective: This study aimed to explore the determinants linked to the prevalence of cancer in Arizona, specifically focusing on breast cancer, skin cancer, and lung cancer.

Methods: Data from reputable sources, including the Centers for Disease Control and Prevention (CDC), Arizona Department of Public Health (ADPH), and the Arizona Department of Health Services (MDPH), were collected and analyzed. The dataset encompassed a comprehensive analysis of trends over the past decade regarding the incidence of breast cancer, skin cancer, and lung cancer. Utilizing descriptive statistics and linear regression analysis, the researchers identified correlations and potential factors associated with these cancer types.

Results: The statistical analysis, with a significance level set at a P-value of 0.05, unveiled intriguing patterns. Both breast cancer and skin cancer exhibited a slight yet statistically significant increase over the studied period ($P < 0.05$). Similarly, skin cancer showed a noticeable upward trend ($P < 0.05$). However, lung cancer did not display any significant variation during the study period 2010-2020 ($P > 0.05$). Notably, the study identified age as a significant factor in lung cancer cases, observing a higher number of incidences in individuals aged 70 and above. The results highlight the need for further exploration and understanding of the factors contributing to the rising incidence of breast and skin cancers, while also suggesting a stable trend in lung cancer cases among the population studied in Arizona.

Conclusions: Based on these findings, the study concluded that there had been an observable increase in the prevalence of breast and skin cancers over the years. These findings underscore the importance of continued research and targeted interventions for specific age groups or cancer types to better address and manage the escalating rates of certain cancers in the region.

Financial Support: The authors are grateful to Ottawa University for providing the necessary support.

Notes:

P118 - Association of pandemic coronavirus disease (COVID-19) with chronic disease burden: predictive model analysis

Demelash Areda[1]

[1]Ottawa University. demelash.biffa@gmail.com

Session: Epidemiology, **2023-01-22, 6:00 - 8:00**

Objective: Chronic non-communicable diseases are widely prevalent worldwide causing significant health problems and economic burden. Many aspects of Covid-19 including its association with underlying medical conditions are not well investigated which otherwise would help healthcare professionals design better plant and strategies to minimize its impacts. This study examines association of pandemic Covid-19 with global burden of major non-communicable diseases namely diabetes and cardiovascular disease

Methods: Data were obtained from open access public repository hosted at https://covid.ourworldindata.org/ (licensed under the Creative Commons BY license and are permissible for public use). Negative binomial regression model and ANOVA were used to determine association between response variables (Covid-19 cases, deaths and fatality) and exposure variables of interest.

Results: People aged 70 years or greater, higher GDP per capita, and diabetes prevalence were significant risk factors for Covid-19. Incidence of Covid-19 cases increased by 1.15% [95%CI=1.05, 1.26] for every one-year increase among individuals aged 70 years or greater. Similarly, rise in GDP per capita was found to be significantly associated (P<0.00) with incidence of Covid-19 cases. Covid-19 cases increased by 1.0% [95%CI=1.00, 1.05] as GDP per capita increased. Diabetes prevalence in a population was significantly linked to incidence of Covid-19 (P<0.05). Incidence of Covid-19 cases increased by 1.1% [95% CI=1.00, 1.12] as diabetes prevalence increased. Likewise, older age (70 years or greater) and GDP per capita were strongly positively associated with incidence of Covid-19 deaths. On the other hand, cardiovascular disease (cvd) death rate and smoking were negatively associated with death due to covid-19.

Conclusions: Diabetes, old age and per capita income were significantly associated with Covid-19 cases and deaths suggesting the importance of designing risk-based control and prevention plans. Particular attention needs to be given to elderly people and people with underlying medical conditions such as diabetes.

Notes:

P119 - Seroprevalence of SARS-CoV-2 in bison and other wildlife species using different serological assays

M. Ardalan[1], K. R. Cool[1], N. N. Gaudreault[1], D. Bold[1], C. Rojas[2], A. Mannix[2], J. F. R. Seetahal[2], J. A. Richt[1], R. M. Pogranichniy[1, 2]

[1]Department of Diagnostic Medicine/Pathobiology, Kansas State University, [2]Veterinary Diagnostic Laboratory, Kansas State University. ardalan@ksu.edu

Session: Epidemiology, **2023-01-22, 6:00 - 8:00**

Objective: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) primarily infects humans, but several animal species including non-human primates are also susceptible to SARS-CoV-2 infection. Cases of SARS-CoV-2 infection have already been reported for large cats, hippopotamus, and gorillas. Not much is known regarding the susceptibility of bison, elk, and other wildlife species to SARS-CoV-2, and an assessment of their SARS-CoV-2 seroprevalence is of utmost importance. Therefore, the objective of this study was to estimate the SARS-CoV-2 seroprevalence for different wildlife species and bison using several serological tests.

Methods: Serum samples from 575 bison collected between 2020 - 2022 from two states (KS and MT), 199 elk samples collected in 2016, 2022, and 2023 from two states (IN and KS), and 147 different wildlife species samples (elephant, fox, llama, camel, koala, alpaca, rhinoceros, lion, addax, giraffe, panda, tiger, goral, porcupine, oryx, cheetah, zebras, hippopotamus, wallaby, cockatiel, macaws, bearcat, eagle, lynx, chevrotain, mick, sea lion, hylobates lar, antelope, caribou, bobcat, hyena, impala, primate, ferret, bear, buck, and gorilla), collected between 2020 - 2023 from sixteen U.S. states were analyzed for the presence of SARS-CoV-2-specific antibodies. The initial testing was performed using two commercial ELISA assays based on: (i) the inhibition of the SARS-CoV-2 receptor-binding domain (RBD)- angiotensin-converting enzyme 2 (ACE2) interaction (cRBD-ELISA); and (ii) the nucleocapsid protein (cN-ELISA) using a commercial kit. Also, the conventional virus neutralization test (cVNT) using the Wuhan-like SARS-CoV-2 USA/WA1/2020 isolate was performed on all positive cRBD-ELISA serum samples as a reference assay to detect neutralizing antibodies.

Results: Our results indicated that 1.2% (7/575) of bison, 2.2% (4/180) of elk, and 4.1% (6/147) of the other wildlife species serum samples were seropositive in the cRBD-ELISA, whereas 4.2% (24/575) of bison and 3.3% (6/180) of elk, and 1.4% (2/147) of the other wildlife species serum samples tested positive by the cN-ELISA. Among the cRBD-ELISA serum samples, 2 samples from bison, 1 sample from elk, and 5 serum samples from other wildlife species (1 cheetah, 1 gorilla, 2 lions, and 1 hippopotamus) had neutralizing antibody titers in the cVNT.

Conclusions: The presence of neutralizing antibodies, in cheetahs, gorillas, lions, hippopotamuses, elk, and bison indicate that they are susceptible to SARS-CoV-2 infection. It is crucial to monitor the impact of SARS-CoV-2 on bison and elk, who exist in the wild and in zoos, and other wildlife species to better assess the reservoir potential of various animal populations for SARS-CoV-2, the risk of transmission in different settings, and the implications for both animal and human health.

Financial Support: This work was supported by the NIH (FDA).

Notes:

P120 - Food types and health outcomes: early findings from the Dog Aging Project

Audrey Ruple[1], Janice O'Brien[1], Katie Tolbert[2]

[1]Virginia Maryland College of Veterinary Medicine, [2]College of Veterinary Medicine, North Carolina State University. janiceobrien@vt.edu

Session: Epidemiology, **2023-01-22, 6:00 - 8:00**

Objective: The Dog Aging Project cohort of over 45,000 dogs located throughout the United States presents a unique opportunity to study nutrition in pets at the population level. Our objective was to describe the first two years of Dog Aging Project nutrition survey results examining correlations between diet types and health outcomes.

Methods: 43,517 owner-reported surveys collected through the Dog Aging Project cohort study were analyzed using logistic regression models to examine potential associations between primary diet component (e.g. kibble, commercial raw, home cooked, organic, grain free, etc.) and health outcome categories (e.g., "cardiac disease"). The logistic regression models were run using diet type as a predictor variable and developed (i.e. non-congenital) health outcomes as the outcome variable. Analyses were run using the health outcomes and the categories of grain-free/not grain-free, cooked/raw, and highly processed/less processed. Odds ratios and 95% confidence intervals were calculated.

Results: Highly processed foods (kibble, canned) were statistically significantly associated with a decreased rate of many developed disease categories: ear OR 0.89 (95% CI 0.85-0.93), cardiac 0.59 (0.56-0.63), eye 0.75 (0.72-0.78), oral 0.77 (0.74-0.79), skin 0.87 (0.84-0.9), respiratory 0.58 (0.54-0.62), gastrointestinal 0.86 (0.82-0.9), liver 0.63 (0.58-0.68), kidney 0.82 (0.77-0.87), reproductive 0.76 (0.68-0.84), orthopedic 0.78 (0.75-0.81), endocrine 0.71 (0.66-0.77), hematologic 0.56 (0.46-0.67), and cancer 0.86 (0.81-0.92). Less processed foods were only statistically significantly associated with decreasing neurological disease (OR for processed 1.27 (1.18-1.36)). Cooked foods (when compared to raw) had a lower association with cardiac OR 0.73 (95% CI 0.67-0.79), skin 0.86 (0.82-0.9), respiratory 0.71 (0.64-0.79), reproductive 0.56 (0.49-0.63), endocrine 0.84 (0.74-0.94), and infectious 0.94 (0.89-0.98) diseases while raw had no decreased associations with any health outcomes. When conducting logistic regression, statistically significant food types for each disease group were identified. For example, oral disease was positively associated with canned food type use (log odds 1.66, $p < 0.0001$), and commercial raw foods were associated with an increase in infectious disease (log odds 1.23, $p < 0.0001$) (in the DAP sample, salmonella is coded as infectious disease instead of gastrointestinal). These findings are not unexpected based on previous research, however interesting and unexpected associations did arise - such as raw diets correlating with an increase in respiratory disease (log odds 1.89, $p < 0.0001$).

Conclusions: Several interesting correlations have been discovered linking diet type to health outcomes in pet dogs. These findings are cross-sectional and therefore not able to draw causal linkages, however this analysis will inform future studies that can be designed to determine causality.

Notes:

P121 - Dog- and owner-level demographic factors associated with dog diet

Amanda Moore[1], Janice O'Brien[1], Audrey Ruple[1]

[1]Virginia Tech. amandam23@vt.edu

Session: Epidemiology, **2023-01-22, 6:00 - 8:00**

Objective: The Dog Aging Project (DAP) is a nationwide, long-term longitudinal study of more than 45,000 dogs that collects diverse information about owners, dogs, and their environments to determine which factors influence lifespan and healthspan in dogs. One present focus is the impact of nutrition on health outcomes. This study examined which dog- and owner-level demographic factors were correlated with dog diet.

Methods: As part of a broader survey, owners were asked to report on the primary diet type, i.e. that comprising >50% of the dog's diet. The possible diet responses were kibble, canned, semi-dry, freeze-dried, commercially prepared raw, home made raw, home cooked, and other. Information was also collected regarding owner and dog demographics. The owner demographics analyzed were owner age, income, and highest education level. The dog demographics included were dog age, size, sex, neuter status, breed type (mixed or pure), activity level, overall health status (from excellent to very poor), primary purpose (e.g. companion, service, etc.), number of dogs in the household, and home environment (rural, suburban, urban). Nutritional information from 43,517 dogs was available. We performed a chi-squared goodness of fit test to determine which owner- and dog-level demographics produced statistically significant differences in diet choices as compared to the survey as a whole.

Results: Across all participants, over 82% of dogs were fed kibble as their primary diet component. The next most common diet choices were canned, commercial raw, and home-cooked, each at 4%. This was consistent across several demographics. Notably, income produced statistically significant but small variation in diet. Across all owner income classes, kibble-fed percentages ranged between 81-85%. Owner age, dog size, and dog health status all produced both statistically significant and the most sizable differences in primary diet. Younger owners were more likely to feed kibble and older owners were more likely to feed non-kibble, especially canned. At the extremes, for owners aged 18-24, 91% fed kibble and 1% fed canned; for owners aged over 75, 75% fed kibble and 8% fed canned. Regarding dog size, smaller dogs (<30lbs) were fed kibble less frequently. For dogs weighing under 10lbs, 64% were fed kibble and 13% were fed canned. As dog health declined, so did the percentage fed kibble. Dogs reported to be in excellent health were fed in proportions similar to those of the survey as a whole. Dogs in very poor health were fed kibble less (67%) and canned and home-cooked more (11% and 9% respectively).

Conclusions: This research found that both dog- and human-level demographic information was associated with the primary component of participant dogs' diets. This information can: 1) aid veterinarians in their discussions about diet with dog owners, 2) inform pet food companies regarding the relationships between various owner and dog demographics and broad categories of diet choices, and 3) help researchers to examine long-held assumptions about how dog and owner demographics affect dog diet. In addition, this hypothesis-generating research lays the groundwork for future studies on the relationship between owner and dog demographics and dog diet.

Financial Support: The Dog Aging Project, The Virginia-Maryland College of Veterinary Medicine

Notes:

P122 - Clinical cases of severe fever with thrombocytopenia syndrome in dogs

Sun-Woo Han[1], Joon-Seok Chae[1]

[1]College of Veterinary Medicine, Seoul National University. jschae@snu.ac.kr

Session: Epidemiology, **2023-01-22, 6:00 - 8:00**

Objective: Severe fever with thrombocytopenia syndrome (SFTS) is a zoonotic disease with a high mortality rate for humans and cats. The clinical course and prognosis of SFTS in dogs remain unclear. In the present study, we investigated the clinical and epidemiological characteristics of SFTS virus (SFTSV) infection in dogs.

Methods: Between April 2019 and December 2020, blood was collected from 448 dogs exposed to hard ticks or exhibiting clinical symptoms similar to those of SFTS at 166 animal hospitals in the ROK. When the SFTSV RNA was confirmed, blood samples for complete blood count (CBC) and serum chemistry, urine samples, and swab samples (rectal, nose, eye, oral) were requested for follow-up. Each sample was tested using nested reverse transcription-polymerase chain reaction (RT-PCR) assays and Real-Time RT PCR to detect the small (S) segment of SFTSV. To confirm the SFTSV PCR products, positive PCR amplicons were directly sequenced.

Results: The blood of 448 dog patients exposed to ticks or suspected to be infected with the SFTS virus was tested for SFTS virus antigen using the PCR methods. As a result, 14 patients were positive, resulting in an infection rate of 3.1%. All evaluated dogs exhibited an acute course and symptoms including fever (57.1%), anorexia (57.1%), depression (42.9%), and vomiting (35.7%). Thrombocytopenia was present in 45.5% of dogs, while jaundice was not observed. C-reactive protein, alanine transaminase, and alkaline phosphatase were elevated in some cases. Viral clearance occurred within 6 to 26 days. Phylogenetic analysis revealed that the SFTSV sequences were consistent with viruses circulating in the Republic of Korea.

Conclusions: In the present study of clinical infection with SFTSV in dogs, all dogs received only symptomatic treatment and survived. The clinical course of the dogs was different to that observed in humans and cats. Further studies should identify the reasons for differences in the clinical course of SFTS among species. Even if the severity of SFTSV infection in dogs is mild to moderate, interspecies transmission is possible, so guidelines for the prevention and diagnosis of SFTS in dogs are warranted. As dogs often live in close contact with humans, awareness of the clinical and epidemiological features of SFTS in dogs is crucial. Further large-scale studies are necessary to investigate SFTSV infection in dogs.

Financial Support: Financial Support: Korea Institute of Planning and Evaluation for Technology in Food, Agriculture and Forestry (IPET 119053-02).

Notes:

P123 - Transboundary avian zoonotic infectious diseases and migratory birds: strengthening and connecting research and surveillance networks

Nisreen AL-Hmoud[1], Mu'men Alrwashdeh[1], Andrew Bartlow[2], Jeanne Fair[2], Annie Elshoff[3], Lara Fakhouri[3], Zurab Javakhishvili[4], Fares Khoury[5], Denys Muzyka[6], Jennifer Owen[7], Jean Tsao[7], Lela Urushadze[8]

[1]Royal Scientific Society, [2]Los Alamos National Laboratory, [3]CRDF GLobal, [4]Ilia State University, [5]American University of Madaba Jordan, [6]National Scientific Center Institute of Experimental and Clinical Veterinary Medicine, [7]Michigan State University, [8]National Centers for Disease and Public Health, Georgia. jmfair@lanl.gov

Session: Epidemiology, **2023-01-22, 6:00 - 8:00**

Objective: Wild birds continue to be hosts and reservoirs for numerous zoonotic pathogens and strains of highly pathogenic avian influenza (HPAI) and migrating birds have led to the introduction of into new regions. Ecological patterns that we once thought were stable are changing, bringing new populations and organisms into contact with one another. The objective of this project is to strengthen global biosurveillance of wild bird populations and build collaborative networks for zoonotic infectious diseases.

Methods: An example of a region with a large gap in knowledge of migration and infectious diseases in the wild birds is along the Mediterranean and Black Sea Flyway (MBSF) that connects Europe and Africa. Focusing on avian influenza, Newcastle disease virus and the microbiome in migratory wild birds along the MBSF, this project seeks to understand the determinants of transboundary disease propagation and coinfection in the region. Through the creation of a Threat Reduction Network for avian diseases (Avian Zoonotic Disease Network - AZDN) conducted in three countries along the MBSF (Georgia, Ukraine, Jordan), the project is strengthening capacities for disease diagnostics, microbiomes, ecoimmunology, field biosafety, proper wildlife capture and handling, experimental design, statistical analysis, and vector sampling and biology.

Results: Here, we cover what is required to build an infectious disease research and wild bird surveillance program, which includes learning the skills in proper bird capture, identification and handling, bird monitoring methods, biosafety and biosecurity, permits, next generation sequencing, leading-edge bioinformatics and statistical analyses, and vector and environmental sampling. We also report the first two years of sampling results for avian influenza and microbial communities for species of birds in Georgia, Jordan, and Ukraine.

Conclusions: With these expanding environmental changes leading to increased uncertainty and emergence of zoonotic infectious diseases in animals, it is even more crucial that regions or counties that previously did not have surveillance programs develop the appropriate skills to sample wild birds and add to the understanding of pathogens in migratory and breeding birds.

Financial Support: This study was implemented under a project funded by Defense Threat Reduction Agency, Grant No. HDTRA-21-1-0021.

Notes:

P124 - Seroprevalence of Foot and Mouth Disease virus infections in livestock in The Gambia

J.M. Matthews[1], A. Secka[2], S. Pauszek[1], S. Lakin[1], V. O'Donnell[1], B. Faburay[1]

[1]USDA-Animal and Plant Health Inspection Service, [2]West Africa Livestock Innovation Center. Jerusha.Matthews@usda.gov

Session: Epidemiology, **2023-01-22, 6:00 - 8:00**

Objective: Foot and mouth disease virus (FMDV) is a highly contagious, viral agent affecting livestock in parts of Africa, Middle East, Asia, and South America. Foot and mouth disease (FMD) impacts cloven hoofed ruminants, like sheep, goats, and cattle and poses a significant economic impact on livestock industries. The epidemiology of FMDV in The Gambia is poorly understood. Sporadic outbreaks of the disease have infrequently occurred in cattle with no apparent clinical cases ever reported in small ruminant populations. This study aimed to investigate the seroprevalence and sero-epidemiology of FMDV in target host populations, small ruminants, and cattle, in an endemic setting of West Africa.

Methods: A cross-sectional sampling of 1,405 serum samples were collected from apparently healthy livestock animals across the five regions of The Gambia/West Africa and tested for the presence of antibodies against the FMDV nonstructural 3ABC polypeptide using a validated competitive enzyme linked immunosorbent assay (ELISA) capable of differentiating infected from vaccinated animals (DIVA). To investigate the antibody prevalence of FMDV in livestock, serum samples were collected from sheep (n= 469) and goats (n = 537), and cattle (n= 399) maintained under the free-range extensive management system.

Results: Of the 1006 small ruminant serum samples collected from sheep and goats throughout the five regions of The Gambia, 137 tested positive for antibodies against the nucleoprotein of FMDV indicating a seroprevalence of 13.6%. With seroprevalence being slightly increased in sheep 16.6% (78/469) compared to goats 10.9% (59/537). For cattle, a higher seroprevalence of 72.7% was detected, with 290 out of 399 cattle testing positive for FMDV antibodies.

Conclusions: The high seroprevalence of FMDV antibodies in cattle coupled with incidents of sporadic outbreaks of the disease in The Gambia, suggest high risk and endemicity of FMD in country. Serological prevalence of FMDV in sheep and goats suggest that small ruminants may play an important role in the epidemiology of FMD in the Gambia, as well as in other endemic settings in West Africa.

Notes:

P125 - Genetic diversity of *Brucella melitensis* isolated from ruminants in Iraq

Fabrizio De Massis[1], Sara Serrani[1], Michela Toro[1], Alessandra Sferrella[1], Nausica D'Aurelio[1], Teresa Romualdi[1], Roberta Di Romualdo[1], Eugenio Felicioni[1], Giuliano Garofolo[1]

[1]Instituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise, Italy. f.demassis@izs.it

Session: Epidemiology, **2023-01-22, 6:00 - 8:00**

Objective: Currently, brucellosis remains a major public health threat, as well as a disease of deep economic impact worldwide. The eradication of the disease is an objective for Veterinary and health Authorities in several countries. This is also the case of Middle East countries, Iraq included, where Competent Authorities need to standardise public and animal health intervention programmes. Efficient surveillance programs are crucial for detecting and managing outbreaks, and they rely on the collection and accessibility of sound epidemiological data. Epidemiological investigations may take advantage from the presence of standardized and efficient molecular typing techniques and analytical tools that enable public health laboratories to identify the origin of an outbreak. The aim of this study was to sequence Brucella *spp.* strains isolated in Iraq from different ruminant species, to verify whether there was a spatial or temporal clustering and, above all, shedding light on how these Iraqi isolates are positioned in the phylogenetic context of *Brucella* spp.

Methods: The isolates under study (N=35) were from abortion, milk, placenta and fetal membrane of sheep, cattle and buffalo. They had been cultured in the center for brucellosis and tuberculosis control in the Iraq CVL, and were isolated during the 2015 - 2017 period. Samples were genotyped using different techniques, namely MLVA-16, Whole Genome Sequencing, MLST and cgMLST.

Results: The comparison of MLVA-16 profiles of the strains analyzed with the profiles obtained from the public databases did not reveal any shared genotypes. All Iraqi isolates from our study clustered within East Mediterranean clade, and, except from one strain, all clustered together in same branch of MST tree. The MST analysis showed the minimum distance of 1 allele between our isolates and the other strains, and the most frequent connection was found with the MLVA profiles assigned to the isolates originating from Syria. One strain, obtained from buffalo, was placed farther away from the rest of the isolates investigated, and was linked to strains from Turkey and Vietnam. The majority of cgMLST sequences grouped together within 35 allele distance. Several more closely related clusters were also observed. Out of these, the biggest, which contained cgMLST profiles 9 and 10, was assigned to isolates from four governorates, Babil, Karbala, Maysan and Wasit. Six strains from this clusters shared the same MLVA-16 profiles.

Conclusions: The findings of this study suggests that even relatively small investigations can provide sufficient evidence to justify improvements in existing control strategies. Breeding practices are similar across the region, therefore, the application of different control measures in specific locations with high prevalence, based on molecular epidemiological studies, would increase the chances to maximize public health benefits and minimize the spread of infection to areas free from the disease or with lower prevalence.

Notes:

P126 - Validation of Ontario swine industry parameters through dynamic simulation modelling

Maggie L. Henry[1], G.W. McDonald[2], Amy L. Greer[1], Robert M. Friendship[1], Zvonimir Poljak[1]

[1]Dept. of Population Medicine, University of Guelph, [2]Dept. of Computer Science, University of Saskatchewan. mhenry12@uoguelph.ca

Session: Epidemiology, **2023-01-22, 6:00 - 8:00**

Objective: The SARS-CoV-2 pandemic created global disruptions in animal agriculture. In the swine industry, these disruptions took the form of swine packing plant closures, as well as inconsistent labor availability. This pandemic highlighted the sensitivity of the Ontario and Canadian swine industry to disturbances caused by human disease and raised concerns as to the robustness of the swine production system in the event of a foreign animal disease. The use of dynamic simulation modelling offers an efficient method to demonstrate how an industry may be impacted by interruptions by exploring several possible scenarios in a risk-free environment. The objective of this research was to construct a discrete event simulation model, which was capable of being scaled from farm level to provincial level, to explore potential mitigation strategies should the Ontario swine flow be disrupted.

Methods: The simulation model runs on a weekly basis, and contains three agents: a Sow Farm, a Pig Farm, and an Abattoir. Each agent reflects a component of the entire Ontario swine flow system, where gilts enter the Sow Farm agent, produce offspring, and then return to breeding. The rate that gilts enter the herd is based on breeding herd size, target litters per sow per year and gilt replacement rate parameters. All three of these parameters are dynamic and are capable of modification based on the users' needs. The resulting piglets from each sow progress through the Pig Farm agent and are then processed within the Abattoir agent. The model concludes with four possible abattoir routes which the pigs are sorted into. These abattoirs reflect the current processing situation within Ontario, Canada and reflect both domestic and exported cuts of pork. Mann-Whitney tests were undertaken to assess statistical differences between the farm and provincial level observed data, compared to the simulation output data.

Results: Simulation validation was completed utilizing real-world data from 6 Ontario farms and observed data from the province of Ontario. For the provincial comparison data, no statistically different values were found between the simulation outputs and the observed provincial data for the number of annual pigs marketed and the annual kilograms of pork exported ($P>0.05$).

Conclusions: Preparation for emergency circumstances is essential to limit the impact of reduced health and welfare in both human and pig populations in the event of disruptions to the current pork marketing situation. The use of this model may be of benefit to policymakers and animal production industries, as it allows the investigation of a decrease in production of the Ontario swine flow in a risk-free environment.

Financial Support: Mitacs, Ontario Pork, and Ontario Ministry of Agriculture Food and Rural Affairs (OMAFRA)

Notes:

P127 - Exploring the swine trading landscape: A network analysis of live swine movement in the Philippines

Toni Rose M. Barroga[1, 2], Michael W. Sanderson[2]

[1]Department of Agriculture-Bureau of Animal Industry, Philippines, [2]Department of Diagnostic Medicine and Pathobiology, Kansas State University. tbarroga@ksu.edu

Session: Epidemiology, **2023-01-22, 6:00 - 8:00**

Objective: The movement of animals plays a pivotal role in disease transmission. In African Swine Fever (ASF)--affected countries such as the Philippines, understanding the trading dynamics and swine movement network are relevant in identifying high-risk areas and potential pathways of disease spread. Social network analysis is used in veterinary epidemiology as a tool to explore patterns and relationships across different livestock holdings in the network.

Methods: The study utilized the information from the local shipping permits (LSP) issued by the country's national veterinary services, Bureau of Animal Industry (BAI). LSP is required to transport livestock from farm to farm and farm to slaughterhouse. All approved live swine shipment records in 2021 were extracted from BAI's online LSP platform. A total of 37,138 inter-city LSP records were used to analyze different metrics for this study. In the network, there are 685 towns (local regions) included which represent 42% of the total towns in the country. Towns were considered nodes while the arcs were defined by the presence of a shipment, weighted by either the number of shipments or the number of pigs per shipment.. Trade communities were also identified using the Walktrap algorithm. R (igraph package) was used in analyzing the network structure.

Results: The LSP records revealed a median travel distance of 102.35 km (IQR: 35.93-417.76) and a median headcount of 50 heads (IQR: 20-80). The number of pig shipments (11,968) and quantity of pigs shipped (682,520) were highest during the last quarter of the year. The majority of the records revealed a destination of slaughterhouse (62.3%). The highest in-degree (ship-in) values were noted in Metro Manila, Regions 3, 4A, 9, 10, and 12. The highest out-degree (ship-out) were recorded in Regions 3,6, 10, 11, and 12. Network-level metrics showed the following results—density (7.9%), transitivity (7.9%), assortativity (0.10), and reciprocity (0.04). A moderate correlation was reflected between the following: (1) number of pigs in the province and out-degree (0.47)/weighted out-degree (0.40), and weighted betweenness (0.41); (2) swine density per km^2 per province and out-degree (0.48)/weighted out-degree (0.43); and (3) cumulative ASF laboratory positive samples from 2019-2021 and in-degree (0.47)/weighted in-degree (0.49). Towns with large swine breeder farms and integrators showed high out-degree values. The study detected 6 large trade communities, each involving more than 20 towns in the network. The largest community was composed of 282 towns actively participating in the swine movement.

Conclusions: The results provided an overview of a sparse trading network of swine in the Philippines. The low values of network-level metrics suggest that there is weak clustering, low preferential connections with towns of similar attributes, and one-sided trade relationships among the towns. Large metropolitan areas received high in-degree due to the high demand for pork and the decreasing swine population due to ASF. The knowledge of the patterns in swine trading is critical in controlling ASF as this can support in formulation of science-based decisions related to risk-based surveillance activities, proper budgetary resource allocation, and appropriate disease management strategies.

Notes:

P128 - African Swine Fever - Epidemiological evolution on farms and wild boars in an eastern-European country, 2018-2022

Beatris Corina Ancuceanu[1], Marius Grigore[1], Cristina Stoica[1], Daniel Donescu[1], Monica Motiu[1], Raluca Badulescu[1], Florica Barbuceanu[2]

[1]Institute for Diagnosis and Animal Health, Romania, [2]Univeristy of Agronomic Sciences and Veterinary Medicine of Bucharest, Romania. ancuceanu.corina@idah.ro

Session: Epidemiology, **2023-01-22, 6:00 - 8:00**

Objective: There is still some debate on the speed of African Swine Fever's (ASF) spread and the relevant variables influencing the disease's epidemiology. In in this context, we used five years' worth of statistics (from 2018 to 2022) to show how it changed in Romania.

Methods: Data on outbreaks at county level by farm size, pigs by their disease status (receptive, infected, dead, killed) and wild boars have been sourced from official reports received by the competent veterinary authority in Romania, submitted to the European Commission through the Animal Disease Information System (ADIS) at Descriptive statistics and time series trend methods were used for analysis.

Results: Excluding the two outbreaks isolated from households in the summer of 2017, in farms, the total number of new outbreaks (not cases) in 2018 amounted to 1164. Subsequently, in 2019 these numbers increased considerably, reaching a total of 1728 new outbreaks. However, the situation took a positive turn as the new outbreak number dropped to 1063 in 2020, only to rise again to 1660 in the following year. Notably, there was a significant decline in new outbreaks in 2022, with only 327 reported, suggesting that epidemics were progressing more slowly. The evolution across 2018-2021 was rather aggressive, despite control measures implemented at national level for both farms and wild boars. For farms, these measures included actions such as animal confiscation, traffic control, safe disposal/incineration, intensified checks on compliance with biosecurity measures, and more. For wild boars, the measures involved increasing hunting quotas, stimulating hunting, limiting the feeding of wild boars, and providing incentives for search, testing, and neutralization of dead animals. These actions targeted a wide range of farms, including large numbers of small-scale farms, with over 70,000 farms sampled in 2020. Additionally, several hundred commercial farms, totaling over 400 annually, were part of this sampling. Interestingly, the passive surveillance approach, as expected, yielded a substantially larger proportion of positive instances than active monitoring. The methods for diagnosis were based on ELISA (most typically), though PCR was also employed. Remarkably, in 2020 alone, there were over 200,000 cases that underwent testing.

Conclusions: ASF has had a rather fast, aggressive evolution in Romania across four years, despite multiple control measures, followed by a decrease in the fifth year.

Notes:

P129 - Addition of the boar stud production type to the National ASF Model

E Britton[1], H Hutchinson[1], S Malladi[1], P Bonney[2], K St. Charles[2], M Culhane[2], T Boyer[1]

[1]USDA-Animal and Plant Health Inspection Service, [2]Department of Veterinary and Biomedical Sciences, University of Minnesota. eric.britton2@usda.gov

Session: Epidemiology, **2023-01-22, 6:00 - 8:00**

Objective: The USDA APHIS Center for Epidemiology and Animal Health (CEAH) has developed a national scale African swine fever (ASF) disease spread model to support emergency preparedness and response activities. The objectives of this project were to 1) expand the capability of CEAH's existing national ASF model by adding a boar stud production type, and 2) to provide a quantitative estimate of the likelihood a boar stud premises located in a regulatory control area becomes infected with ASF. The results of this project will support an ASF boar stud risk assessment being developed by the University of Minnesota Secure Food Systems Team (UMSFST).

Methods: Addition of boar stud premises required determination of the number, geographic distribution, and sizes of premises across the United States. In addition, potential routes of transmission and distances between boar studs and other swine premises were defined by type and frequency of contacts. The UMSFST formed a boar stud working group with participants from industry which allowed definition of these parameters. Modelers from the University of Minnesota provided output from a within-herd ASF disease spread model that defined the probability of transmission given contact between an infectious boar stud premises and a susceptible premises, and the probability of detection of an infected boar stud premises under varying surveillance schemes. The national ASF model was used to run 12 scenarios that varied the location of ASF introduction in order to investigate differences in starting production type (commercial grower finisher vs commercial farrow to wean), the state where it was introduced (Iowa, North Carolina, or Oklahoma), and surrounding density of swine premises (high versus low). For each scenario one premises was selected to introduce ASF and each scenario was run for 500 iterations.

Results: The context at the start of the outbreak, consisting of production type, state, and surrounding density had an impact on outbreak size and duration. Outbreaks were larger and had longer duration when the outbreak began in a farrow to wean operation, compared to a grower finisher operation. There was additionally a larger number of infected farms and longer outbreaks when the outbreak was started in areas of high swine density compared to areas of low swine density. Outbreak size by state of introduction were largest in North Carolina followed by Iowa then Oklahoma. Across all scenarios boar studs were unlikely to become infected. The percent of iterations where any boar stud premises was infected ranged from 4% to 14%. In iterations where a boar stud was infected, the median number of infected boar stud premises was 1 (95^{th} percentile = 2). The probability that a boar stud located in a control area become infected ranged from 0 to less than 0.1 across all scenarios.

Conclusions: This project contributes to the overall ASF preparedness efforts by evaluating a possible route of spread and providing quantitative information on the severity of this specific disease pathway. This project will also support a risk assessment that will support ASF emergency preparedness for the boar stud sector.

Notes:

P130 - Brucellosis among swine in the Republic of Armenia

Hrant M. Danelyan[1], Pertsh G. Tumanyan[1], Ashkhen A. Hovhannisyan[1]

[1]Reference Laboratory of Especially Dangerous Pathogens. danelyanh@mail.ru

Session: Epidemiology, **2023-01-22, 6:00 - 8:00**

Objective: Brucellosis is a serious agricultural and public health problem in Armenia. Testing of brucellosis among the swine population is not currently conducted, but swine can be a source of infection for cattle, sheep, goats, and dogs (particularly when different species of farm animals coexist in the same environment), as well as humans. We have conducted a screening study to determine the presence of brucellosis among swine in several regions of Armenia with high and low prevalence of brucellosis among livestock.

Methods: In 2023, blood samples (150 total) from sexually mature swine on small, mixed animal farms from 25 villages of 4 regions of Armenia (Armavir, Shirak, Gegharkunik, and Tavush) were collected. Blood samples were collected in duplicate in two different vacutainer tubes (red cap for serology and purple cap for the polymerase chain reaction (PCR)). All serum samples were tested by the Rose Bengal Test (RBT), serum agglutination test (SAT), enzyme-linked immunosorbent assay (ELISA), fluorescent polarization assay (FPA), and PCR. We utilized the AmpliSens PCR test kit which is designed for identification of *Brucella* spp. in human and animal samples.

Results: Out of the 150 samples, 9 tested positive for *Brucella* by RBT and 8 samples were positive by SAT, ELISA, and FPA, of which 6 samples were positive by RBT and 2 samples were negative by RBT. There was no inconsistency between SAT, ELISA, and FPA results. Distribution of 8 confirmed positive samples/samples tested by region was: Gegharkunik 4/36, Armavir 3/36, and Shirak 1/48. All 150 samples were negative by PCR. Due to the small positive sample size, there were no comparisons performed between the testing methods.

Conclusions: This screening study indicates the presence of brucellosis among swine in some regions of Armenia. The discrepancy in the testing results between RBT and the other assays can possibly be explained by differences in sensitivity and specificity of the tests but a larger study sample is needed to verify this. More study is needed to determine assay standardization for brucellosis in swine. According to the diagnostic algorithm used in Armenia among animals, all positive samples by RBT must be confirmed by at least one confirmatory test (in this case, ELISA or FPA). The negative results of PCR are possibly due to the species tested or timing of the samples, which should be taken into consideration in future studies. Additional studies among swine populations in all regions of Armenia will provide an opportunity to develop a more comprehensive overview of the epidemiological situation of brucellosis among swine, as well as all livestock.

Notes:

P131 - Presence and diversity of *Leptospira* in domestic animals, rodents, and mongooses in Puerto Rico communities

Ammar Yasir[1], Luis J. Santiago Ramos[2], Natalia Coriano [2], Ralph Rivera Gutierrez [2], Ilana J. Schafer[3], Melissa Mueller[4], Jarlath E. Nally[5], Camila Hamond[6], Karen LeCount[6], Tammy Anderson[6], Renee Galloway[3], Claudia Munoz-Zanzi[1]

[1]School of Public Health, University of Minnesota, [2]Medical Sciences Campus, University of Puerto Rico, [3]National Center for Emerging and Zoonotic Infectious Diseases, Centers for Disease Control and Prevention, [4]Clinical and Translational Science Institute, University of Minnesota, [5]USDA-Agricultural Research Service, [6]USDA-Animal and Plant Health Inspection Service. yasir005@umn.edu

Session: Epidemiology, **2023-01-22, 6:00 - 8:00**

Objective: Leptospirosis is a zoonotic disease caused by the spirochete bacterium *Leptospira* and is endemic in Puerto Rico, affecting people and a wide variety of animal species. Wild and domestic animals are reservoirs of pathogenic *Leptospira*. The objective of this study was to describe the presence and diversity of *Leptospira* in domestic animals, rodents, and mongooses in various communities across Puerto Rico with a history of human cases.

Methods: Enrollment of targeted sites (households or farms) occurred between March and May 2021 in 10 sampling areas. Sampling consisted of rodent and mongoose trapping, as well as collecting specimens from domestic animals. Questionnaires were used to collect information at animal and site levels. Laboratory methods included PCR, culture, and Fluorescent antibody test (FAT) for the detection of *Leptospira,* and the Microscopic agglutination test (MAT) for the detection of antibodies. Logistic regression, with random effects for the sampling area, was used to examine risk factors associated with MAT seropositivity in rodents and in dogs.

Results: Overall, leptospirosis prevalence, defined as positive by any test, was 62% (32/52) in mice, 50% (21/42) in rats, 30.2% (19/63) in cattle, 35.7% (20/57) in dogs. Other species sampled included cats (1/5 =20% positive), goats (2/8 = 25% positive), and horses (5/5 = 100% positive). Successful isolation identified predominantly serogroup Ballum in mice and Icterohaemorrhagiae in rats. Dogs, cattle, and horses had the highest MAT titers to serogroup Icterohaemorrhagiae. The type of site (farm vs residential household), and presence of dogs on the site were positively associated with leptospirosis in rodents. Among unvaccinated dogs, living in a site located in a flood-prone area was positively associated with leptospirosis.

Conclusions: The high prevalence of animal leptospirosis, notably in rodents, is a significant public health finding. This study provided new surveillance data on animal leptospirosis in Puerto Rico and highlights the need for further studies to understand the local ecology and risk factor.

Financial Support: This study was funded by the CDC Cooperative Agreement No. 1 NU1ROT000005-01-00

Notes:

P132 - Modeling host behavior and environmental transmission of chronic wasting disease

Ian McGahan[1], F Mandujano[1], D. Storm[2], Marie Gilbertson[1], S Lichtenberg[3], J Powell[4], W Turner[1], D Walsh[5], J Zhu[1]

[1]University of Wisconsin - Madison, [2]Wisconsin Department of Natural Resources, [3]University of Minnesota, [4]Utah State University, [5]University of Montana. imcgahan@wisc.edu

Session: Epidemiology, **2023-01-22, 6:00 - 8:00**

Objective: Understanding disease transmission is difficult when infection occurs due to both direct contact between individuals and indirect exposure to pathogens in the environment. For example, chronic wasting disease (CWD) is a fatal disease of deer spread by infectious proteins called prions. Understanding and managing CWD spread requires sorting out complex habitat use, deer movement, and behavior that affects direct transmission, as well as tracking infectious prions in the environment and how the deer may encounter them. The objective of this project is to quantify relative contribution to spread between direct and environmental transmission for the ongoing CWD epidemic in central Wisconsin.

Methods: This project aims to develop a general modeling framework, linking landscape features, white-tailed deer (*Odocoileus virginianus*) movement, prion contamination and transmission routes, describing how individual behavior affects disease spread in space and time. We determine the extent and infection pressure of prions in the environment. Modern observation methods reveal how deer behavior, movement, and interactions with infectious prions contribute to infection across habitat patches. Mathematical models link deer social behavior and movement with prion retention, transport, and infection potential in complex landscapes. Application of homogenization, a technique that averages many small processes into broader net effects, links fine-scale infection pathways with large-scale population impacts on deer in Wisconsin. We statistically test our models to assess the relative strength of disease pathways and the impacts of population management and landscape disturbance.

Results: We anticipate several results from this work. First, our general modeling framework will synthesize diverse, unexplored spatiotemporal influences on disease transmission. Homogenization applied to candidate models will incorporate detailed multi-scale data, and improve computational efficiency. Second, our development and application of new diagnostic methods will elucidate the retention, transport, extent, and infection potential of prions in heterogeneous soil and plant environments. Third, using deer movement and behavioral data we will determine landscape structuring of host habitat preference and aggregation sites, potential environmental prion reservoirs, and deer preference for and risk behavior at these and other host aggregation sites. Finally, by specifying models with multiple transmission pathways in our general modeling framework and fitting these models with both existing and newly collected data, we will determine the relative contribution to infection between direct transmission and environmental transmission.

Conclusions: We aim to develop broadly applicable mechanistic models uniting direct and environmental transmission processes across scales. We use a novel application of homogenization techniques to mechanistic models, allowing us to analytically integrate the impact of small-scale variability on large-scale population processes. Consequently, our proposed approach is well-suited to modeling the multi-scale data streams and underlying drivers governing disease dynamics and provides critical advances beyond standard compartmental modeling approaches. By integrating processes including landscape structure, host movement ecology, pathogen distribution, and transmission modes in a tractable, homogenized model framework, we link underlying transmission mechanisms with emergent disease processes (e.g., spatial spread, observed prevalence).

Financial Support: We are thankful that this work is funded by USDA-NIFA through an EEID grant.

P133 - Extensive SARS-CoV-2 genomic survey supporting farmed white-tailed deer health

Jamin Dreyer[1], Francisco Ferreira[2], Carlos Rodriguez[3], Gabriel Hamer[2], Ashbeel Roy[1], Sarah Hamer[4]

[1]Ginkgo Bioworks, [2]Department of Entomology, Texas A&M University, [3]Texas A&M University Veterinary Medical Diagnostic Laboratory, [4]School of Veterinary Medicine & Biomedical Sciences, Texas A&M University. jdreyer@ginkgobioworks.com

Session: Epidemiology, **2023-01-22, 6:00 - 8:00**

Objective: Since late 2019, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has swept around the world leaving millions of dead and diseased humans in its wake. While the specific zoonotic origin of this virus has yet to be identified, multiple new animal reservoirs have become established. White-tailed deer (*Odocoileus virginianus*, "WTD") in North America is one of the most conspicuous secondary zoonotic SARS-CoV-2 reservoirs, with very high seroprevalence and viral PCR testing positivity rates across North America. Furthermore, studies have demonstrated that WTD are highly susceptible to infection and can transmit SARS-CoV-2 by contact. Genomic surveillance of wild WTD populations revealed viral variants unique to these populations, and also allowed the potential detection of "spillback" transmission events from deer to humans. Still, genomic sequencing from WTD is lacking and hampers our ability to understand chains of transmission within farmed WTD populations and between captive and wild WTD or other conspecific animals. The Extensive Deer Genomic Surveillance (EDGeS) Project will conduct large-scale SARS-CoV-2 testing and whole genome sequencing (WGS) in farmed and wild WTD to improve our understanding of SARS-CoV-2 impacts on farmed animal health and towards future countermeasure development.

Methods: We aim to test ~6000 samples from farmed and wild WTD, other closely associated species, and integrated environmental samples (e.g., farm wastewater or effluent) and sequence up to 1500 SARS-CoV-2 genomes from samples that test positive.

Results: To date (Aug 2023) we have executed Materials Transfer Agreements to obtain >5000 lymph node samples from white-tailed deer, mule deer, and other cervids (captive and wild) from across all Texas ecoregions for use in the project. An initial 72 mule deer samples have been screened, with none testing positive for SARS-CoV-2 via PCR.

Conclusions: The goal of this effort is to help identify routes of SARS-CoV-2 transmission in WTD and provide genomic information necessary for future animal coronavirus therapy or vaccine countermeasures. Our results will aim to help protect farmed WTD and humans that interact with them, and develop infrastructure to respond to unforeseen developments to the current SARS-CoV-2 pandemic as well as future zoonotic disease outbreaks impacting livestock in the United States. This project is also a unique collaboration between industry and academia, in this case sample collection and testing at Texas A&M University with subsequent SARS-CoV-2 whole genome sequencing at Ginkgo Bioworks in Boston, MA USA.

Financial Support: This project was supported by Agriculture and Food Research Initiative Competitive Grant no. 13696499 from the USDA National Institute of Food and Agriculture.

Notes:

P134 - Retrospective analysis of farmed cervid respiratory samples, 2016-2023

Philma Glora Muthuraj[1], Annabelle Burnum[1], Solomon Odemuyiwa[1], Tamara Gull[1]

[1]University of Missouri. philmagloramuthuraj@gmail.com

Session: Epidemiology, **2023-01-22, 6:00 - 8:00**

Objective: Respiratory disease is a significant cause of losses in farmed deer, especially during fawning season. Very little is known about infectious agents associated with respiratory disease in farmed deer. A retrospective study was conducted investigating farmed deer with respiratory lesions.

Methods: We analyzed data from 119 lung tissues or lung swabs from farmed deer submitted to the University of Missouri - Columbia Veterinary Medical Diagnostic Laboratory from 2016 to 2023. Based on lesions and client request, aerobic /anaerobic bacterial culture and molecular analysis for epizootic hemorrhagic disease virus, bluetongue virus, infectious bovine rhinotracheitis, *Mycoplasma* and *Salmonella* were performed.

Results: On aerobic culture of the lung samples, 16.8% of the samples isolated *Pasteurella multocida*, 11.7% and 2.5% of samples isolated *Bibersteinia trehalosi* and *Mannheimia* spp respectively. Coliform bacteria were isolated from 44.5% of the samples, 20.2% yielded *Trueperella pyogenes*, 10.1% grew *Pseudomonas* spp and 5.9% yielded *Streptococci* spp. *Enterococci* spp were isolated from 5.0% samples, and 12.0% samples resulted in the isolation of other aerobes (including postmortem contaminants). Anaerobic bacteria including *Fusobacterium* and *Clostridium* were isolated from 10.0% of the samples. On molecular analysis of the samples, 11.7% samples were positive for epizootic hemorrhagic disease virus, 9.2% samples for *Mycoplasma* spp, 1.7% samples for bluetongue virus and 0.8% samples for infectious bovine rhinotracheitis virus. Only one of the lung samples tested positive for *Salmonella* by PCR and the liver sample from the same animal also isolated *Salmonella* group C1. One sample tested positive for both epizootic hemorrhagic fever and bluetongue viruses.

Conclusions: Overall, 7.6% of the samples had coinfections with epizootic hemorrhagic disease virus along with *Pasteurella multocida, Bibersteinia trehalosi,* or *Mycoplasma* spp. The condition of samples and treatment of animals with antimicrobials could have precluded isolation of additional respiratory pathogens. The data suggest that farmed deer could have a respiratory disease complex as seen in cattle, but further studies are warranted to study viral and bacterial respiratory co-infection patterns.

Notes:

P135 - Benchmarking of computer vision algorithms on cloud platforms for the detection of digital dermatitis in dairy cows

Srikanth Aravamuthan[1], Emil Walleser[1], Dorte Dopfer[1]

[1]University of Wisconsin-Madison. aravamuthan@wisc.edu

Session: Biosecurity / Infection Control, 2023-01-22, 6:00 - 8:00

Objective: Digital dermatitis (DD) is a bovine claw disease responsible for ulcerative lesions on the coronary band of the foot. DD is associated with massive herd outbreaks of lameness and influences cattle welfare and production. Early detection of DD can lead to prompt treatment and decrease lameness. Computer vision (CV) provides a unique opportunity to improve early detection. The study aims to train lightweight CV models for cloud deployment and compare the implementations for the detection of DD in dairy cows. CV models were trained for the detection of DD lesions and scoring of M-stages, compared using performance metrics and inference time, and automated for real-time detection using images and videos on cloud platforms.

Methods: Images were collected from commercial dairy farms while facing the interdigital space on the plantar surface of the foot. Images were scored for M-stages by a trained investigator using the M-stage classification system. The dataset contained 240 M0, 17 M2, 51 M2P, 114 M4H, and 108 M4P images. A YOLOv5 model was trained to detect and score DD lesions and evaluated for average precision (AP) and mean average precision (mAP). The model was exported and deployed on multiple cloud platforms, then compared using Cohen's kappa for agreement between the implementations and a trained investigator and inference time using frame per second (FPS) on live streaming videos.

Results: YOLOv5s achieved an mAP of 0.946 with high AP for all five M-stages during model training. The Cohen's kappa for Google Colab was determined to be 0.706 and interpreted as "substantial" agreement between the two raters ($z = 11.4$; $p < 0.001$). The Cohen's kappa for Docker using an IP camera via HTTP was determined to be 0.691 and interpreted as "substantial" agreement between the two raters ($z = 9.9$; $p < 0.001$). The Cohen's kappa for Docker using an IP camera via RTSP was determined to be 0.568 and interpreted as "moderate" agreement between the two raters ($z = 9.24$; $p < 0.001$). The Cohen's kappa for TensorFlow.js was determined to be 0.763 and interpreted as "substantial" agreement between the two raters ($z = 9.83$; $p < 0.001$). The Docker container using an IP camera via RTSP stream outperformed all other implementations at a maximum of 25 FPS followed closely by the Docker container using an IP camera via HTTP stream at a maximum of 22 FPS. All deployments exceeded the minimum threshold for image processing by a human visual system at approximately 10 FPS. The prediction accuracy for TensorFlow.js was 0.842 with high detection for M0, M4H, and M4P and perfect detection for M2 and M2P.

Conclusions: The CV models were able to detect DD lesions and classify all five M-stages on the TensorFlow.js application with the highest performance and sufficient speed. The proposed CV tool can be used for early detection and prompt treatment of DD in dairy cows. This result is a step towards applying CV algorithms to veterinary medicine and implementing real-time DD detection on cattle farms.

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

Notes:

P136 - Real-time digital dermatitis detection in cows on an Android/iOS app using computer vision techniques

A. Dwivedi[1], M. Henige[1], D. Doepfer[1], S. Aravamuthan[1]

[1]University of Wisconsin-Madison. aravamuthan@wisc.edu

Session: Biosecurity / Infection Control, 2023-01-22, 6:00 - 8:00

Objective: The aim of the study is to deploy Computer Vision (CV) models for real-time detection of Digital dermatitis (DD) lesions in cows using Android or iOS mobile applications. Early detection of DD lesions in dairy cows is crucial for prompt treatment and animal welfare. Android and iOS apps can facilitate routine and early DD detection in cows' feet on dairy and beef farms. Upon detecting signs of DD, dairy farmers can implement preventive and treatment methods, including foot baths, topical treatment, hoof trimming, or quarantining cows affected by DD to prevent its spread. Administering early treatment decreases the severity of the condition but also enhances the overall productivity of cows.

Methods: We applied transfer-learning to DD image data for 5 classes, M0, M4H, M2, M2P, and M4P, on pretrained YOLOv5 model architecture using COCO-128 pretrained weights. The combination of localization loss, classification loss, and objectness loss was used for the optimization of prediction performance. This custom DD detection model was trained on 363 images of size 416x416 pixels and tested on 46 images. During model training, data were augmented to increase model robustness in different environments. The model was converted into TFLite format for Android devices and CoreML format for iOS devices. These models were deployed as Android/iOS applications for real-time DD detection using Android Studio and XCode software. Techniques such as quantization were implemented to improve inference speed in real-world settings.

Results: The DD models achieved an average mAP (mean Average Precision) of 0.95 on the test dataset. When tested in real-time, iOS devices resulted in Cohen's kappa value of 0.57 averaged across 5 classes denoting the moderate agreement of the model detection with human investigators. The Android device resulted in a Cohen's kappa value of 0.38 denoting fair agreement between model and investigator. Combining M2 and M2P classes and M4H and M4P classes resulted in a Cohen's kappa value of 0.65 and 0.46, for Android and iOS devices respectively. For the two-class model (lesion vs. non-lesion), a Cohen's kappa value of 0.74 and 0.65 was achieved for iOS and Android devices. iOS achieved a good inference time of 20ms, compared to 57 ms on Android.

Conclusions: iOS performs better than Android in terms of Cohen's kappa value, inference time, and confidence score because models are fine-tuned for Apple device architecture and hardware such as Neural Engine that boosts CoreML's inference speed. These mobile apps can perform routine DD detection in cow's feet on dairy farms. If signs of DD are seen, treatment lists can be generated. The apps do not require internet access and can be used in remote farms with no reliable internet. In the future, microcontrollers such as Jetson AGX Orin can be used to automate DD detection and send data directly to veterinarians through Cloud services such as AWS and Google Cloud.

Notes:

P137 - New England livestock stakeholders' perspectives on emergency animal disease preparedness

Julia M. Smith[1], Michael Moser[2]

[1]Dept. of Animal & Veterinary Sciences, University of Vermont, [2]Center for Rural Studies, University of Vermont. julie.m.smith@uvm.edu

Session: Biosecurity / Infection Control, 2023-01-22, 6:00 - 8:00

Objective: As part of an effort to review the New England Secure Milk Supply Plan, this project sought veterinary and producer perspectives in relation to foreign animal disease response planning and preparedness. Nationally, Secure Milk Supply guidance describes biosecurity performance standards for dairy producers, haulers, and milk receiving plants in the event of a foot-and-mouth disease outbreak.

Methods: Interviews were completed with 6 producers and 8 private veterinarians. The interviews were conducted via Teams and recorded for later transcription and analysis. All research activities were approved by the University of Vermont Institutional Review Board under exemption category 2.

Results: Stakeholders provided their perspectives on having an enhanced biosecurity plan, identifying a biosecurity manager, establishing a line of separation, and operating a cleaning and disinfection stations. Stakeholders also indicated their level of interest in using an app to simplify the process of creating an enhanced biosecurity map.

Conclusions: Continuing education and outreach efforts in support of foreign animal disease preparedness can take into account the perspectives of veterinarians and producers. In addition, by understanding stakeholders' perspectives on meeting expectations of Secure Food Supply plans, state animal health authorities can better prioritize steps to improve readiness.

Financial Support: Funding for this project is/was provided from the USDA Animal and Plant Health Inspection Service through the National Animal Disease Preparedness and Response Program (VS NADPRP VT01.20). It may not necessarily express APHIS's views.

Notes:

P138 - Dial it down: Effective load reduction of aerobic bacteria on cellphones in a veterinary teaching hospital

DN Barnes[1], BA Burgess[1]

[1]Department of Population Health, University of Georgia. yvonne.barnes@uga.edu

Session: Biosecurity / Infection Control, 2023-01-22, 6:00 - 8:00

Objective: In the healthcare world, cellular phone use has become an efficient means to not only communicate, but may also be used in the diagnosis and treatment of patients in both emergency and outpatient settings. Cyclical contamination is commonly observed when healthcare providers encounter patients and subsequently access their cellular phones, or vice versa. As such, these devices may become reservoirs for potential pathogens and are likely important factors in this contamination cycle. Use of disinfectant wipes on such devices has shown variable results, and typically damages or degrades electronic devices over time, therefore, alternative methods should be considered. One such solution is the use of commercially available UV-C light sanitation boxes which uses irradiation at ~250 nm to disinfect a device and to do so without damaging the equipment. This study aims to 1) evaluate the efficacy of 3 commercially available UV-C irradiation products to commercially available disinfectant wipes (70% alcohol [ALC], accelerated peroxygen [AHP], quaternary ammonium [QAC]) to reduce aerobic bacterial load on cellphone keypad areas; and 2) characterize current cell phone disinfection practices among personnel in a veterinary teaching hospital.

Methods: An experimental study was undertaken where all hospital personnel in clinical roles were eligible to participate. For each cell phone (N=84), an aerobic culture was performed by pressing a contact plate (RODAC™) on the key pad area, then randomly received 1 of 6 treatments - 1 of 3 disinfectant wipe treatments (ALC, AHP, or QAC) for 10-seconds, allowed to dry for 3-minutes; or 1 of 3 UV-C light sanitation boxes (applied per manufacturer recommendation) - and then re-cultured. All plates were incubated at 35°C for 24-hours. Each participant completed a brief survey regarding current cell phone use and disinfection practices. Data were analyzed using multivariate linear regression to control for hospital type (small animal or large animal) and personnel position within the facility.

Results: We expect that UV-C irradiation at ~250nm will provide greater reduction of bacterial counts, followed by AHP, QAC, and then ALC wipes. Additionally, we expect access to cleaning and disinfection products will be key to instigating use by clinical personnel.

Conclusions: This study will provide information on alternative cleaning methods that can be used throughout the hospital without potentially damaging essential equipment nor contribute to the development of resistant organisms. Additionally, these results will inform policies, practices, and educational resources regarding cleaning and disinfection of cellular phones used in the healthcare setting.

Financial Support: Veterinary Teaching Hospital, University of Georgia

Notes:

P139 - Effect of *Brucella* spp. infection on the milk microbiome in cattle and goats: implications for disease transmission

Beatus M Lyimo[1], Charles Mayenga[2], Samson Lyimo[1], Lidia Munuo[1], Daniel Malkiadi[3], Amina Abdalla[3], Waziri Mlewa[3], Stephanie Bierly[4], Erin K Box[5], Cheryl D Gleasner[5], Lingling Li[4], Maurice Byukusenge[4], Isabella Cattadori[4], Erika Ganda[4], Earl Middlebrook[5], Jessica Radzio-Basu[4], Andrew Bartlow[5], Peter Hudson[4], Coletha Mathew[3], Zachariah E Makondo[2], Jeanne M Fair[5], Vivek Kapur[4], Joram Buza[1], Robab Katani[4]

[1]Nelson Mandela African Inst. of Sci. and Technology, [2]Tanzania Vet. Lab. Agency, [3]Sokoine University of Agriculture, Tanzania, [4]Pennsylvania State University [5]Genomics and Bioanalytics, Los Alamos National Laboratory. beatus.lyimo@nm-aist.ac.tz

Session: Biosecurity / Infection Control, **2023-01-22, 6:00 - 8:00**

Objective: Brucellosis, caused by bacterial pathogens belonging to the genus *Brucella*, is a significant zoonotic disease whose transmission occurs through several avenues, such as contact with aborted material and consumption of contaminated animal by-products—most notably milk. Despite its significance, the role of milk in transmitting and maintaining infections within livestock herds is not well understood. Furthermore, bacterial microbiomes play a critical role in determining the overall health of animals. Advanced microbiological tools can identify coexisting pathogens that may compound the infection. However, investigation of the milk microbiome presents methodological challenges due to its inherent complexity, particularly in the sequencing and analysis phases. In this study, we seek to explore the bacterial microbiomes present in milk samples collected from both cattle and goats. Our primary objective is to ascertain whether a distinguishable difference exists in the microbiomes of *Brucella*-positive animals compared to those that have tested negative for the pathogen.

Methods: To execute the study, we procured milk and serum samples from 139 cattle and 106 goats, distributed across seven mixed-species herds. Antibodies specific to *Brucella* spp. were assayed in the serum samples utilizing Enzyme-Linked Immunosorbent Assay (ELISA). Concurrently, the presence of *Brucella* spp. infection in the milk samples was determined through Real-Time Polymerase Chain Reaction (RT PCR). Following the optimization of DNA extraction procedures and Illumina Library preparation protocols, we employed 16S ribosomal RNA sequencing to characterize the bacterial microbiome present in milk samples. Subsequently, we conducted a comparative analysis of the bacterial communities in milk specimens that tested positive for *Brucella* spp. against those that tested negative.

Results: The investigation yielded statistically significant disparities in Shannon diversity ($P < 0.01$) and bacterial composition ($P < 0.01$) between cattle and goats. Additionally, noteworthy differences in both diversity ($P < 0.01$) and bacterial composition ($P < 0.01$) were observed among the seven mixed- herds under study. Animals that tested positive for Brucella spp. antibodies via ELISA exhibited a significantly reduced microbial diversity compared to those that tested negative ($P = 0.04$). While animals that were qPCR-positive for *Brucella* spp. displayed higher Shannon diversity than their qPCR-negative counterparts; this difference did not reach statistical significance ($P = 0.09$). Our findings collectively indicated that infection with *Brucella* spp. exerts a tangible influence on the microbial composition of milk. These results suggest that *Brucella* spp. infection leads to alterations in the microbiomes present in milk, thereby providing valuable insights into the epidemiology of this zoonotic disease.

Conclusions: Moving forward, a thorough microbiome analysis is underway, with the expectation that the complete results will be disclosed during the conference. Gaining a detailed understanding of the conditions that facilitate the transmission of *Brucella* spp. via milk in livestock is imperative for the effective management of the disease and for the accurate identification of sources of infection. By examining the complex interactions between *Brucella* spp. and the microbial communities present in milk, our research aims to make substantive contributions to the advancement of brucellosis management strategies, ultimately aiming to mitigate the disease's adverse consequences on both livestock and human health.

Financial Support: This research is supported, by a grant from the U.S. Department of Defense, Defense Threat Reduction Agency, Biological Threat Reduction Program, Project # HDTRA12110039 to Robab Katani and Joram Buza.

Notes:

P140 - Qualitative studies on farmers' perception and implementation of biosecurity measures on UK poultry farms in relation to HPAIV

Paniz Hosseini[1], Ivo Syndicus[2], Eve Houghton[2], Guillaume Fournie[2, 3], Javier Guitian[2], Ashley Banyard[4], Andy Paterson[4], Richard Hepple[4], Ian Brown[4], Pablo Alarcon Lopez[2], James Wood[1]

[1]Department of Veterinary Medicine, University of Cambridge, [2]Department of Pathobiology and Population Sciences, Royal Veterinary College, [3]Department of Pathobiology and Population Sciences, National Research Institute for Agriculture, Food and Envrionment, France, [4]Animal and Plant Health Agency, United Kingdom. ph586@cam.ac.uk

Session: Biosecurity / Infection Control, 2023-01-22, 6:00 - 8:00

Objective: (1) Explore biosecurity and disease management on commercial poultry farms in the UK. (2) Research into farmers' experiences and perceptions of diseases such as avian influenza (AI). (3) Understand how the spread of avian influenza can be prevented in the future amongst poultry farms.

Methods: A total of 13 qualitative interviews with farm managers across various poultry farms in the UK were conducted, consisting of "case" farms (farms that have had an AI outbreak since 2021) and "non-case" farms (farms that have not had an AI outbreak before). Interviews were semi-structured and allowed for in-depth discussions on farming practices and biosecurity, including any associated challenges. Ethnographic observations were also conducted on each farm, completed during and after each interview to help identify which biosecurity measures were physically being used on the farm, allowing for analysis of both farmers' reported experiences and physical evidence of any biosecurity management/mismanagement. Interviews, observation notes/diary entries and other information gathered were analysed using NVivo 12 to generate codes, patterns and themes amongst the data.

Results: Biosecurity measures such as the use of foot-dips, changing wellies and regular disinfection routines were some of the most popular and adhered to measures across a majority of the farms. Some of the weaker biosecurity measures related to the old age and poor maintenance of sheds and buildings; poor structure and layout of the farms and poor biosecurity surrounding wild birds. Additionally, farm managers expressed high levels of stress and anxiety surrounding the threat of AI. Analysis of the findings suggest that the level of adherence to biosecurity was impacted by factors such as the farmers own perceptions of risk; general knowledge surrounding the spread of AI; financial limitations; communication with other actors and the age/length of experience of the staff members on the farm.

Conclusions: We recommend further guidance and support services that acknowledge the mental health impacts which farmers may face during times of disease outbreaks. Providing services where farmers can speak to other individuals in the industry that have had experiences of an AI outbreak would be beneficial, as would access to further training and educational materials to better understand biosecurity and the risks associated with AI. Finally, further research with other actors in the poultry industry would help to better understand the factors influencing biosecurity implementation, and how best to approach this in the future from an industry perspective.

Financial Support: Thank you to the Biotechnology and Biological Sciences Research Council (BBSRC) and the Department for Environment Food and Rural Affairs (DEFRA) for funding this project and the wider Flu-MAP study.

Notes:

P141 - Unraveling the nexus of farm-level biosecurity and disease reporting: Evidence from regret minimization models

Richmond Baye[1], Asim Zia[1], Scott Merrill[1], Eric Clark [1], Julie Smith [1], Christopher Koliba[2]

[1]University of Vermont, [2]University of Kansas. rbaye@uvm.edu

Session: Biosecurity / Infection Control, **2023-01-22, 6:00 - 8:00**

Objective: Farm-level activities and operations have potential implications for disease outbreaks in the entire global food value chain. This is particularly true concerning decisions and choices made by farmers regarding their biosecurity investments and disease reporting. In the face of this challenge, policymakers must devise appropriate strategies to encourage or trigger a middle-ground solution to mitigate the negative consequences of transboundary animal diseases. This paper aims to exploit the potential for minimizing regret under different biosecurity investment options and examines how a potential policy response of conditional indemnity could affect the biosecurity investment response and disease reporting response of swine producers in the US.

Methods: We aim to launch a survey and collect data from swine producers across the US and analyse the responses using the random regret minimization (RRM) and the generalized regret minimization (GRRM) models to examine the various choices that drive conditional biosecurity adoption and disease reporting.

Results: We anticipate that regret aversion may not have a dominant effect on the proportion of choices, even if delayed reporting is considered.

Conclusions: In conclusion, our findings hold power in shaping policy discourse. By understanding the drivers that shape conditional biosecurity practices, we contribute to a more resilient and safe agricultural system that guards against the impact of a tier 1 disease.

Notes:

P142 - Registered cases of Anthrax in the Gegharkunik region of the Republic of Armenia in june 2021

Hayk Serobyan[1], Pertsh Tumanyan[2]

[1]FSIB, [2]RLEDP. serhaykvet@gmail.com

Session: One Health / Public Health, 2023-01-22, 6:00 - 8:00

Objective: Anthrax is an acute infectious disease caused by the gram-positive, spore-forming bacterium *Bacillus anthracis*. Anthrax is included in the list of diseases subject to compulsory notification of the World Organization for Animal Health (WOAH). The aim of this work is to present cases of animal deaths in 2021 caused by incomplete preventive vaccinations in historically endemic areas during the coronavirus disease (COVID-19) global pandemic in 2020-2021.

Methods: The data was collected from the Food Safety Inspection Body of the Republic of Armenia (RA), Center of Agricultural Services of the Ministry of Economy, and from the expertise protocols of the Reference Laboratory for Especially Dangerous Pathogens of the Republican Veterinary and Phytosanitary Laboratory Service Center. Between June 22 and June 29, 2021, 14 animals in the villages of Torfavan and Vanevan in the Gegharkunik Region of the RA died. Samples from these animals were collected and sent to the laboratory for research. Gram staining and Ascoli precipitation testing were used as evidence of infection in accordance with the methods defined by WOAH. Following the death of the first animal displaying clinical signs of anthrax, samples from the remaining 13 deceased animals were subsequently analyzed.

Results: Among 14 samples evaluated, 8 tested positive for anthrax by both gram stain and Ascoli reaction. Positive samples were from the villages of Torfavan (1 head of cattle) and Vanevan (2 head of sheep and 5 head of cattle). Based on the clinical signs and laboratory examination, we organized and conducted vaccinations (live-attenuated vaccine) for 252 head of cattle in Torfavan, as well as 281 head of cattle and 407 head of sheep in Vanevan. Anthrax cases were reported in a region where anthrax has occurred in the past. Vaccination of farm animals, including vaccinations against anthrax under the program in the spring of 2021 was incomplete, likely due to COVID-19 among veterinarians, the flow of many animals in emergency conditions, and the lack of implementation of the animal registration system. In addition, from the end of 2020 to the first months of 2021, 81 head of cattle were imported to the village of Torfavan, and 96 head of cattle and 116 head of sheep were imported to the village of Vanevan, which made it difficult to plan and implement vaccination programs.

Conclusions: To prevent future anthrax outbreaks, it is necessary to implement an emergency vaccination plan for animals; introduce a system of numbering and registration of animals; develop mapped livestock cemeteries, fence the territory of livestock cemeteries, and place information signs to exclude the entry of people and animals into the territory; improve disinfection of contaminated burial sites; and improve quarantine measures for imported animals.

Notes:

P143 - Genomic evaluation of *Salmonella enterica* ser. Dublin in cattle and humans in the United States: 1986-2022

Sophia M. Kenney[1], Nkuchia M. M'ikanatha[2, 3], Erika Ganda[1]

[1]Dept. of Animal Science, Penn State University, [2]Dept. of Food Science, Penn State University, [3]Pennsylvania Dept. of Health.
smk459@psu.edu

Session: One Health / Public Health, 2023-01-22, 6:00 - 8:00

Objective: The emergence of increasingly antimicrobial resistant (AMR) pathogens is a global threat to human and animal health, therefore requiring a One Health approach to comprehensively understand pathogen evolution across host and environmental reservoirs. *Salmonella enterica* serovar Dublin (*S.* Dublin), specifically, is a bovine-adapted and multi-drug resistant zoonotic pathogen. Capable of evading detection and causing severe, invasive disease in both humans and cattle, its increasing antimicrobial resistance and dissemination throughout the United States threatens human and animal health, and food safety and security. As such, an updated understanding of its evolution over time and across pathogen-reservoirs is timely. To this end, existing multi-agency biosurveillance infrastructure of the National Antimicrobial Resistance Monitoring System (NARMS) and the NCBI Pathogen Detection Project were leveraged to evaluate the genomic evolution of previously sequenced *S.* Dublin strains.

Methods: A combined 2,180 *S.* Dublin strains were identified through NCBI's *Pathogen Isolate Browser* and NARMS BioSample identifiers. Isolate collection spans 1986-2022 and 37 states. Isolate source includes clinical samples for both humans and cattle, pre- and post-harvest environmental samples, and retail meats. Raw reads were filtered and trimmed prior to *de novo* genome assembly. Following two-fold *in silico* serotype confirmation for inclusion in downstream analyses, the plasmid content, virulence factor genes, and antimicrobial resistance determinants of all strains were characterized. Core SNPs were used to construct a maximum-likelihood phylogeny.

Results: 2,079 of 2,180 strains passed all sequence quality, assembly quality, and -*in silico* serotyping inclusion criteria. Among these strains, resistance determinants for 17 antimicrobial classes were identified. Genes for rifampin, MLS, and colistin resistance were found in exclusively 3 S. Dublin strains isolated from bovine clinical submissions. Between-strain AMR gene diversity varied significantly by host ($p < 0.001$) and, within bovine-associated strains, by collection period ($p < 0.001$). Regardless of host, virulence genes associated with effector delivery, adherence, metabolic factors, motility, and stress survival were identified. These consisted of primarily chromosomally encoded type III secretion system components. Multidrug resistance plasmid IncA/C2, as well as IncX1- and IncFII- type plasmids were present in over 90% of strains.

Conclusions: These findings indicate a substantial degree of genomic similarity between bovine and human associated isolates as it relates virulence potential and plasmid profiles. While antimicrobial resistance gene diversity does vary significantly by host association, the genetic traits examined here are largely conserved over time and geography.

Financial Support: Funding support for this research was provided by the USDA APHIS NBAF Scientist Training Program Fellowship (AP23VSD&B000C001).

Notes:

P144 - Comparative genomics of *Listeria monocytogenes* isolated from small specialty crop farms in northeast Ohio

Shonna Smith[1], Menuka Bhandari[1], Mike Kauffman[1], Jelmer Poelstra[1], Greg Habing[1], Gireesh Rajashekara[1]

[1]The Ohio State University. smith.15648@osu.edu

Session: One Health / Public Health, 2023-01-22, 6:00 - 8:00

Objective: *Listeria monocytogenes* is an invasive, zoonotic, foodborne pathogen that causes human listeriosis. *L. monocytogenes* plays a significant risk to food safety due to its ability to grow and multiply at refrigeration temperatures (Osek et al., 2022). According to the CDC, *L. monocytogenes* is the third leading cause of death associated with foodborne illness in the USA (Smart, 2023). Small specialty crop farms (SSCFs) are an important and growing sector for crop production in the USA. Produce from SSCFs is considered fresh, local, and safe for consumption.Therefore, many people are relying on SSCFs produce (Debalis et al., 2023). Characterized by a mixture of animal and vegetable farming practices, these farms pose concerns related to foodborne pathogens, particularly *Listeria monocytogenes*, which are found commonly in livestock feces (Sharma and Reynnells, 2016). There is little information on the prevalence and genetic landscape of the *L. monocytogenes* from SSCFs. Bridging this knowledge gap would help understand potential public health risks associated with the *L. monocytogenes* coming from these farms.

Methods: The goal of our study is to understand the prevalence and genomic diversity among the isolates in the SSCF niche by characterizing the pangenome, serotypes, antimicrobial resistance genes, and virulence genes. We hope our results may facilitate the implementation of effective management practices and improve food safety and mitigate public health outbreaks. We collected, tested, and processed dairy and poultry manure, soil, water, and produce samples (n = 1848) for *L. monocytogenes* from 15 SSCF between 2016 to 2020. Samples were analyzed through Whole Genome Sequencing (WGS).

Results: The overall prevalence of *L. monocytogenes* on SSCF was 7.14% (132/1848 samples). From these 132 positive samples, 347 isolates were obtained and were subjected to whole genome sequencing (WGS). Based on the sample type, the prevalence of *L. monocytogenes* was 40.2% in dairy manure, 21.2% in soil, 23.5% in produce, 0.6% in water, and 0.05% in poultry manure. Pangenome analysis detected the presence of 2,036 core genes. In silico serotyping of *L. monocytogenes* detected the presence of four serogroups including 1/2a, 1/2b, 1/2c, and 4b, serotypes implicated in human infections. Analysis of antimicrobial resistance (AMR) genes identified the presence of 9 genes: *abc-f, fox(x), vanZ, mprF, vga(G), ampC, Group_142, TetR,* and *Ide*. In addition, an arsenic-resistant cassette was detected in one of the isolates from a produce sample. Analysis of virulence genes revealed 68 virulence genes with six common virulence genes present: *prfA*(transcriptional regulator), *plcA, hly, mpl, plcB, actA.* These genes were present in all 347 (100%) of our genomes except for the *actA* gene which was present in 243 (70.3 %) of our genomes.

Conclusions: Overall, our results showed that SSCF harbored *L. monocytogenes* serotypes that are of public health significance and carried critical AMR and virulence genes. Therefore, regular monitoring is required to prevent the transmission of *L. monocytogenes* from SSCF to humans. Thus, our research will enhance food safety, protect public health, sustain the economic viability of SSCFs, and contribute to environmental quality by promoting prudent farming practices.

Notes:

P145 - Virtual reality technology as a training tool on personal protective clothing and equipment use for dairy farms

Elizabeth Kim[1], Pedro Boscan[1], Isaac Fraire[1], Morgan Jones[1], Cyane Tornatzky[2], Aracely Diaz[1], Marie Vans[3], Wenjing Jiang[4], Sangeeta Rao[1]

[1]Department of Clinical Sciences, Colorado State University, [2]College of Liberal Arts, Colorado State University, [3]Department of Systems Engineering, Colorado State University, [4]College of Veterinary Medicine Information Technology, Colorado State University. elizabeth.kim@colostate.edu

Session: One Health / Public Health, 2023-01-22, 6:00 - 8:00

Objective: Dairy workers are integral to global food security. However, despite their importance, their working conditions create hazards such as physical injuries, exposure to chemicals, and infectious disease. Training to prevent exposure to these risks includes the use of appropriate personal protective clothing and equipment (PPE) while working on the farm. Although there are various methods of training, the method where individuals best process and utilize what they learned into practice is through an engaging and interactive learning experience. Therefore, this study aims to utilize interactive virtual reality (VR) as a training tool to educate livestock personnel on the use of appropriate PPE. This tool surrounds the One Health concept incorporating two important focuses - reducing the risk of occupational illness and supporting a safe food supply.

Methods: To develop the VR training tool, a concept process algorithm was developed. The VR training followed the algorithm by placing participants in a locker room within a dairy farm. Participants have the option to choose from a variety of clothing and protective equipment to wear. They would need to choose the appropriate items in their correct sequence to progress further into the farm. To test the likeability and applicability of the VR training tool, a questionnaire was administered after the training was completed. The questionnaires were in English and Spanish to give participants the option to choose either language.

Results: Preliminary data from five dairy farm personnel showed that all five participants responded positively to enjoying the VR training game with an average ± SD of 9.8 ± 0.45, from a 1-10 scale with 1 being "Not at all enjoyed" to 10 being "Strongly enjoyed". Assessment on how helpful the tool would be to train new workers received a score of 9.8 ± 0.45 on a 1-10 scale with 1 being "Not helpful" to 10 being "Very helpful". Participants were asked to compare the method they used for their original training, which was hands-on practice, to the VR method on a 1-10 scale with 1 being "Worse" to 10 being "Better". This question received a score of 7.5 ± 2.52. Comments from participants included having equipment descriptions within the VR training game in Spanish and the inclusion of topics such as appropriate milking practices and vaccination techniques.

Conclusions: Preliminary data showed favorable effectiveness of VR technology in training dairy workers. Further data is necessary to learn and advance VR training in implementing more effective biosecurity and biosafety practices within the farm environment. Doing so will help reduce the risk of exposure on farm personnel and farm animals to hazards.

Financial Support: This research was supported by High Plains Intermountain Center for Agricultural Health and Safety (HICAHS). Student support was provided by the National Institute of Food & Agriculture at the USDA through the Veterinary Summer Scholars Program (VSSP) at Colorado State University.

Notes:

P146 - Examining air component dynamics in dairy farm milking parlors and cross-ventilated barns

Afrin Jannat[1], Diego Manriquez[1]

[1]Department of Animal Sciences, Colorado State University. afrin.jannat@colostate.edu

Session: One Health / Public Health, 2023-01-22, 6:00 - 8:00

Objective: Dairy farming demands daily human-cow interactions within indoor spaces such as milking parlors and barn areas. These environments expose both humans and dairy cows to potentially harmful air components originated from milking procedures and inherent biological processes of cows. Dairy operations have been identified as sources of airborne microbes, methane, nitrous oxide, ammonia, and particulate matter. These air components might impact both worker well-being and the health, productivity, and sustainability of the dairy industry. Considering these challenges, this research is designed to comprehensively evaluate indoor air quality (IAQ) in dairy farms. The objective was to describe air component dynamics in the milking parlor and cross-ventilated barn in a commercial dairy farm.

Methods: Air component measurements were performed at a commercial dairy farm located in Northern Colorado, milking around 6,000 Holstein cows. Two automated sensors (IAQbio system, IEQMax, CO, USA) were installed at an approximate height of 4 meters from the ground in the barn area, whereas the sensor in the milking parlor was in the center of the rotary milking machine. These sensors provided real-time indoor air component measurements every one-minute interval. Data collection began on August 23rd, 2023, and it finished on September 4th, 2023. We collected a total of 21,541 air component measurements. Descriptive statistics of the overall concentration within the observation period were calculated. In addition, we compared the mean concentrations of PM2.5, CH_4, and CO_2 between the two study areas using the multiple comparisons Tukey test.

Results: In the milking parlor, PM2.5 averaged 12.70 ppm (SD: 9.76), with a range from 1.52 to 71.2 ppm. The CO_2 levels in this area averaged 518.52 ppm (SD: 32.36), ranging from 372 to 653 ppm, while CH_4 averaged 5.60 ppm (SD: 7.87), with a range from 1 to 88.1 ppm. Within the cross-ventilated barn, PM2.5 averaged 3.45 ppm (SD: 1.84), with a range from 1.52 to 26.6 ppm. The CO_2 levels averaged 582.36 ppm (SD: 50.24), with values ranging from 362 to 928 ppm. The CH_4 averaged 12.41 ppm (SD: 5.09), ranging from 2.6 to 136 ppm. We observed that the milking parlor had higher volumes of PM2.5 compared with cross-ventilated barn ($P<0.0001$). In addition, we determined that the milking parlor had higher CO2 concentration than the cross-ventilated barn ($P<0.03$). Finally, we did not detect significant differences of CH4 between milking parlor and cross-ventilated barn.

Conclusions: We identified patterns of the overall production of PM2.5, CH_4, and CO_2 within the milking parlor and cross-ventilated barn areas, and significant differences among the study areas. These values serve as a foundational step for ongoing investigations into air quality within dairy facilities. Understanding these dynamics will allow us to develop mitigation strategies for healthier, more productive, and sustainable dairy operations.

Notes:

P147 - A systematic review of mitigation strategies to reduce veterinary teaching hospital zoonotic disease transmission

Nichelle V. Jasper[1], Brandy A. Burgess[1], Emily Feyes[2], Tamara Gull[3], Gillian Perkins[4], Jason Stull[5], J. Trenton McClure[5]

[1]Dept. of Population Health, University of Georgia, [2]Dept. of Veterinary Preventive Medicine, The Ohio State University, [3]Veterinary Medical Diagnostic Laboratory, University of Missouri, [4]Dept. of Clinical Sciences, Cornell University, [5]Dept. of Health Management, University of Prince Edward Island. Nichelle.Jasper@uga.edu

Session: One Health / Public Health, 2023-01-22, 6:00 - 8:00

Objective: To provide veterinarians with evidence-based guidelines on the prevention of zoonotic diseases.

Methods: A systematic review of published articles was conducted using the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) method, assessing mitigation strategies investigated to reduce zoonotic disease transmission in veterinary educational settings. A search string was developed in collaboration with a science librarian and searches were performed in four databases (Agricola, CAB Abstracts and CAB Archive, Global Health, and Web of Science). Data were compiled, managed, and stored using Microsoft Excel®, Endnote®, and Covidence® software. Descriptive statistics were conducted on the data.

Results: Twenty-four studies met the review's criteria reporting different mitigation strategies. The study designs used varied with various levels of evidence. The study designs included observational cohort (n=2), experimental (n=12), qualitative-cross-sectional (n=1), and observational cross-sectional (n=9). There were also varied study settings of academic (n=18), Private practices (n=5), and one research facility.

Conclusions: These results suggest that while veterinarians attempt to prevent zoonotic disease transmission, there is a lack of robust evidence to determine which mitigation strategies are effective. The reporting from the studies conducted was inconsistent with high levels of heterogeneity highlighting the absence of a standard. Therefore, no meta-analysis was conducted. This study highlights the need for evidence-based, best practices infection prevention strategies in veterinary teaching hospitals and within the profession in general.

Notes:

P148 - Isolation and characterization of *Pseudomonas aeruginosa* bacteriophage to treat canine skin infections

S. Ojha[1], C. Pye[2]

[1]Dept. of Pathology and Microbiology, University of Prince Edward Island, [2]Companion Animal Medicine, University of Prince Edward Island. shojha@upei.ca

Session: One Health / Public Health, 2023-01-22, 6:00 - 8:00

Objective: Bacterial skin infections are highly presented in small animal practice; among which, *Pseudomonas aeruginosa* is a highly represented pathogen associated with skin infections and otitis in canine species. Treatment challenges are significant for the biofilm producer and inherently drug resistant, *P. aeruginosa* infections. The present study is aimed at the isolation and characterization of bacteriophages against *Pseudomonas aeruginosa* with a primary goal of developing a topical bacteriophage therapy to tackle antibiotic resistance.

Methods: In this study, three lytic anti-*Pseudomonas* bacteriophages were isolated from the sewage wastewater using canine clinical isolates of *P. aeruginosa* as the propagating hosts. Briefly, untreated wastewater was filtered through 0.45 µM membrane filters. The filtrate was incubated with *P. aeruginosa* as the phage propagating host. Next day, the phage enriched culture filtrate was spotted in double agar overlay assays on the lawn culture of *P. aeruginosa*. Individual lytic zones (plaques) were harvested in salt-magnesium phage buffer. The dislodged filtered phages from plaques were subjected to 5x amplifications in similar spot assays to obtain a high titre of 10^8 PFUs (plaque forming units)/ml. The characterization of one virulent (lytic) phage is presented in this study. In the growth kinetics of phage versus *P. aeruginosa*, the trypticase soy broth was infected at a multiplicity of infection of 1. The OD at 600 nm wavelength, colony forming units (CFUs) and PFUs of the incubating culture were examined over 4.5 hours period. The lytic activity of the phage was tested on dermatitis and otitis clinical isolates of *P. aeruginosa.*

Results: The biological activity of the phage generated clear, approximately 1 mm lytic zones. In vitro growth kinetics of *P. aeruginosa* versus phage exhibited pronounced clearing at 4.5 h. While a high turbidity was observed at 1 h, the lowest OD of 0.328 at 4.5 h exhibited the lowest CFUs and the highest yield of PFUs. The lytic efficiency of phage was observed on 75% of the clinical isolates of *P. aeruginosa* tested so far. The phage was effective at inhibiting enrofloxacin and amikacin resistant *P. aeruginosa* isolates.

Conclusions: In this study, three anti-*P. aeruginosa* bacteriophages were isolated with a primary goal of formulating therapeutic phage cocktail to target *P. aeruginosa* infection in canine patients. The analyses of one of the phages demonstrated high lytic efficiency against the clinical isolates of the pathogen. At present, the sequencing of the bacteriophage genome is underway, which will provide further information on its taxonomic placement and genetic characteristics.

Financial Support: Zoetis Innovation Fund, AVC start-up and AVC Companion Animal Trust Fund (CATF)

Notes:

P149 - Agricultural animal ownership in Michigan's most urban counties: an exploratory assessment

Courtney Marsh[1], A.E. Bauer[2]

[1]University of Detroit Mercy, [2]Department of Biology, University of Detroit Mercy. marshca@udmercy.edu

Session: One Health / Public Health, 2023-01-22, 6:00 - 8:00

Objective: Zoonotic diseases are caused by the spread of pathogens between vertebrate animals and humans. Zoonotic diseases pose significant worldwide public health concerns because of the close connection between humans and animals. To help plan zoonotic disease prevention strategies in urban environments, researchers must understand the relationship between the presence of agricultural animal species and the human population in these environments. This research study was conducted to determine the prevalence and trends in ownership of certain agricultural animal species within the two most populated counties of Michigan: Oakland County and Wayne County.

Methods: The data used for this study came from the National Agricultural Statistics Service (NASS) Census of Agriculture from the years 2002, 2007, 2012, and 2017. The proportion of premises in each county reporting the presence of each of 10 species or species groups was calculated for each year. Statistically significant changes in the proportion of ownership of each species between 2002 and 2017 for each county, and between the two counties in 2017 were determined by use of Pearson's chi-square test in SPSS statistical software.

Results: There were statistically significant increases in the proportions of Oakland County premises that reported ownership of poultry ($p<0.001$), camelids ($p=0.035$), and hogs ($p=0.014$) between 2002 and 2017. Although there were no statistically significant changes in the proportion of premises owning cattle between 2002 and 2017, 10% of the premises in Oakland County reported ownership of cattle or calves. In Wayne County, there were statistically significant increases in the proportion of premises reporting ownership of sheep ($p<0.001$), goats (<0.001), and poultry ($p=0.005$) between the years 2002 and 2017. Although there were no statistically significant changes in the proportion of premises owning cattle between 2002 and 2017, 11.7% of the premises in Wayne County reported ownership of cattle or calves.

Conclusions: While Oakland and Wayne counties have the densest human populations in the state of the Michigan, agricultural animal ownership is not uncommon in these counties. People living Oakland county are potentially at risk of exposure to zoonotic diseases associated with poultry, cattle, camelids and hogs. People living in Wayne county are potentially at risk of exposure to zoonotic diseases associated with poultry, cattle, and small ruminants. In the future, researchers should investigate the differences in zoonotic risks of animal agriculture in these counties, as well as the role that human demographics such as race, income, and education may play in access to information about zoonotic diseases in urban premises.

Financial Support: This work was supported by a Faculty Research Award sponsored by the McNichols Faculty Assembly of the University of Detroit Mercy.

Notes:

P150 - Comparing ownership of agricultural animals in Macomb county and Genesee county, Michigan

Jannath Aurfan[1], A. E. Bauer[2]

[1]University of Detroit Mercy, [2]Biology Department, University of Detroit Mercy. aurfanja@udmercy.edu

Session: One Health / Public Health, 2023-01-22, 6:00 - 8:00

Objective: Urban agriculture is a vital movement worldwide. There is an increase in cultivation of both plant and animal species within or adjacent to cities. Different animal species can impact the urban lifestyle in multiple ways - as sources of food, products for sale, or fertilizer. The integration of agricultural species into the urban ecosystem creates the risk of introducing zoonotic diseases, diseases transmitted between vertebrate species and humans. The focus of this research is to examine trends in agricultural animal ownership in Macomb and Genesee counties in Michigan, to better understand which zoonotic diseases have the potential for affecting the people living in these counties.

Methods: Data on agricultural animal ownership came from the National Agricultural Statistics Service (NASS) Census of Agriculture. The data gathered focused on the 2002, 2007, 2012, and 2017 censuses. The proportion of premises in each county reporting the presence of each of 12 species or species groups was calculated for each census year and compared between 2002 and 2017. Statistically significant changes in the proportion of ownership between 2002 and 2017 for each county, and between the two counties in 2017 were determined by use of Pearson's chi-square test using SPSS statistical software.

Results: In Macomb County, the only statistically significant increase in the proportion of farms reporting ownership of agricultural species between 2002 and 2017 was for camelids ($p<0.001$) from 0.9% to 1.5%. However, in 2017 10.9% of participating farms in Macomb county reported owning poultry, 14.6% reported owning cattle, and 24.5% reported owning equids (horses, donkeys, or mules). In Genesee county, there was a statistically significant increase in the proportion of farms reporting ownership of poultry ($p=0.007$) from 12.7% to 17.2%. As with Macomb county, although there were not statistically significant increases when compared to 2002, 21.9% of premises in Genesee county reported owning cattle in 2017 and 33.0% reported owning equids.

Conclusions: While there were few statistically significant increases in the species investigated in this study between 2002 and 2017 in either county, this does not mean that zoonotic disease risks from these species should be considered negligible. Future research on zoonotic disease risks to the human populations of Macomb and Genesee counties should focus on diseases associated with poultry, cattle and equids. In future, survey data taken at the local level will help to clarify if the county-level trends are reflected at a finer geographical scale within the counties of interest.

Financial Support: This work was supported by a Faculty Research Award from the McNichols Faculty Assembly at the University of Detroit Mercy.

Notes:

P151 - Testing for heavy metals in drinking water collected from the Dog Aging Project

Courtney Sexton[1], Sam Rodgers[1], Janice O'Brien[1], Amber Keyser[2], Mandy Kauffman[2], Matthew Dunbar[2], Audrey Ruple[1]

[1]Virginia-Maryland College of Veterinary Medicine, Virginia Tech, [2]University of Washington. sextonc@vt.edu

Session: One Health / Public Health, 2023-01-22, 6:00 - 8:00

Objective: Access to potable water is essential for animals' survival, yet drinking water source, quality, and availability all vary greatly across species and populations. For most companion animals, access to drinking water is provided by the people with whom they live. And pet dogs and cats typically drink from the same source as humans in the household. Because non-municipal water supplies are not subject to regular, standardized testing, little is known about the quality of these supplies or potential rates of contamination. Heavy metals are commonly found in groundwater, which can affect the quality of drinking water, specifically if from a well. In this study, we analyzed the quality of drinking water for dogs participating in the Dog Aging Project (DAP), a large cohort study, who lived in homes not served by a municipal water supply. Our aim was to determine whether or not the composition of dogs' drinking water is related to long-term health and wellness outcomes.

Methods: We selected a subset of DAP participants who had previously reported having a non-municipal water supply. In order to capture diverse and localized environmental factors which may affect drinking water, 200 owners of DAP dogs located in one of 10 selected states were invited to participate. Dog owners filled out a pre-sampling survey with any known information about their dog's drinking water. They were sent kits via mail including collection vials and instructions for collecting water samples from their dog's primary water source, and returned the kits via pre-paid mailing labels. We used the EPA-certified Inductively Coupled Plasma Optical Emission Spectrometry (ICP) test to analyze the samples and determine the presence and amount of 28 different elements, including eight heavy metals with EPA-designated maximum contamination levels (MCL), in the water samples.

Results: A total of 180 of the 200 (90.0%) sample kits sent were returned by dog owners. There was wide variability in the types and amounts of metals present in dogs' drinking water. There were 13 instances where values of arsenic, lead, or copper were above the MCL. Additional analyses are in-progress.

Conclusions: Drinking water toxicity from heavy metals and other contaminants can lead to both acute and chronic health conditions including organ failure in dogs. Pet dogs, who have little awareness of the quality or control over their drinking water sources, may be especially susceptible to such risks. And, because previous and emerging research supports dogs' role as sentinels of human health and wellbeing, heavy metal toxicity should also be regarded as a concern for humans living in the same households as the dogs and also drinking contaminated water.

Financial Support: NIH

Notes:

P152 - Impact of micro-/nanoplastics on natural transformation efficiency of *Campylobacter jejuni*

Liu Cao[1, 2], Ximin Zeng[2], Jun Lin[2]

[1]Gannon University, [2]University of Tennessee. cao003@gannon.edu

Session: One Health / Public Health, 2023-01-22, 6:00 - 8:00

Objective: *Campylobacter jejuni* is a prevalent and significant zoonotic bacterial pathogen in food animal production systems, including poultry, swine, and cattle. *C. jejuni* is naturally competent, which allows this organism to take up DNA fragments and acquire genes to better adapt to diverse environments. Recent studies suggest that microplastics and nanoplastics (MNPs), the ubiquitous pollutants, could interact with microbial communities and facilitate horizontal gene transfer. In this study, we aim to explore the impact of MNPs on the natural transformation efficiency of *C. jejuni*.

Methods: Biphasic method was used to determine the natural transformation frequencies of the *C. jejuni* NCTC 11168 that was exposed to the MNPs with different sizes (25 nm - 125 µm), concentrations (10, 20, and 50 µg/mL), and types (polystyrene, polyethylene, and polyvinyl chloride). The genomic DNA isolated from the kanamycin-resistant and erythromycin-resistant derivatives of *C. jejuni* NCTC 11168 were used as donor DNA for standard natural transformation assay.

Results: Presence of polystyrene (50 µg/mL) with size of 25 nm, 1 µm, and 100 µm significantly ($P < 0.05$) increased natural transformation frequencies of kanamycin-resistant gene for 1.6, 2, and 11 folds, respectively. However, no significant difference was observed in the presence of lower concentration of polystyrene, or the same high concentration of polyethylene and polyvinyl chloride. Regarding erythromycin resistance marker that is mediated through a point mutation, both polystyrene and polyethylene (50 µg/mL) significantly ($P < 0.05$) increased transformation frequency for 3 and 6 folds, respectively; however, polyvinyl chloride did not affect the natural transformation frequency ($P > 0.05$).

Conclusions: These findings suggested that MNPs may enhance natural transformation efficiency of *C. jejuni* in a dose- and size-dependent manner.

Notes:

P153 - Imported and traveling dogs as carriers of *Dirofilaria* spp. from Central and South America

Theresa Quintana[1], Grace Schieferecke[1], Breck Aguinaga [1], Jessica Mitchell[1, 2], Janine Seetahal[1, 2], Jeba Jesudoss Chelladurai[1]

[1]College of Veterinary Medicine, Kansas State University, [2]Veterinary Diagnostic Laboratory, Kansas State University. taq@ksu.edu

Session: One Health / Public Health, 2023-01-22, 6:00 - 8:00

Objective: An increased risk of vector-borne disease (VBD) spread exists among dogs, as rising temperatures allow disease-carrying vectors, like mosquitoes, to expand their geographic range and increase their reproductive rates. Importing infected dogs from Central and South American countries further enhances threats of VBD transmission throughout U.S. dog populations. Furthermore, two species, *Dirofilaria immitis* and *Dirofilaria repens*, have been characterized as the principal agents of human dirofilariosis. Current U.S. importation laws require only proof of rabies vaccination or the detection of rabies antibodies in imported dogs, creating potential gaps for VBDs to enter and spread to vulnerable populations.

Methods: Canine serum samples originating from South America were obtained from the rabies division of the Kansas State Veterinary Diagnostic Laboratory (KSU VDL). Samples were sorted by country and pooled into groups. Pooled sizes were optimized based on recorded prevalence in each country using epidemiology software (EPITOOLS). Pooled samples were tested for *D. immitis* and *D. repens* using DiroCheck (Zoetis), with and without heat treatment. Spectrophotometry was used to quantify the ELISA reactions colorimetrically.

Results: This study is ongoing. We present data from Central and South American countries in samples collected from September 2022 to October 2023. We have tested 118 pools (~1,500 serum samples) from submission sites throughout Central and South America that were banked at the KSU VDL after rabies antibody testing. Several positive heat-treated pools were detected using theD. immitis ELISA, despite being negative with unheated sera.

Conclusions: Unlike *D. immitis*, *D. repens* is not endemic in the U.S. Despite increased prevalence in endemic countries and expanding distribution into new territories, the U.S. does not screen imported dogs for any *Dirofilaria spp.*

Financial Support: This study was funded by start-up funds provided to JJC by the Kansas State University College of Veterinary Medicine.

Notes:

P154 - Using a passive sampling technique to quantify environmental chemical exposures in dogs enrolled in the Dog Aging Project

Rylee Matheson[1], Janice O'Brien[1], Courtney Sexton[1], Audrey Ruple[1]

[1]Virginia-Maryland College of Veterinary Medicine. rlhaga18@vt.edu

Session: One Health / Public Health, 2023-01-22, 6:00 - 8:00

Objective: The Dog Aging Project (DAP) is a long-term, longitudinal study of over 45,000 dogs located throughout the United States. Dogs provide valuable insights as sentinels for human disease related to environmental exposures because dogs share human exposures and have shorter lifespans. The objective of this pilot study was to determine if passive samplers could be deployed to DAP participants to attach to their dog's collars to quantitatively assess chemical exposures within the shared home environment.

Methods: Precleaned passive silicone monitoring devices were worn by DAP dog study participants (N=15) on their collar for 5 full days. Once removed, they were placed in a clean piece of aluminum foil and sealed in an airtight bag. All samplers and field blanks (N=3) were processed and analyzed for a suite of target compounds using gas chromatography-mass spectrometry. Field blanks were used to determine the method detection limits (MDLs) of each analyte and median values were calculated for all 120 specific compounds detected. Owner reported data regarding the household environment was summarized and descriptively analyzed.

Results: All sampling devices sent to dog owners were returned to the laboratory for analysis. Analytes belonging to these chemical classes were detected using silicone dog tags placed on dog's collars: brominated flame retardants, organophosphates, PAHs, polychlorinated biphenyls, pesticides, phthalates, and personal care products. Differences in both the types and amounts of analytes detected varied significantly between participants.

Conclusions: The data in this study indicate that silicone dog tags are an effective means to measure chemical exposure in pet dog's household. This supports the value of using silicone tags with dogs to investigate health impacts on humans from shared environmental exposures.

Financial Support: The Dog Aging Project is supported by U19AG057377 from the National Institute on Aging, a part of the National Institutes of Health, and by additional grants and private donations.

Notes:

P155 - Monitoring Coccidiomycosis and *Giardia* trends among pet dogs to inform public health

Janice O'Brien[1], Courtney Sexton[1], Audrey Ruple[1]

[1]Virginia Maryland College of Veterinary Medicine. janiceobrien@vt.edu

Session: One Health / Public Health, **2023-01-22, 6:00 - 8:00**

Objective: Dogs have previously been used as sentinels of human disease, for both toxic exposures and vector-borne diseases, and are a well-established model for human cancer outcomes. Dogs may be important for investigating shared exposures for infectious diseases as well. Here we examine use of dogs as sentinels for two such diseases: coccidiomycosis, an environmental fungal organism common to the southwestern United States, and *Giardia*, an agent that is not commonly directly shared between humans and dogs but which requires similar environmental conditions for transmission.

Methods: Pet insurance claims from a major North American insurance provider were sorted for Coccidiomycosis and Giardia disease claim codes from 2008-2020. These claims were normalized by region (state or province), year, and seasonally. The seasonal trends and yearly rates were compared to human-level data from the Centers for Disease Control and Prevention (CDC) for the same timeframe. Annual rates among dogs and humans were graphed relative to the initial starting rate for the species, so all initial rates started at 1 for 2008, and every year after was recorded as a change relative to the first year's rate.

Results: The geographic distributions of *Giardia* and Coccidiomycosis in both the human and canine populations are very similar. The incidence rate of both diseases is higher in dogs than in people. Giardia's seasonality in dogs is recorded earlier in the year and is more spread out than for humans. However, outbreaks within the canine population in February/March may forecast outbreaks within the human population occurring in July/August, which could make dogs a good sentinel species for predicting increased rates of infections in humans before they occur.

Conclusions: Using pet insurance data, we can monitor trends for environmental diseases that dogs and people both share. This initial investigative work warrants further exploration into both these diseases - to both confirm that seasonal temporality among dogs precedes human disease and that pet insurance data can be useful in forecasting infections in human populations and to examine more shared environmental exposure diseases among pet dogs and people.

Notes:

P156 - Detection of multiple enteric bacterial pathogens in sheep and goats in a live animal show in Mid Atlantic US

Eunice Ndegwa[1], Ivan Odur[2], Amy Pruden[2], Patrick Pithua[2], Chyer Kim[1], Dahlia O'Brien[1]

[1]Virginia State University, [2]Virginia Tech. endegwa@vsu.edu

Session: One Health / Public Health, 2023-01-22, 6:00 - 8:00

Objective: Livestock shows are often cited as sources of pathogens of public health importance but may also serve as avenues for inter-animal and inter-farm transmission of diseases of economic importance. In the US, studies targeting risks posed by small ruminant live animal shows are scarce despite their high appeal to the general public and producers. In this study we evaluated the animal and farm prevalence of *E. coli* shigatoxin (STEC) and intimin protein genes, *Campylobacter*, *Salmonella* and *Mycobacterium avium subsp paratuberculosis (MAP) spp* in sheep and goats participating in a live animal show in Mid Atlantic US using molecular methods.

Methods: Fecal samples were collected from animals from different farms presented at the annual sheep and goat show and transported in ice to the laboratory. Forty-four animals were sampled from 16 different farms. DNA was extracted from fecal samples using commercial kits and from a tryptic soy broth (TSB) overnight enrichment using a simple boiling method. The DNA was consequently used in PCR for pathogen detection applying pathogen specific primers. Fecal DNA was used for singleplex PCR detection of *Campylobacter, Salmonella*, and MAP while TSB DNA was used for *E.coli* virulence gene detection using a multiplex PCR.

Results: All animals appeared healthy at the time of sampling. Potentially pathogenic strains of bacteria were detected by molecular methods in both sheep and goats at the show. Overall the animal and farm prevalence of STEC was each 20%. Of these, five animals (11%) harbored the shiga toxin (*stx*) 1 gene, nine animals (20%) harbored the *stx2* gene, and five animals (11%) harbored both *stx1* and *stx2* genes. The intimin gene was detected in eight animals (18%) from six different farms (38%). In six of these animals the gene was detected alongside shiga toxin genes in the same animal. *Campylobacter* was detected in 45% of the animals from thirteen of the sixteen farms (81%) while 11% of animals were positive for MAP from five different farms (31%). No *Salmonella* positive animals were detected in this study.

Conclusions: The pathogens evaluated in this study are of economic and public health importance in small ruminant agrosystems. These results indicate presence of potentially pathogenic enteric bacteria that pose risks to the public and other animals at the livestock show. The findings underscore the importance of continued education of the general public and livestock producers participating in these events including hygiene and biosecurity protocols to prevent spread of the pathogens.

Notes:

P157 - The future of SARS-CoV-2 in ecological communities - project outline

Andrew Kramer[1], Barbara Han[2], Payel Das[3], Michael Letko[4]

[1]University of South Florida, [2]Cary Institute of Ecosystem Studies, [3]IBM Research, [4]Washington State University. amkramer@usf.edu

Session: One Health / Public Health, 2023-01-22, 6:00 - 8:00

Objective: It now appears certain that SARS-CoV-2 will persist in animals as long as it is prevalent in humans. The implications of multiple mammal species contracting SARS-CoV-2 are unclear, but mounting evidence suggests that some animals may experience fatal disease, and that animal species will serve both as stable reservoirs of viral variants that were previously dominant in humans, and as new reservoirs for new variants. Many of these new reservoirs are common wildlife species, such as white-tailed deer, deer mice and red fox, which occur in relatively high abundances in some systems and highlight pathways of secondary spillover transmission to humans living in close proximity. To understand the future of SARS-CoV-2 in mammal communities, this recently funded project will advance our currently limited capacity to predict animal host species, novel zoonotic variants, and the potential interactions among multiple species that comprise mammal communities.

Methods: This project will integrate leading edge advances in artificial intelligence, virology, and ecological theory in four specific aims: Aim 1 makes predictions about future zoonotic variants of SARS-CoV-2 and their evolutionary trajectories by advancing methods in controlled generative AI. Aim 2 predicts which particular mammalian species are suitable hosts for existing and future zoonotic variants, extending predictions even to species for which host cell receptor (ACE2) sequences are still unknown. Aim 3 conducts safe and efficient empirical validations of predictions made in Aims 1 and 2 by testing cell entry using a pseudotyped virus platform for a subset of zoonotic variants and animal cell receptors. Aim 4 advances theory for disease transmission in wildlife communities by building a framework that accounts for species distributions, their ecological interactions (such as predation and competition), and fine-scale contact patterns that influence transmission among individual animals.

Results: Together these methods should enable spatially explicit predictions about SARS-CoV-2 dynamics in mammal communities. This model will be applied to the northeastern forest community using 30 years of data for multiple mammal species and ongoing SARS-CoV-2 surveillance. Collectively, the tools developed in this project will lead to the rapid prediction and validation of animal hosts for current and future variants of SARS-CoV-2.

Conclusions: Given the persistent and growing risk of zoonotic emergence from animal hosts, the innovations developed in this project are designed to advance risk assessment capacity for wildlife, domestic animals, and humans by helping to predict and target particular viruses, their variants, and their multiple host species, while also integrating the dynamic ecological context in which species interactions and transmission culminate to inform zoonotic risk in complex systems.

Financial Support: Funding was provided by the joint NIFA-NSF-NIH Ecology and Evolution of Infectious Disease award 2023-70432-40381.

Notes:

P158 - SARS-CoV-2 WA1 induces distinct transcriptome profiles in human and deer primary respiratory epithelial cells

Luis Gimenez-Lirola[1], Laura Miller[2], Bryan Bellaire[3], Rachel Ruden[1], Rahul Nelli[1], Kruttika Phadke[3], Yongming Sang[4], Kaitlyn Sarlo Davila [5]

[1]Dept. Veterinary Diagnostic and Production Animal Medicine, Iowa State University, [2]Dept. Diagnostic Medicine/Pathobiology, Kansas State University, [3]Dept. Veterinary Microbiology and Preventive Medicine, Iowa State University, [4]Dept. Agricultural and Environmental Sciences, Tennessee State University, [5]National Animal Disease Center, USDA-Agricultural Research Service. rknelli@iastate.edu

Session: One Health / Public Health, **2023-01-22, 6:00 - 8:00**

Objective: The potential infectivity of SARS-CoV-2 in animals raises a public health and economic concern, particularly the high susceptibility and transmission in white-tailed deer (WTD). The disparity in the disease outcome between humans and WTD is very intriguing, as the latter are often asymptomatic, subclinical carriers of SARS-CoV-2 WA1. No studies have evaluated the innate immune factors responsible for the contrasting SARS-CoV-2-associated disease outcomes in these mammalian species. A comparative transcriptomic analysis in primary respiratory epithelial cells of humans (HRECs) and WTD (Deer-RECs) infected with SARS-CoV-2 WA1 was assessed throughout 48 hours post inoculation (hpi).

Methods: The SARS-CoV-2 WA1 strain (BEI Resources; SARS-Related Coronavirus 2, Isolate USA-WA1/2020) was propagated and titrated in Vero-E6 cells using plaque assays. Primary respiratory epithelial cells from humans (HRECs; ATCC) and white-tailed deer (Deer-RECs) were isolated and cultured in collagen-coated plates with a Dulbecco's Minimum Essential / Ham's F-12 medium with GlutaMAX supplemented with various growth factors. SARS-CoV-2 WA1 infection dose was pre-determined for both HRECs and Deer-RECs, and infections were performed for 6, 24, and 48 hpi. Cell pellets and supernatants were collected in Trizol reagent for virus titration and transcriptomic analysis. In addition, the presence of SARS-CoV-2 N protein in infected Deer-RECs and HRECs was confirmed by immunocytochemistry staining using a specific antibody and a chromogenic detection system. For transcriptomic analysis, total RNA was extracted from the cells, and samples having RNA Integrity Numbers (RIN) ranging from 9.7 to 10 were used to prepare sequencing libraries using a 3' QuantSeq kit. A 100 bp single-end reads were generated using Illumina Hiseq 6000 and aligned to the human and deer reference genomes, respectively. Differential gene expression analysis was performed using DeSeq2 and identified significant genes based on the interaction effect of treatment and time point at a Benjamini-Hochberg False Discovery Rate (FDR) of 0.15. Following gene annotation, pathway analysis was performed using Qiagen Ingenuity Pathway Analysis software.

Results: Both HRECs and Deer-RECs were susceptible to SARS-CoV-2, with significantly ($P < 0.001$) lower virus replication in Deer-RECs. The number of differentially expressed genes (DEG) gradually increased in Deer-RECs but decreased in HRECs throughout the infection. The ingenuity pathway analysis of DEGs further identified that genes commonly altered in HRECs and Deer-RECs during SARS-CoV-2 infection mainly belong to cytokine and chemokine response pathways mediated via IL-17 and NF-κB signaling pathways. SARS-CoV-2 activated *AP-1/JUN* transcription factor in both HRECs and Deer-RECs. Meanwhile, Deer-RECs also showed delayed upregulation of genes related to wound repair, such as *CXCL8, MAP4K4, p21, VEGFA*, and *HBEGF.*

Conclusions: The inhibition of the NF-κB signaling in the Deer-RECs was predicted as early as 6hpi may have contributed to comparatively less proinflammation than in HRECs. The findings from this study could partly explain the lack of clinical signs reported in WTD in response to SARS-CoV-2 WA1 as opposed to the severe clinical outcomes reported in humans.

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Notes:

P159 - Impact of heat stress on the chicken cecal bacterial metagenome and luminal serotonin

Xinglin Jia[1], Gregory Phillips[2], Valentina Caputi[3], Karrie Daniels[1], Mark Lyte[3], Joshua Lyte[3]

[1]Iowa State University, [2]University of Georgia, [3]USDA=Agricultural Research Service. joshua.lyte@usda.gov

Session: Omics / Microbiome, **2023-01-22, 6:00 - 8:00**

Objective: Host stress is a contributing factor to the emergence of avian pathogenic Escherichia coli in the broiler chicken. As a leading cause of avian stress, heat stress has been reported to alter chicken gut microbial diversity. Yet, the impact of heat stress on the cecal bacterial metagenome and luminal concentrations of serotonin has received little attention. As the microbiome and gut serotonin affect *E. coli* pathogenesis, we sought to determine the role of heat stress in driving changes in serotonin and the microbial metagenome.

Methods: Broiler chicks (n=12/group/timepoint) were randomly allocated to control or heat stress (HS) groups. At 4 wks/age, the control group was kept at standard conditions, while the HS group was subjected to a 12-h daily cyclic HS (35°C) for either 1 or 6 consecutive days. Birds were sacrificed at 1 or 6 day(s) following HS. Cecal content was collected for determination of serotonin and microbiome sequencing. Serotonin was quantified using ultra high performance liquid chromatography with electrochemical detection (n=12/group/timepoint). Shallow whole-genome shotgun sequencing was performed using the extracted DNA samples (n=10/group/timepoint) on the Illumina NovaSeq 6000-S4 platform at a depth of ~18million reads/sample. The sequencing data was profiled for metagenomic diversity with bioBakery4 and analyzed using Welch's two sample t-test and linear models. Serotonin concentrations were analyzed using two-way ANOVA with tukey post-hoc test.

Results: Cecal content serotonin concentrations were significantly elevated ($p<0.05$) in the heat stress group compared to control following 6 days of heat stress. Heat stress caused a significant ($p<0.05$) change at the family-level in relative abundances of Lactobacillaceae, Ruminooccaceae, and Bacteriodaceae. Alpha diversity measurements indicated greater ($p<0.05$) diversity and development in the microbiome of control group chickens compared to those of the heat stressed group. Principal component analysis revealed separation between the microbiomes of control and heat stressed chickens. Functional enrichment analysis of differentially abundant microbial gene families is ongoing.

Conclusions: Heat stress impacted the chicken cecal microbiome, with changes observed in enteric serotonin concentrations. Further investigation is warranted into determining how these changes may impact avian pathogenic *Escherichia coli* infection in the broiler chicken gut.

Financial Support: This project was supported by Agriculture and Food Research Initiative Competitive Grant no. 2021-67015-34530 from the USDA National Institute of Food and Agriculture.

Notes:

P160 - A commensal bacterium protects chickens against necrotic enteritis

Isabel Tobin[1], Jing Liu[1], Melanie Whitmore[1], Markus Kim[1], Zijun Zhao[1], Jiaqing Guo[1], Joy Scaria[2], Glenn Zhang[1]

[1]Department of Animal & Food Sciences, Oklahoma State University, [2]Department of Veterinary Pathobiology, Oklahoma State University. isabel.tobin@okstate.edu

Session: Omics / Microbiome, 2023-01-22, 6:00 - 8:00

Objective: Necrotic enteritis (NE), caused by *Clostridium perfringens,* is an economically significant disease, costing the global poultry industry $6 billion annually. With the withdrawal of in-feed antibiotics in the U.S. since 2017, NE has become more prevalent, necessitating more effective mitigation strategies. This study aimed to investigate the potential of microbiome-mediated colonization resistance to NE in chickens.

Methods: Thirteen representative bacterial isolates were selected from a library of cecal bacteria isolated from healthy feral chickens and screened for their ability to inhibit the growth of *C. perfringens* using an *in vitro* competition assay. After 24-h co-culture with each bacterium, the *C. perfringens* counts were enumerated by serially plating on *C. perfringens*-selective medium agar plates. Furthermore, the culture supernatants of these 13 commensal bacteria were screened for their capacity to induce the synthesis of a group of critical effector molecules of innate immunity known as host defense peptides (HDPs) using a novel high-throughput assay. The assay employed a chicken macrophage cell line expressing a luciferase reporter gene driven by a chicken HDP gene promoter. Lastly, a chicken model of NE was utilized to confirm the protective efficacy of a newly-identified commensal bacterium with dual *C. perfringens*-inhibiting and HDP-inducing activities. Chickens were orally administered with the selected bacterium on day 0 and day 1 of age, followed by challenge with *Eimeria maxima* and *C. perfringens* on day 10 and day 14, respectively, to induce NE. Animal behavior and mortalities were observed twice daily. On day 17, all surviving chickens were euthanized and necropsied to score small intestinal lesions.

Results: In the *in vitro* competition assay, six commensal bacteria showed no activity, while seven were capable of inhibiting the growth of *C. perfringens*. The most potent bacterial isolate showed approximately 62% inhibition after co-culturing with *C. perfringens*. Furthermore, the supernatant of the same potent bacterium was the most effective in inducing HDP gene expression in a dose-dependent manner in the high-throughput assay. In the chicken model of NE, 52% of chickens died without intervention, but oral administration of the selected bacterium improved the survival rate to 98%. Moreover, the bacterium significantly alleviated the severity of intestinal lesions.

Conclusions: The results of this study clearly demonstrate the potential of a beneficial commensal bacterium to mitigate NE through direct competition with *C. perfringens* and simultaneous augmentation of host innate immunity.

Financial Support: This research was funded by the USDA National Institute of Food and Agriculture (2022-67016-37208). Also, I.T. is supported by the Graduate Research Training Initiative for Student Enhancement (G-RISE) from the National Institutes of Health to Oklahoma State University (T32 GM140953).

Notes:

P161 - Fecal microbiota transplant (FMT) as a tool for the mitigation of respiratory virus infections in nursery pigs

Yale Deng[1], Pratiksha Khanal[2], Aubrey Deavours[2], Ada G. Cino-Ozuna[3], Megan Niederwerder[2, 4], Noelle Noyes[1]

[1]University of Minnesota, [2]Kansas State University, [3]Oklahoma State University, [4]Swine Health Information Center. nnoyes@umn.edu

Session: Omics / Microbiome, 2023-01-22, 6:00 - 8:00

Objective: Gut microbiome has been shown to have associations with the immune response, chronic and acute infectious diseases, autoimmune diseases and growth performance of both humans and animals. In swine, microbiome studies have found similar effects, including associations with the development of immune system, viral shedding, weight gain, and clinical disease after pathogenic infection. The objective of this study is to investigate gut microbiome modulation as a potentially preventative tool for migrating the effects of the co-infections of two most common viral pathogens in swine production worldwide, namely, porcine reproductive and respiratory syndrome virus (PRRSV) and porcine circovirus type 2 (PCV2), with the ultimate goal to maintain swine health and welfare while lessening the antimicrobials use and economic loss of respiratory diseases on pork producers.

Methods: Weaned piglets (N = 100) were randomly assigned to two treatment groups, with one group orally administered with sterile saline (Ctrl group), and another group with fecal microbiota transplant material (FMT group). Pigs from both groups were orally inoculated with either saline or FMT for 7 consecutive days prior to virus infection with PRRSV and PCV2. Blood samples, feces, and individual body weight data were collected on weekly base over the 6-week post-infection growth trial. All pigs were euthanized either at the day they have severe clinic signs or at 42 day post infection to perform complete necropsies and sampling of tissues including lung, lymph node, spleen, thymus, kidney and intestinal tract.

Results: Analysis of the collected samples is ongoing. Objectives of this study include investigating the effects of FMT on outcome of swine with respiratory disease, including PRRSV and PCV2 replication, morbidity and mortality, average daily weight gain, lung and lymphoid pathology, and humoral immune response. Further, a second objective includes the outcome on gut microbiome and identification of beneficial gut microbes within the FMT material, which are associated with improved health outcomes in growing pigs under a porcine respiratory disease complex model.

Conclusions: As results are pending, conclusions for this study are not yet available. 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.

Financial Support: This project is supported by Agriculture and Food Research Initiative Competitive Grant no. 2020-67015-31808 from the USDA National Institute of Food and Agriculture.

Notes:

P162 - Analysis of the vaginal and fecal microbiota of pregnant elk following *Brucella abortus* challenge

Bienvenido Tibbs-Cortes[1], Faith Rahic-Seggerman[2, 3], Stephan Schmitz-Esser[2, 3], Paola Boggiatto[1], Steven Olsen[1], Ellie Putz[1]

[1]USDA-Agricultural Research Service, [2]Interdepartmental Microbiology Graduate Program, Iowa State University, [3]Department of Animal Science, Iowa State University. paola.boggiatto@usda.gov

Session: Omics / Microbiome, 2023-01-22, 6:00 - 8:00

Objective: The North American elk (*Cervus canadensis*) is a natural reservoir of *Brucella abortus*, the causative agent of brucellosis. In cattle, brucellosis results in abortion and costly reproductive issues, and transmission of *B. abortus* from wild elk to domestic cattle presents a serious threat to herds in the American Northwest. Vaccination of elk against *B. abortus* may therefore help mitigate cattle brucellosis outbreaks in this region; however, the *B. abortus* vaccine strain RB51 is comparably less protective in elk than cattle. As part of ongoing research on RB51 efficacy in elk, we investigated the fecal and vaginal microbiota of unvaccinated and RB51-vaccinated pregnant elk challenged with *B. abortus*.

Methods: Unvaccinated (n=8) and RB51-vaccinated (n=8) pregnant elk were challenged with *B. abortus*. Vaginal swabs and fecal samples were collected two weeks before challenge, at challenge, before calving, post-calving, and at necropsy. Library preparation for amplicon sequencing of the 16S rRNA gene V4 region was performed on extracted DNA, and samples were sequenced using the Illumina MiSeq platform. Reads were cleaned, aligned, and classified at 99% similarity using Mothur, and *in silico* contaminant removal was performed using the Decontam package in R. Alpha diversity metrics and differential taxa abundance were calculated in R using DivNet and Corncob, and time-effects were analyzed in SAS.

Results: Analysis of the microbiome revealed significant differences between the vaginal and fecal microbiota at the community level, and the community structure of the vaginal, but not the fecal, microbiota was temporally dynamic. However, vaccination status did not affect the community structure of either microbiota. At the phylum level, *Firmicutes* were highly abundant in both fecal and vaginal samples. While the vaginal community was primarily composed of *Firmicutes*, the phylum *Bacteroidota* was present at a similar abundance to *Firmicutes* in the fecal microbiota. The abundance of *Firmicutes* in the vaginal samples also varied over the course of the experiment. Differences between the vaginal and fecal microbial communities were also observed at the genus level. Highlighting the comparatively less-studied vaginal microbiome, the most abundant genera in the vaginal microbiota were uncharacterized, belonging to families including *Lactobacillales* and *Bacteroidales*. Genera prevalent in fecal samples included those belonging to the families *Prevotellaceae* and *Lachnospiraceae* which are known for their short-chain fatty acid-producing members.

Conclusions: Research indicates that RB51 vaccination has minimal efficiency for preventing abortion in elk compared to cattle. This is consistent with the findings of this study which show non significant changes across the vaginal and fecal microbiota between vaccination groups in elk in response to *B. abortus* challenge. This experiment, however, is the first to assess the elk vaginal microbiota and indicates that the elk vaginal microbiota is temporally dynamic from late pregnancy to after parturition. Our results also confirm that the elk fecal and vaginal microbiota are similar to that of other ruminants. These results increase our understanding of the elk microbiome and may inform non-invasive strategies to monitor the health of wild elk populations.

Financial Support: This project was solely funded by the USDA.

Notes:

P163 - Development and optimization of bovine ileum organoid models

Carl Yeoman[1]

[1]Montana State University. carl.yeoman@montana.edu

Session: Omics / Microbiome, **2023-01-22, 6:00 - 8:00**

Objective: Organoid systems have recently been at the forefront of human research, ranging from drug development to personalized medicine. Organoid models for production animals have been developed in the last 5 years, but progress is lacking, specifically in disease prevention and reproduction. We have developed bovine uterine, and ileum organoids from regional cattle for use as in-vitro models to study host-microbiome interactions.

Methods: Ileum tissues were collected from each of three Angus-crossbred cattle immediately following slaughter in a local processing plant and used to harvest stem cells. Organoids were developed following Miyoshi & Stappenbeck et. al.. 2013. Organoid growth conditions were optimized and organoid growth dynamics were documented. Organoids were in several experiments, exposed to common gut microbes and the microbial toxin, lipopolysaccharide A (LPS) and the expression of common immunoproteins measured by Reverse transcription Quantitative PCR (RT-qPCR).

Results: Organoids show that the growth is consistent with literature, ranging in size from 40 um to 500 um. Folds are observed within the 3-dimensional spheroids replicating *in vivo* morphology. Cultivation media and growth conditions were optimized and unique to uterine and ileum organoid models. RT-qPCR results show the organoid models are responsive to microbes and the microbial toxin, LPS analogous to what is observed in vivo.

Conclusions: Organoids developed have shown to grow consistently, offering a physiological and morphological insight in in vitro studies of host - microbiome interactions and reproduction. Confirmation of cultivation protocols will allow us to experiment in these models with microbial immune modulation and optimization of in-vitro fertilization and artificial insemination.

Financial Support: This work was funded by the United States Department of Agriculture through NIFA award #2020-67016-31676

Notes:

P164 - Characterization of the respiratory microbiome and virome associated with Bovine Respiratory Disease Complex

Tara McDaneld[1], Bernadette Earley[2], Louise Cosby[3], Kerrie Duffy[2], Mark McGee[2], Larry Kuehn[1], Aspen Workman[1], Gavin Conant[4], Paul Cormican[2], Matthew McCabe[2], Catherine Duffy[3], Hannah Turkington[3], Ken Lemon[3], Michael McMenamy[3], Victoria Smyth[3], Timothy Smith[1]

[1]US Meat Animal Research Center, [2]Teagasc Animal Bioscience Centre, Ireland, [3]Agri-Food and Biosciences Institute, [4]North Carolina State University. tara.mcdaneld@usda.gov

Session: Omics / Microbiome, **2023-01-22, 6:00 - 8:00**

Objective: Bovine respiratory disease (BRD) is one of the most significant health problems in cattle and the most expensive animal disease afflicting herds worldwide. 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. 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 short and long-read sequencing platforms, bioinformatic technologies, and high throughput sensitive and rapid diagnostics to identify respiratory viral and bacterial agents associated with BRD; 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/Teagasc/USMARC).

Methods: Research for Objective 3 will be completed in 2023 with the infection of calves with either bovine respiratory syncytial virus (BRSV) or bovine herpes virus-1 (BHV-1) and subsequent sampling of the respiratory tract and select tissues. The bacterial and viral pathogens will be identified in the respiratory tract samples and the immune repertoire will be evaluated in response to infection.

Results: To date, nasal swabs have been collected from herds in the US and Ireland for year one and year two (Objective 1), and evaluation of the bacterial and viral populations through 16S rRNA profiling and virus-directed qPCR (Objective 2), respectively, has been completed. Untargeted-metagenomic sequencing also allowed for identification of unexpected viral and bacterial species such as *Mycoplasma bovirhinis*, *Mannheimia varigenia*, Bovine rhinitis virus A/B.

Conclusions: Analysis of these specific respiratory pathogens will present a clearer picture of the distribution of bacterial and viral populations in cattle.

Financial Support: This project was supported by Agriculture and Food Research Initiative Competitive Grant no. 2019-67015-29847 from the USDA National Institute of Food and Agriculture.

Notes:

P165 - Pathogenomics of the respiratory *Mycoplasma bovis* strains circulating in cattle

Ethan Dudley[1], Sal Lamsal[1], Paul S. Morley[1], Matthew Scott[1], Lee Pinnell[1], Hatem Kittana[1, 2], Robert Valeris-Chacin[1]

[1]Veterinary Education Research and Outreach, Texas A&M University, [2]Texas Veterinary Medical Diagnostic Laboratory. edudley01@exchange.tamu.edu

Session: Omics / Microbiome, **2023-01-22, 6:00 - 8:00**

Objective: Bovine respiratory disease (BRD) is a major economic and animal welfare issue in the beef industry. *Mycoplasma bovis* is one of the main bacterial pathogens associated with BRD, especially with chronic cases. However, there is a lack of information about the genomic characteristics of the strains circulating in the Texas panhandle due to the difficulties of isolating *M. bovis* in clinical specimens. Therefore, the objective of this study is to describe the genomic features of field *M. bovis* strains isolated from cattle in the Texas panhandle.

Methods: *Mycoplasma bovis* isolates (n=52) recovered from lung lesions in cattle by the Texas A&M Veterinary Diagnostic Lab (TVMDL) were used in this study. DNA extraction from the isolates was performed using the DNeasy UltraClean microbial kit. In order to increase DNA yield and meet the requirements for the library preparation, a whole genome amplification was carried out using REPLI-g UltraFast Midi kit. DNA library preparation was performed using the Oxford Nanopore Technologies native barcoding kit v14. We used R10.4.1 flow cells for sequencing the DNA libraries. Sequencing was done in a MinION Mk1C device. Long reads quality will be assessed using LongQC. *De novo* assemblies will be generated using Flye. Annotated genomes will be obtained with Prokka from the Flye-derived scaffolds. The pangenome will be estimated using Roary. The presence of single nucleotide polymorphisms (SNPs) in the core genome will be inferred using Snippy. Antibiotic resistance genes will be identified *in silico* using ABRicate. The pangenome generated from Roary will be searched for previously identified genes associated with virulence in Mycoplasma species. The prevalence of antibiotic resistance genes in *M. bovis* isolates will be inferred from the proportion of isolates carrying antibiotic resistance genes detected via ABRicate. Scoary will be used to assess the association between the presence of antibiotic resistance genes in *M. bovis* isolates and the host characteristics.

Results: We expect to find an enrichment of virulence and antimicrobial resistance genes in *M. bovis* isolates from cattle with lung lesions. In addition, we expect to detect differences in the gene prevalence by type (beef or dairy), age, and location of cattle. These methods have been validated using a known *M. bovis* strain, American Type Culture Collection 255523.

Conclusions: The knowledge obtained from these studies will help improve current molecular diagnostics of BRD.

Financial Support: Texas A&M AgriLife Research

Notes:

P166 - Update on the molecular epidemiological assessment of beef cattle management systems

Bradly Ramirez[1], Hudson McAllister[1], Sarah Capik[2], Robert Valeris-Chacin[1], Kelsey Harvey[3], Brandi Karisch[3], Amelia Woolums[4], Paul S. Morley[1], Lee Pinnell[1], Matthew Scott[1]

[1]VERO Program, Texas A&M University, [2]Tumbleweed Veterinary Services, [3]Department of Animal and Dairy Sciences, Mississippi State University, [4]Department of Pathobiology and Population Medicine, Mississippi State University. mascott@cvm.tamu.edu

Session: Omics / Microbiome, **2023-01-22, 6:00 - 8:00**

Objective: Our hypothesis is that cattle management decisions related to reducing BRD risk influence host immunity and cellular activity and alter the composition of microbial communities. To test this hypothesis, we will analyze the 1) whole blood transcriptomes and 2) microbial DNA and RNA from the upper respiratory tract of cattle in a time-course, multi-omics approach.

Methods: *Objective 1:* To analyze the influence of vaccination and marketing strategy on host gene expression through whole blood transcriptomics, we have selected jugular blood samples from 73 individual cattle enrolled in a whole-plot, split-plot study for mRNA sequencing. These samples were from six time points: modified attenuated viral vaccination or not during the cow-calf phase (T1), seven-days post-vaccination (T2), booster administration or not (T3), weaning and enrollment into marketing strategies (auction versus direct sale; T4), arrival to the backgrounding facility (three-days post-weaning; T5), and end of the backgrounding period (45-days post-arrival; T6). Clinical treatment and production data were collected throughout the study. RNA extracted from blood will be sequenced for mRNA via Illumina NovaSeq 6000 S4 chemistry (150 PE bp; ~30M reads/sample). Sequenced reads will be bioinformatically processed via HISAT2/StringTie2 reference-guided assembly, where gene counts will be analyzed for differential expression and dynamic expressional trends with edgeR, glmmSeq, and Trendy (FDR<0.10). *Objective 2:* To utilize a bacterial and viral DNA/RNA extraction and bioinformatic analysis pipeline for exploring the host transcriptome - upper respiratory microbiome interface, we will enroll nasopharyngeal swab samples collected from the same cattle as Objective 1 at T1, T4, and T5. Microbial DNA and RNA will be extracted with a developed automated dual nucleic acid extraction protocol, where bimodal high-throughput shotgun sequencing will allow for simultaneous microbial metagenomic and metatranscriptomic analyses. Following cDNA synthesis of extracted RNA, nucleic acid will be sequenced via Illumina NovaSeq 6000 S4 chemistry (150 PE bp; ~50M reads/sample). Bioinformatic processing of sequenced reads will follow k-mer-based assembly, Swiss-Prot annotation, and coverage-weighted contig-abundance estimation for both bacterial and viral reads. Redundancy and random forest analyses will determine the level of association with co-expressed genes from Objective 1.

Results: *Objective 1*: RNA was successfully extracted from 337 blood samples across all six timepoints (mean concentration: 169.6 ± 104.5 ng/µl; mean RIN 9.0 ± 0.4). RNA library preparation and sequencing is underway, with expected results by fall 2023. *Objective 2:* Dual DNA/RNA extraction protocols are successfully developed on nasopharyngeal swabs (DNA: mean concentration: 322.6 ± 124.4 ng/µl; mean DIN 7.2 ± 1.6; RNA: mean concentration: 42.3 ± 22.8 ng/µl; mean RIN 5.3 ± 1.0). Extractions for all samples is expected to be finished by fall 2023.

Conclusions: Analysis work for Objective 1 is expected to be complete by fall 2023, with manuscript submission by spring 2024. Preliminary benchtop work for Objective 2 has been successful, with sequencing and analysis work to begin in spring 2024.

Financial Support: This work is supported by the USDA NIFA Grant No. 2023-67015-39711.

Notes:

P167 - Big-data genomic investigation to improve dairy cattle health

Li Ma[1], Zhengguo Xiao[1], Curt P. VanTassell[2], Christian Maltecca[3], Kristen P. Gaddis[4], Pamela R. Adkins[5]

[1]University of Maryland, [2]USDA-Agricultural Research Service, [3]North Carolina State University, [4]CDC, [5]University of Missouri. lima@umd.edu

Session: Omics / Microbiome, 2023-01-22, 6:00 - 8:00

Objective: Animal health is important for the sustainability and profitability of the dairy industry. While host genetics contributes only a small variation in disease risk, genetic and genomic selection provides an economic and sustainable solution that can accumulate in the long term. Leveraging the US dairy genomics database and other functional genomic resources, we aim to uncover the host genetic mechanism of cattle disease and to apply these genomic discoveries to improve disease resistance and profitability of dairy production.

Methods: The CDCB and USDA AGIL Lab has included six common diseases in the national genomic evaluation since 2018. Cow and heifer livability was also developed and added to the system. We use GWAS to identify genomic regions and apply imputation and fine-mapping to pinpoint candidate genes and mutations associated with cattle health. We integrate transcriptome, methylome, and other functional genomics data to find health-relevant SNPs for genomic selection.

Results: We recently conducted GWAS analyses for heifer livability and early calving. In this study, we included two datasets for GWAS analyses, a discovery dataset including millions of animals imputed to 80K SNPs and a fine-mapping dataset including 27K bulls genotyped by SNP chips and imputed to sequence level. As a result, we found one QTL region for each trait. For heifer livability, we obtained 118 genes near the associated SNPs with several genes exhibiting biological relevance for livability. For early calving, we identified 596 genes near the top associated SNPs in the bovine MHC region on chr23. By focusing on the regions covering leading variants, we fine-mapped 90 and 136 genes for heifer livability and early calving. We further investigated the enrichment of variants with respect to their genomic locations and the annotations (CDS, conserved, intron, and UTR). For heifer livability, we observed a significant enrichment of CDS and protective variants. For early calving, we observed the significant enrichment of variants in CDS, conserved and UTR.

Conclusions: This project is one of the largest such genomic studies of disease resistance in dairy cattle. The results provide useful information on the genetic architecture of cattle health and reveal candidate genes and SNP markers of disease resistance to be used in future genomic selection of health traits.

Financial Support: This project is supported by the Agriculture and Food Research Initiative Competitive Grant no. 2020-67015-31398 from the USDA National Institute of Food and Agriculture.

Notes:

P168 - Transcriptome analysis of bovine abomasal mucosa following experimental infection with *Ostertagia ostertagi*

Clarissa Boschiero[1, 2], George Liu[1], Wenbin Tuo[1]

[1]USDA-Agricultural Research Service, [2]University of Maryland. george.liu@usda.gov

Session: Omics / Microbiome, **2023-01-22, 6:00 - 8:00**

Objective: *Ostertagia ostertagi* is a cattle stomach nematode that causes significant disease; however, little is known about host responses to infection. Due to the emergence of drug resistance in these parasites, there is a need for alternative treatments.

Methods: To understand host responses to *O. ostertagi* infection in the gastric mucosa, gene expression in fundic and pyloric mucosa was compared between infected and uninfected calves for 21 days post-infection.

Results: The number of differentially expressed genes (DEGs) increased over time with more upregulated than downregulated DEGs at each time point. Fundic mucosa has more DEGs than pyloric mucosa across all time points. The largest changes occurred between days 7-9 and 10 post-infection. Most DEGs are associated with immunity, cellular reorganization, cell migration, and proliferation. Tuft/epithelial cell response to the infection was atypical, lacking key cytokines. Numerous genes associated with T cell exhaustion were upregulated.

Conclusions: The data collectively indicate that *O. ostertagi* infection elicited host immune responses, yet also suggest parasite-induced immunosuppression is present. This may explain why cattle are slow in developing protective immunity to *O. ostertagi*. Understanding mechanisms of parasite evasion will facilitate the rational design of protective vaccines against complex nematode parasites.

Financial Support: GEL is supported in part by AFRI grant numbers 2019-67015-29321 and 2021-67015-33409 from the USDA National Institute of Food and Agriculture (NIFA).

Notes:

P169 - Dual RNA-seq in Mycoplasma bovis and mammary epithelial cells following cell line and intramammary infection

Aga E. Gelgie[1], Benti D. Gelalcha[1], Oudessa K. Dego[1]

[1]University of Tennessee. agelgie@vols.utk.edu

Session: Omics / Microbiome, 2023-01-22, 6:00 - 8:00

Objective: *Mycoplasma bovis* (*M. bovis*) is notoriously contagious pathogen of cattle which has multiple predilection sites including udder, lung, uterus, joint, heart and an eye. Mastitis due to *M. bovis* has a global occurrence and causes significant economic losses and animal welfare concerns. Since *M. bovis* is extremely resistant to antimicrobials, vaccination is a likely attainable prevention and control strategy. However, there is no effective commercial vaccine against *M. bovis* infections to date. Multiple studies have reported whole cell *M. bovis* bacterin vaccines without satisfactory protection upon challenge. Furthermore, bacterin vaccines, do not only have limited protection but also have adverse reactions which strongly indicates a dire need for highly immunogenic and protective recombinant proteins. On the other hand, the pathogenic mechanism of *M. bovis* infections and the host immune response is poorly understood which partly contributed to the lack of prevention and control strategies such as vaccines. To that end, we proposed to use RNA-seq to analyze differentially expressed genes by profiling mRNAs in *M. bovis* and mRNAs, long non-coding RNAs (lnRNAs) and microRNAs (miRNAs) in mammary epithelial cells.

Methods: Bovine mammary epithelial cells were seeded 24 h prior to the infection in 75 cm^2 flask in triplicates at a concentration of 2.0×10^6 cells/mL. *M .bovis* was grown in a friis Mycoplasma broth for 48 h until exponential phase was reached (1.2×10^9 cfu/mL). Mammary epithelial cells were infected for 24 h in a multiplicity off infection (MOI) of ˜ 500. Similarly, bovine mammary epithelial cells were seeded in triplicate at a concentration of 2.0×10^6 cells/mL in a 75 cm2 tissue culture without *M. bovis* as a control. Three Holstein dairy cows were challenged with ˜10^6 cfu/mL of *M. bovis* grown to exponential phase 24 h before the challenge. After monitored for 7 days, cows were euthanized according to standard guidelines and mammary tissue samples were collected from the higher secretory region of all quarters. Tissue samples were immersed in a 10 mL RNALater and stored in -80^{o}C until further use. Mammary tissue sample was also collected from one control cow which received 5 mL sterile 1×PBS as a placebo. Total RNA was extracted using RNeasy Mini Kit according to manufacturer's instructions and library were prepared using Zymo-Seq RiboFree Total RNA Library Kit. The resulting reads from sequencing were checked for quality, adapters removed and mapped to reference genomes. The differential expression of genes (DEGs) were performed in R-Package.

Results: Characterization of up-regulated or down-regulated *M. bovis* genes at the mRNA level is anticipated to bring forward certain clues about the important virulence genes playing key role during infection.

Conclusions: TBD

Financial Support: We would like to thank 10x Genomics Single Cell Grant Program and US-Israel Binational Agricultural Research and Development Fund for their financial support to make this work a reality.

Notes:

P170 - Molecular markers of early equine post-traumatic osteoarthritis

Annette McCoy[1], Troy Trumble[2], Alexander Lipka[1]

[1]University of Illinois Urbana-Champaign, [2]University of Minnesota. mccoya@illinois.edu

Session: Omics / Microbiome, 2023-01-22, 6:00 - 8:00

Objective: Post-traumatic osteoarthritis (PTOA) is a common cause of lameness in agricultural animals, particularly horses, and represents a significant economic and welfare burden on the animal agricultural industry. However, development of effective tools for early diagnosis and therapeutic intervention for PTOA remains elusive. The objective of this project is to identify the earliest morphological and molecular events that occur in the joint after induction of PTOA in a novel equine experimental model, focusing on the role of the synovium, and to correlate these events with synovial fluid protein signatures. We hypothesize that altered synovial tissue gene expression secondary to surgical induction of PTOA results in measurable changes in protein secretion into the synovial fluid. Further, these molecular changes will correlate with clinical signs.

Methods: This project utilizes a novel non-destabilization osteochondral fragment model in the horse that recapitulates early naturally-occurring PTOA, reliably inducing disease with repeatable mild joint pathology (superficial cartilage wear lines, synovial subintimal fibrosis) and clinical signs (mild joint effusion and gait asymmetry) over 16 weeks. An osteochondral fragment will be created in one randomly chosen metacarpophalangeal joint (MCPJ), while the other MCPJ will serve as a sham-operated control. Horses will be evaluated weekly for joint effusion, range of motion, and gait asymmetry. Synovial tissue biopsies will be taken from the dorsomedial aspect of each joint at the time of surgery for gene expression analysis via RNAseq and histopathology. Starting two weeks after surgery, horses will be exercised on a treadmill 5 days a week for 14 weeks. Synovial fluid samples will be collected every 8 weeks and labeled tandem mass spectroscopy (MS-MS) will be performed to quantify proteins in the fluid. 16 weeks after fragment creation, a second arthroscopic procedure will be performed, and synovial biopsies will be collected from both injured and sham-operated joints. The second half of the study protocol is identical to the first half. Fragments will be removed from all horses at the time of the third surgery, 32 weeks after creation and synovial biopsies will be collected. Outcome measures will be compared between injured and sham joints at each time point. Correlation between clinical and morphological parameters and quantified gene or protein expression (either absolute expression or fold-change between injured and uninjured joints) will be determined.

Results: As this is the first year of the study, results have not yet been generated.

Conclusions: The identification of diagnostically relevant correlations is a critical prerequisite for the development of tests that can classify individual disease risk. In future work, the mechanisms behind these correlations will be explored, facilitating development of novel treatments for PTOA.

Financial Support: This project was supported by Agriculture and Food Research Initiative Competitive Grant no. 2023-67015-39656 from the USDA National Institute of Food and Agriculture.

Notes:

P171 - Identification of gene by environment interactions influencing equine metabolic syndrome genomic risk alleles

Elaine Norton[1], Molly McCue[2], Cyprianna Swiderski[1]

[1]Department of Animal and Comparative Biomedical Science, University of Arizona, [2]Department of Comparative and Molecular Biosciences, University of Minnesota. elainenorton@arizona.edu

Session: Omics / Microbiome, 2023-01-22, 6:00 - 8:00

Objective: Equine metabolic syndrome (EMS), the leading cause of laminitis in horses, is influenced by both genetics and the environment yet management strategies are still focused on a generic set of diet and exercise modifications for which many horses fail to respond and continue to suffer from metabolic complications leading to laminitis. With up to a quarter of the population of high-risk breeds being affected with EMS, there is a critical need to identify gene by environment interactions (GxE) impacting EMS etiology to allow for individualized management programs for horses diagnosed or at high risk for EMS, increasing treatment efficacy and preventing laminitis. The overall objective of this project is to identify a set of targetable GxE in EMS horses.

Methods: We will achieve this objective by using a layered multi-omics approach in genetically homogeneous lines of adult Arabian horses, a high-risk breed for EMS. Populations selected for this study were chosen based on our preliminary data showing breeding lines with a high inbreeding coefficient, an EMS prevalence of 20-40% at the farm level, and the unique ability to target known environmental risk factors, including: pasture access, high non-structural carbohydrate diets, and lack of exercise. We will use this cohort to (1) identify genetic risk alleles for impacted by the environment; (2) determine molecular pathophysiology factors influenced by the environment; and (3) identify epigenetic modifications that impact EMS clinical phenotypes.

Results: The expected outcome of this proposal is the identification of genetic risk alleles, molecular phenotypes and epigenetic signals associated within known environmental risk factors for EMS.

Conclusions: This is the first necessary step to determining specific EMS environmental triggers leading to GxE, and will provide a foundation for future work, including: (1) evaluation of GxE across additional environmental risk factors, (2) identification of modifiable GxE via controlled environmental challenges and (3) identification of GxE secondary to an adverse fetal or early life environment. Ultimately, our findings will provide specific genomic regions for targeted environmental interventions in horses with EMS.

Financial Support: This project was supported by Agriculture and Food Research Initiative Competitive Grant no. 2023-67016-40110 from the USDA National Institute of Food and Agriculture

Notes:

P172 - Transcriptomic analysis of *Campylobacter jejuni* survival under aerobic conditions

Anand B. Karki[1], Elise Delaporte[1], Mohamed K. Fakhr[1]

[1]The University of Tulsa. mohamed-fakhr@utulsa.edu

Session: Omics / Microbiome, 2023-01-22, 6:00 - 8:00

Objective: *Campylobacter* remains a leading cause of foodborne illness around the globe. This microaerophilic microorganism encounters various stressors such as aerobic conditions and low temperature during retail meat processing and storage. In recent years, multiple studies have reported high prevalence of aerotolerant *Campylobacter* strains in various retail meats including poultry. While known to harbor genes involved in tolerance to oxidative stress, genes specific for *Campylobacter* aerotolerance during survival under aerobic conditions are yet to be identified. The objective of this study was to conduct transcriptomic analysis using RNA-Seq Illumina technology for the survival under aerobic conditions of two selected *Campylobacter jejuni* strains previously isolated from retail meats and sequenced in our laboratory. These strains possessed different aerotolerancy levels: *C. jejuni* T1-21 (aerosensitive) and *C. jejuni* WP2-202 (aerotolerant).

Methods: Strains were exposed to aerobic conditions in Mueller Hinton Broth media and triplicate samples were taken at 0, 0.5, 6, 12, and 24 hrs for RNA isolation. Directzol RNA miniprep kit was used for RNA isolation and DNA was removed using the Turbo DNA free kit. cDNA libraries prepared using TruSeq stranded mRNA library preparation kit (Illumina) was sequenced in a Hiseq 4000 platform and sequence reads were analyzed using the CLC Genomic Workbench 20.

Results: Results indicated that prolonged aerobic incubation induced significant expression of various genes among the two tested strains. More differentially expressed genes (DEGs) were observed in *C. jejuni* WP2-202 compared to *C. jejuni* T1-21. Despite the variation in genomic expression among the two *Campylobacter jejuni* strains, several genes including oxidative stress response genes and other stress response genes were found to be significantly upregulated at 12 hrs and 24 hrs compared to 0 hr. Higher number of genes related to inorganic iron transport and metabolism were found to be upregulated and more genes related to ribosomal proteins were downregulated for the two strains at most incubation times. The majority of upregulated genes for both strains belonged to categories with unknown functions.

Conclusions: In conclusion, genomic expression of *Campylobacter jejuni* survival under aerobic condition varied among the two tested strains and appeared to be altered as time of incubation increases.

Financial Support: This work was supported by the USDA National Institute of Food and Agriculture, AFRI grant # 2020-67018-33240.

Notes:

P173 - Functional characterization of a microbial consortium for the competitive exclusion of *Salmonella* in chickens

Dylan Harris[1], Carmen Wickware[1], Christopher Anderson[1], Torey Looft[1]

[1]USDA-Agricultural Research Service. dylan.harris@usda.gov

Session: Omics / Microbiome, 2023-01-22, 6:00 - 8:00

Objective: *Salmonella* is one of the leading causes of intestinal disease globally with poultry being a major source for human infection. While undefined communities of bacteria, particularly from the cecal contents of healthy birds, have been shown to limit the ability of *Salmonella* to colonize young birds, a fully defined consortium has potential, as it would improve efficacy and reproducibility. This project sought to construct a defined consortium of commensal bacteria derived from the chicken gut microbiome to reduce and prevent the colonization of *Salmonella* within young birds. An animal study was performed using this defined consortium of 15 commensal bacteria, and when compared to a negative control, showed inhibition of *Salmonella*. However, the consortium exhibited reduced inhibition against *Salmonella* when compared to the administration of an undefined cecal content control. To examine the functional capacity of the bacteria within the consortium, predicted functions of individual consortium members were compared against *Salmonella* Heidelberg (SH2813) to evaluate competition potential and examine any redundancy or gaps in function to refine and increase the competitive nature of the consortium.

Methods: To examine potential functions within the consortium, individual members of the consortium were sequenced via Oxford Nanopore with basecalling performed via Guppy's Super accurate model. Each member's genome was assembled using Flye, Minasm+Minipolish, and Raven. Trycycler was used to obtain a consensus whole-genome sequence for each consortium member through the reconciliation and alignment of the sequences generated by the three assemblers. Genome annotation was performed with Distilled and Refined Annotation of Metabolism (DRAM) and Anvi'o was used to visualize the functional characteristics of assembled genomes under a pangenomic comparison. Potential functions were compared to the available genome for SH2813 along with other *Salmonella* spp.

Results: Pangenomic comparisons between consortium members and *Salmonella* spp. showed some overlap in terms of gene clusters, but most organisms appeared unique in terms of gene content. Functional genomic comparisons of the consortium members to *Salmonella* spp. revealed overlap in predicted function amongst many of the consortium members in terms of carbohydrate metabolism with some members contributing more functionality than others. Gaps in metabolic pathways that *Salmonella* may be utilizing were also revealed such as TMAO reductase and thiosulfate reduction.

Conclusions: While administration of undefined cecal contents had a much stronger effect on the overall inhibition of *Salmonella* colonization, the consortium still managed to inhibit *Salmonella* over time. The delay in *Salmonella* inhibition in animals colonized by the consortium suggests some niches within the community are not being occupied by consortium members. Functional genomic comparison shows that there are gaps within the metabolic network that could be filled by adding strains to the consortium to improve inhibition of *Salmonella* such as non-pathogenic TMAO reductase and thiosulfate reductase producers. Further characterization of the functional capacity of our consortium members will inform key additions of new consortium members to compete with *Salmonella* and reduce its colonization in poultry.

Financial Support: ORISE USDA-ARS Microbiology Graduate Student Research Opportunity

Notes:

P174 - Hypothalamic disruptions in response to maternal infection with Porcine Reproductive and Respiratory Syndrome Virus

S.I.M Alsegehy[1], B.R. Southey[1], A.M. Antonson[1], R.A. Nowak[1], R.W. Johnson[1], S.L. Rodriguez-Zas[1]

[1]University of Illinois at Urbana-Champaign. southey@illinois.edu

Session: Omics / Microbiome, 2023-01-22, 6:00 - 8:00

Objective: The immune response of the sow to porcine reproductive and respiratory syndrome virus (PRRSV) infection during gestation can affect the developing pig brain. DNA methylation is a frequent epigenetic modification that typically depresses gene expression, affects mammalian development, and presents sex-dependent patterns.

Methods: The effects of maternal PRRSV infection and sex on the methylation patterns of 22-day-old pig hypothalami were investigated to understand the potential impacts of these factors on neurohormonal pathways. Reduced representation bisulfite sequencing enabled measuring DNA methylation at single base resolution in regions of high CpG density on 24 pigs. Twenty-four pig libraries were made with the NuGEN system and high-throughput DNA sequenced. The reads were mapped to the Sscrofa11.1 genome using the software Bismark, and differential methylation sites were identified using the MethylKit R package.

Results: Among the differentially methylated sites (FDR-adjusted P-value < 0.05) between PRRSV and control groups, two sites were located in the promoter regions of SIRT3 and NRBP1, most sites were over-methylated, and seven were in the proximity of genes known to participate in regulation of transcription either via methylation (e.g., CYP3A29), and histone or RNA polymerase activity (e.g., NR5A2). Among the differentially methylated sites (FDR-adjusted P-value < 0.05) between females and males, most sites were over-methylated, one gene was located in the promoter region of TNC, half were located in CpG islands or shores, and two genes were in the proximity of genes known to participate in the regulation of transcription via RNA polymerase activity (e.g., LHX2). Aligned with the methylation patterns, CYP3A29 and NR5A2 presented differential expression (FDR-adjusted P-value < 0.05) between PRRSV and sex groups. Among the genes proximal to the differentially methylated sites, CYP3A29 and NR5A2 participate in hormonal signaling, and ITG85 and IFITM3 participate in an inflammatory response.

Conclusions: Our results suggest that postnatal methylation-informed therapies can play a role in reducing the prolonged effect of virally elicited inflammation on pig physiology and behavior.

Financial Support: This study is supported by USDA NIFA AFRI, grant number 2018-67015-27413.

Notes:

P175 - Alternative splicing in the hypothalamus exposed to prenatal Porcine Reproductive and Respiratory Syndrome Virus and weaning

Y. Shen[1], B.R. Southey[1], A.M. Antonson[1], R.A. Nowak[1], R.W. Johnson[1], S.L. Rodriguez Zas[1]

[1]University of Illinois at Urbana-Champaign. southey@illinois.edu

Session: Omics / Microbiome, 2023-01-22, 6:00 - 8:00

Objective: The distinct transcript isoforms from a gene produced by alternative splicing can result in proteins that differ in function, and these processes are prevalent in the brain. Exposure to inflammatory signals and environmental stressors can alter the relative abundance of isoforms, dependent upon sex. The objective of this study was to investigate the disruption of alternative splicing patterns associated with prenatal inflammatory signals, environmental stress associated with weaning, and sex differences.

Methods: The hypothalamic transcriptome of 48 pigs from both sexes was analyzed across two treatment groups; prenatal inflammation and weaning. Prenatal inflammation was induced in using infection of the porcine reproductive and respiratory syndrome virus and compared to a saline injection. At 21 days of age, half of the pigs were weaned, and the rest continued nursing until sampling at 22 days of age. Sequences were aligned to the pig reference genome using the package STAR v2.7, and differential splicing was tested using the package rMATS v.4.

Results: At False Discovery Rate-adjusted P-value < 0.05, the number of genes presenting differential alternative splicing ranged from 812 to 923 and the most common splicing category was exon skipping (58%) followed by mutually exclusive exons (22%) across the three factors studied. Gene Ontology enrichment analysis for genes presenting significant differential alternative splicing in response to prenatal inflammation identified pathways of neurodegeneration (e.g., GRIN2A), Herpes simplex virus 1 infection (e.g., STAT1), and mRNA surveillance (e.g., members of the UPF family). Analysis of the genes presenting alternative splicing associated with weaning and sex identified enrichment of the regulation of circadian rhythm, ephrin receptor signaling pathway, mRNA processing, and regulation of alternative mRNA splicing.

Conclusions: Our results suggest that strategies to reduce the effect of viral and environmental challenges on pig physiology and behavior, such as genetics and gene-targeting treatments, require the consideration of the alternative isoforms resulting from gene splicing.

Financial Support: This study is supported by USDA NIFA AFRI, grant number 2018-67015-27413.

Notes:

P176 - Wildfire smoke impacts on dairy cattle performance and health and the molecular and cellular mechanisms involved

Amy Skibiel[1], Pedram Rezamand[1], Denise Konetchy[1], Jenifer Cruickshank[2], Jeffrey Abbott[3]

[1]University of Idaho, [2]Oregon State University, [3]Washington State University. askibiel@uidaho.edu

Session: Physiology, **2023-01-22, 6:00 - 8:00**

Objective: Wildfires are becoming more frequent and destructive. Large catastrophic wildfires are common occurrences in the western US, home to almost 4 million dairy cows that are producing 40% of the nation's milk. Wildfires not only destroy land and vegetation in the immediate burn path but release harmful pollutants into the atmosphere through smoke. Fine particulate matter ($PM_{2.5}$) is considered one of the most hazardous components of wildfire smoke. In humans, wildfire-$PM_{2.5}$ exposure is linked to cardiopulmonary impairments and disease, and premature mortality. Preliminary work by our group found that dairy calves and cows show signs of an inflammatory response and cows produce less milk during and in the days following acute exposure to wildfire-$PM_{2.5}$. However, the mechanisms contributing to these physiological effects are unclear and there are currently limited guidelines and strategies for producers to employ to mitigate risk to their herds. The overarching goal of our project is to investigate the short and long-term effects of exposure to wildfire-$PM_{2.5}$ during lactation and postnatal life on pulmonary function, animal health, and production and to test the efficacy of large outdoor air purifiers to reduce cattle exposure to wildfire smoke.

Methods: We will use observational and experimental approaches and advanced laboratory techniques to quantify $PM_{2.5}$-induced changes in pulmonary and systemic health, performance, and expression and mobilization of inflammatory mediators.

Results: We anticipate that wildfire smoke exposure will impair pulmonary function and contribute to pulmonary disease through oxidative stress and inflammation in both lactating cows and postnatal calves. Further, these effects are expected to be reduced using outdoor air filters designed to trap fine particles.

Conclusions: We expect the results of this work will lead to further development and implementation of strategies to reduce the occurrence of cattle health issues stemming from wildfire smoke inhalation.

Financial Support: This project was supported by Agriculture and Food Research Initiative Competitive Grant no. 2023-67016-39658 from the USDA National Institute of Food and Agriculture.

Notes:

P177 - UCP1 expression in adipose tissue of Holstein dairy cows

Ursula Abou-Rjeileh[1], Adam L. Lock[2], G. Andres Contreras[1]

[1]Dept. Large Animal and Clinical Sciences, Michigan State University, [2]Dept. Animal Science, Michigan State University. abourje2@msu.edu

Session: Physiology, **2023-01-22, 6:00 - 8:00**

Objective: Characterize mitochondrial uncoupling protein 1 (UCP1) in adipose tissue (AT) of periparturient dairy cows.

Methods: Subcutaneous AT (SCAT) samples were collected from multiparous Holstein cows (n=12) at 11 ± 3.6 d before calving (PreP), and 6 ± 1.0 d (PP1) and 13 ± 1.4 d (PP2) after parturition. The averages (mean ± SD) for BCS, MEq, and parity were 3.53 ± 0.22, 14597 ± 1702 kg, and 2.67 ± 0.65, respectively. All animals received a common diet during the close-up (30 d pre-calving to parturition) and fresh (1-15 DIM) periods to meet NRC (2001) requirements. For histology analysis, SCAT were fixed in 4% paraformaldehyde, blocked in paraffin, and then sectioned (4 µm). Sections were stained with an antibody against UCP1 (1:800) followed by rabbit on rodent HRP-polymer. Imaging was performed using an Olympus SLIDEVIEW VS200 Research Slide Scanner. UCP1 expression was quantified using ImageJ software (particle count/adipocyte count). UCP1 protein expression was quantified by capillary electrophoresis. NGS RNA sequencing was used to evaluate the transcriptomic profile of bioenergetics gene pathways. Statistical analyses were performed using a mixed-effect model including the random effect of cow and fixed effect of time where time was included as a repeated measure.

Results: We observed a time effect on UCP1 expression reflected by higher HRP signal at PP1 and PP2 (2.09 and 2.86 ± 0.48) compared to PreP (0.72 ± 0.48; $P<0.05$). Similarly, UCP1 protein expression increased at PP2 (0.0057 ± 0.001) compared to PreP and PP1 (0.0027 and 0.0031 ± 0.0003; $P=0.03$). Simultaneously, β_3-adrenergic receptor gene expression increased at PP1 and PP2 compared with PreP ($P < 0.01$).

Conclusions: Our results provide novel evidence for the browning of white AT in dairy cows after calving. Whether this reflects an increase in thermogenesis or an upregulation of the mitochondrial bioenergetic capacity is unknown. Further genomic and proteomic analysis are warranted to better understand UCP1-mediated energy expenditure in bovine AT especially during times of intense lipolysis and metabolic stress such as during the periparturient period.

Financial Support: USDA grants (2019-67015-33386, 2021-67015-29443); Michigan Alliance for Animal Agriculture (AA18-028)

Notes:

P178 - Early-gestation nutrient restriction in dairy heifers may impair duodenal development in the offspring

D. Edache[1], A. Frau[2], A. Gallo[3], F. Mossa[2], A.S. Atzori[1]

[1]Department of Agricultural Sciences, University of Sassari, Italy, [2]Department of Veterinary Medicine, University of Sassari, [3]Department of Animal Science, Food and Nutrition, Università Cattolica del Sacro Cuore, Italy. edachedave@vet.k-state.edu

Session: Physiology, **2023-01-22, 6:00 - 8:00**

Objective: In cattle, early life experiences can profoundly influence phenotypic, reproductive, and physiological performances in the progenies, with long lasting repercussions later in life. However, less is known about the relationship between in utero nutrient stress and organ morphology and development. Herein, we investigated the effects of feeding replacement dairy heifers a limited amount of their maintenance energy requirements from preconception to early gestation on the development of the gastrointestinal tract in their offspring.

Methods: From 11 days before conception to early gestation, 28 Holstein Friesian heifers (initial BW±SD; 371.6±42.1, age±SD; 15.7±1.1 months) were randomly assigned to one of three feeding programs and differentially fed as following: (i) 0.6 of their maintenance energy (M) requirement during the first 80 days of pregnancy (NR80, n=11), (ii) 0.6 M during the first 120 days of pregnancy (NR120, n=11), and (iii) 1.8 M during the first 120 days of pregnancy (Control, n=6). All heifers were sired by a single bull from sex-sorted semen. After the differential feeding period ended, all pregnant heifers were grouped fed with *ad libitum* access to feed until calving. From the 28 single calves born, 22 female calves were retained. Calves received similar nutritional management during the pre and post weaning phases. Calves were euthanized at d 135±3 of postnatal life to obtain organs weight. Data was analyzed in R with One-way ANOVA and mean contrast separated with Tukey post-hoc test.

Results: At birth, calves born to control-fed heifers weighed 480g more than NR80 calves (P=0.019) and tended to weigh 260g more than NR120 calves (P=0.102). Similarly, calves born to heifers offered 1.8 M had a larger thoracic circumference than their peers (P=0.05). Exposure to maternal energy restrictions during early gestation had no effect on several immunological and gastrointestinal tract organ weights. However, the weight of the duodenum tended to be lower in calves born to nutrient restricted mothers (P=0.07). In addition, no association was detected between the low birth weight and duodenal mass at slaughter (r=0.29, P=0.481).

Conclusions: Our results demonstrate that altering maternal energy requirements in dairy heifers during early gestation reduces the birth weight of their offspring and may impair duodenal development later in life. Continued research is necessary to determine the long-term implications of impaired duodenum development in the offspring.

Financial Support: The Italian ministry of Education, University, and Research (Ministero dell'Istruzione dell'Università e della Ricerca, MIUR). “DESTINE PROJECT”, grant (Fondi PRIN: Progetti di Ricerca di Rilevante Interesse Nazionale - Bando 2017, Prot. 20172N2WL3).

Notes:

P179 - Evaluation of a novel antioxidant formulation for the improvement of canine gastrointestinal health

David Atuahene[1, 2], Annalisa Costale[3], Elisa Martello[4], Alessandro Mannelli[1], Elisabetta Radice[5], Garret Suen[2], Ibrahim Zuniga-Chaves[2], Davide G. Ribaldone[6], Biagina Chiofalo[7], Bruno Stefanon[8], Giorgia Meineri[1]

[1]Dept. Veterinary Sciences, University of Turin, [2]Dept. Bacteriology, University of Wisconsin-Madison, [3]Dept. Science and Pharmaceutical Technology, University of Turin, [4]School of Medicine, University of Nottingham, [5]Dept. Surgical Sciences, University of Turin, [6]Dept. Medical Sciences, University of Turin, [7]Dept. Veterinary Sciences, University of Messina, [8]Dept. Agrifood, Environmental and Animal Science, University of Udine. datuahene@wisc.edu

Session: Physiology, **2023-01-22, 6:00 - 8:00**

Objective: Reactive oxygen species (ROS) play a significant role in inducing an imbalance of redox homeostasis, leading to oxidative stress, which is associated with various pathological conditions in companion animals, including several inflammation-related gastrointestinal disorders. The potential therapeutic value of nutritional supplementation with antioxidant substances is a subject of ongoing research. The aim of this study was to evaluate *in vitro* through chemical assays the antioxidant properties of three natural substances, namely bromelain (B), quercetin (Q), and powdered shiitake - *Lentinula edodes* - mushroom (LE); and to create a formulation for a feed supplement integrating the three ingredients in the quest to improve gastrointestinal health in dogs in an *in vivo* study.

Methods: In this study, we assessed the total phenolic content (TPC) and antioxidant activity of the three natural substances, B, Q, and LE, as well as a mixture containing these components using a TPC chemical assay, DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic diammonium salt acid)) assays respectively, each determination was done in triplicates. The mixture was obtained using the same extract concentration for each individual substance when tested. A novel complementary feed supplement, based on a mixture of the natural substances B, Q, and LE was developed. The safety and efficacy of the complementary feed were evaluated in a 35-day *in vivo* dog study involving 30 dogs randomized into a placebo-controlled (no. 15) and treatment (no. 15) group, and faecal markers of inflammation and immunity were analysed using linear mixed models, where treatment, time, and treatment-by-time interaction were included as fixed-effect predictors, A random intercept was included and pairwise comparisons were made with pairwise t-test. The significance threshold was set at $P< 0.05$

Results: Our findings revealed varying antioxidant activities among the individual natural substances. Remarkably, the formulated combination exhibited the highest ($P<0.0001$) TPC (4±0.2 mg GAE/g DM (dry matter)) and ABTS (125±3 µmol TE/g DM) while Q displayed the lowest ($P<0.0001$) TPC (2±0.2 mg GAE/g DM) and ABTS (11±3 µmol TE/g DM). Additionally, Q demonstrated the lowest ($P<0.0001$) DPPH• activity (EC50: 1±10 µg/mL), with the formulation exhibiting an EC_{50} of 138±10 µg/mL, LE with an EC_{50} of 231±10 µg/mL, and Bromelain with an EC_{50} of 434±10 µg/mL. Analysis of faecal markers of inflammation and immunity revealed a significant reduction in calprotectin, cortisol, histamine, and indole/skatole levels ($p < 0.05$). In contrast, short-chain fatty acid (SCFA) levels increased over time. This reduction suggests a decrease in intestinal inflammation, oxidative stress, and harmful bacterial metabolites, in contrast, the increase in SCFA levels implies an improved gut microbiota composition and enhanced fermentation of dietary fibres.

Conclusions: The study's outcomes have culminated in the creation of an innovative complementary feed supplement, formulated from a blend of the natural constituents, B, Q, and LE. Its efficacy showcased promising outcomes in enhancing canine gastrointestinal well-being. These findings substantiate the viability of this novel natural feed supplement as a dietary modality for ameliorating gastrointestinal health and overall psycho-physical well-being in dogs. Future studies could investigate its long-term effects on a larger and more diverse population of dogs.

Financial Support: This research was funded by the Dept. of Veterinary Sciences, University of Turin (Italy).

Notes:

P180 - Evaluation of Nanopore 16S rRNA gene sequencing for taxonomic identification of *Mycobacterium* spp. in MGIT cultures

Olga Andrievskaia[1], Amalia Garceac[1], Kristin Arnold[1], Dara Lloyd[1], Mirjana Savic[1]

[1]Canadian Food Inspection Agency, Canada. Olga.Andrievskaia@inspection.gc.ca

Session: Diagnostic Testing, 2023-01-22, 6:00 - 8:00

Objective: Bovine tuberculosis (bTb) is an important zoonotic infectious disease that poses a risk to public health, livestock and wildlife. The effective management and control of bTb depends on the bacterial isolation and taxonomic confirmation of the infectious agent, *Mycobacterium bovis*, which is a time-consuming process. While the automated liquid culture system BACTEC MGIT is an efficient method for recovery of *M. bovis* from diagnostic specimens, non-tuberculous *Mycobacterium* and other bacterial species, that survive decontamination and antibiotics in media, can trigger positive MGIT (MGIT+) signals leading to a confirmation process and delays in final reporting. We explored the utility of the Oxford Nanopore Technology (ONT) sequencing of the 16S rRNA gene for fast and affordable taxonomic identification of bacterial species in primary MGIT+ cultures.

Methods: Aliquots of MGIT+ cultures (n=84) of bovine granulomas were processed for DNA extraction within 2-3 days of signaling positive; further *Mycobacterium* confirmation was done by standard microbiology methods. Sequencing libraries were constructed by using 1) Nanopore 16S barcoding kit SQK-RAB204, 2) customized 16S rRNA gene primer sets and Nanopore kits SQK-LSK109 and EXP-NBD104. Sequencing was done on ONT MinION Mk1B using Flongle flowcells R9.4. and live high accuracy base calling. ONT EPI2ME Fastq 16S on-line platform was used for data analysis and taxonomic classification of 16S rRNA gene amplicons.

Results: Universal primers supplied in the Nanopore SQK-RAB204 kit did not efficiently amplify the *Mycobacterium* 16S rRNA gene due to 1-2 nucleotide mismatches in the binding sites. Therefore an alternative strategy was applied using customized primers and SQK-LSK109 kits. Within 2 h of ONT Flongle 16S rRNA gene sequencing, an accurate taxonomic identification of *Mycobacterium* spp. was achieved in 18 of 28 (64.3%) primary MGIT+ cultures from which *Mycobacterium* spp. were later isolated; notably all 5 *Mycobacterium tuberculosis* complex organisms were correctly identified. In the 10 remaining primary MGIT+ cultures of confirmed *Mycobacterium* isolates the following bacteria were identified in various combinations: *Rhodococcus hoagii*, *Acinetobacter johnsonii*, *Brevibacterium casei*, *Clostridium haemolyticum*, and *Mycoplasma alkalescens*. *Mycobacterium* spp. were not identified in all 56 (100%) primary MGIT+ cultures confirmed as *Mycobacterium*-negative by bacterial isolation; the MGIT false-positive signals could be attributed to *Rhodococcus hoagii* (29.8%), *Mycoplasma bovis* (14.3%), *Mycoplasma* spp (not *bovis*) (5.2%), *Fusobacterium necrophorum* (5.2%), *Pseudomonas* spp (7.1%).

Conclusions: These results suggest that the Nanopore 16S rRNA gene sequencing of MGIT+ primary cultures can be considered as a complementary tool in taxonomic identification of *Mycobacterium* and other species, especially in high-profile cases when preliminary information on the nature of the pathogen can provide guidance in the initiation of investigative activities, or when definitive isolation of the pathogen can be accelerated or optimized by an informed choice of specimen decontamination, antibiotic supplementation, and isolation strategy.

Notes:

P181 - In silico performance of a targeted enriched metagenomics approach to infer *Mycoplasma bovis* strains

M.M. Biesheuvel[1], P.S. Morley[2], L. Pinnell[2], E. Doster[2], H.W. Barkema[1], R. Valeris-Chacin[2]

[1]Faculty of Veterinary Medicine, University of Calgary, [2]VERO program, Texas A&M University. marit.biesheuvel@ucalgary.ca

Session: Diagnostic Testing, 2023-01-22, 6:00 - 8:00

Objective: *Mycoplasma bovis* infections in dairy herds can lead to clinical symptoms such as mastitis, arthritis, and pneumonia. Additionally, certain cows become subclinical intermittent shedders. Moreover, recent research revealed significant variation in transmissibility among 20 clinically infected dairy herds. This variability is not well understood, but it is hypothesized that strain differences could explain most of this variation, rather than external farm management factors. However, identifying strains after culture is subjective to numerous biases, and becomes especially challenging without culture. Until now, an efficient and accurate method to distinguish and identify *M. bovis* strains in metagenomics samples is lacking. Therefore, the objective of this study was to evaluate the *in silico* performance of a targeted enriched metagenomics method to infer the number and identity of milk-derived *M. bovis* strains.

Methods: Milk-isolated *M. bovis* genomes (n=162) were downloaded from NCBI. The genomes were categorized into phylogenetic cluster variants (PCVs), defined as having >97% genomic similarity, to assess the strain-level diversity. Genomes from distinct clusters were selected to model various scenarios of *M. bovis*co-infections, with the number of co-infecting PCVs set at 1, 3, 6 and 9. The enrichment of *M. bovis*-specific reads was simulated at four different percentages (30, 50, 70 and 90%). The sequencing depth was randomly drawn from a Poisson distribution with a mean of 20 million reads. For each combination of parameters, 1,000 iterations were performed. Metagenomic datasets were simulated using a non-parametric bootstrapping method during each iteration and were subjected to two rounds of classification using Kraken2. The first round was performed with the default database and the second round with a custom *M. bovis*-specific database to infer the number and identity of the PCVs, which were then compared with the true values.

Results: At a 90% enrichment of *M. bovis*-specific reads, the targeted enriched metagenomic approach consistently allowed for the accurate inference of both the correct number and identity of PCVs in all scenarios, including 1, 3, 6 and 9 PCVs, providing that the sequencing depth was not extremely low. Accuracy in distinguishing both the number and identity of PCVs diminished when the enrichment percentage was set at a lower value, such as 30%, particularly when there were six or more PCVs present in the simulated sample.

Conclusions: Strain differentiation of *M. bovis* is extremely important when it comes to controlling and eradicating infections in dairy herds, as it facilitates the tailoring of farm-specific control measures for efficiently reducing the spread of the pathogen and the number of affected animals in a herd. This targeted enriched metagenomic approach, which enriches *M. bovis* reads, has proven to be successful at identification of strains based on PCVs. Next steps involve applying this approach to field samples taken from animals with various disease presentations and outbreak farms to determine the PCVs associated with distinct clinical outcomes and transmission characteristics.

Financial Support: Industrial Research Chair in Infectious Diseases of Dairy Cattle, funded by Canada's Natural Sciences and Engineering Research Council (NSERC) Industrial Research Chair Program (Ottawa, ON, Canada).

Notes:

P182 - Proof-of-concept computer vision identification of canine external ear canal lesions

Srikanth Aravamuthan[1], Samuel Murray[1], Marlee Henige[1], Dörte Döpfer[1], Neoklis Apostolopoulos[1]

[1]University of Wisconsin-Madison. aravamuthan@wisc.edu

Session: Diagnostic Testing, 2023-01-22, 6:00 - 8:00

Objective: Otitis externa (OE) is a common and multifactorial disease accounting for up to 20% of consultations in small animal practice. Early diagnosis of OE and contributing factors is crucial for proper management, but can often be challenging. As a result, many patients suffer from recurrent otitis, which can lead to antimicrobial resistance, otitis media, otitis interna, and/or irreversible narrowing of the ear canal. Artificial intelligence (AI) is used in human dermatology with great potential and can also be a valuable tool used in veterinary dermatology. We aim to create a proof-of-concept computer vision model for OE detection in the canine ear canal (healthy, ear canal mass, and OE).

Methods: All images were from client-owned dogs from the University of Wisconsin Veterinary Care - Dermatology service. The images were labeled as "Healthy", "Mass", or "Otitis", by a board-certified veterinary dermatologist. We used four datasets to evaluate the effects of dataset size and duplicate images on performance.

- Dataset A (small dataset with duplicates): 2,963 images
- Dataset B (small dataset without duplicates): 251 images
- Dataset C (large dataset with duplicates): 4,342 images
- Dataset D (large dataset without duplicates): 626 images

The model was trained in YOLOv5. The data were split into training (90%) and validation (10%) datasets. The prediction performance metrics used to evaluate the models include mean average precision (mAP), precision (P), recall (R), and average precision (AP) for accuracy.

Results: The existence of duplicates interfered with the model producing high accuracy results due to overfitting (Dataset A and C had mAP50 of 0.96 and 0.958, respectively). The accuracy of the large dataset without duplicates (Dataset D) was better than the small dataset without duplicates (Dataset B). Dataset D had P = 0.703, R = 0.768 and mAP50 = 0.733, and was the most clinically relevant model. Dataset D can be further finetuned to improve accuracy for the detection of otitis.

Conclusions: These results show that this novel object detection model has the potential for clinical application in veterinary medicine. The addition of more images would make fine-tuning of the model. Further work will incorporate additional classes and clinical recommendations.

Notes:

P183 - Development of an Ion Torrent Targeted NGS panel for canine infectious neurological and reproductive pathogen detection

Jobin J. Kattoor[1], Rebecca P. Wilkes[1]

[1]Department of Comparative Pathobiology, Purdue University. jkattoor@purdue.edu

Session: Diagnostic Testing, 2023-01-22, 6:00 - 8:00

Objective: The etiology of diseases affecting the reproductive and central nervous systems (CNS) in canines is often multifaceted and largely unknown. In this study, we aimed to develop a targeted next-generation sequencing panel to identify infectious agents related to neurologic and reproductive disease in canines so that infectious causes of CNS and reproductive diseases can be ruled out in a single comprehensive test.

Methods: The devised assay utilizes a targeted amplicon sequencing approach to detect the causative agent while providing valuable genomic data about the pathogen. To achieve this, a complex primer panel for Ampliseq DNA library preparation was meticulously designed in collaboration with ThermoFisher Scientific. This panel comprised 672 primers divided into two separate pools. Following the manufacturer's protocol, barcoded DNA libraries were prepared using the Ion Ampliseq Library kit plus. The normalized libraries were subsequently loaded onto an Ion 530 chip for sequencing on an Ion S5 sequencing system. The raw sequencing data was processed by mapping the reads to the reference genome using SPAdes in the Torrent suite software (v: 5.16.1). The aligned BAM files were then scrutinized using Genious Prime software to perform an initial identification of pathogens. Among the aligned reads, only those reads ≥ 100bp length corresponding to the mapped pathogens were used to confirm the results using the online BLAST tool hosted in the NCBI web server. Additionally, the assay was employed to screen a limited number of clinical samples, which consisted of cerebrospinal fluid (CSF) samples from cases of unknown meningoencephalitis.

Results: This assay was able to detect a panel of seventeen different pathogens known to affect the reproductive and nervous systems, including viruses, bacteria, and parasites such as Rabies virus, Canine distemper virus (CDV), Canine parvovirus (CPV), Canine herpesvirus (CHV), Eastern equine encephalitis virus (EEE), West Nile virus (WNV), Canine adenovirus 1, *Toxoplasma*, *Leptospira*, *Neospora caninum*, *Anaplasma* spp., and *Babesia* spp. The analytical sensitivity was evaluated by comparing the Cycle threshold (Ct) values of the tested pathogens through the dilution of positive test samples. All pathogens exhibited a detection limit corresponding to a Ct value in the mid-thirties, except Canine Influenzavirus (23.2) and Bluetongue virus (25). Among the 22 screened clinical specimens, 1 sample revealed the presence of Am-5 genotype of CDV. This assay was not only capable of pathogen detection but also could identify specific genotypes in certain pathogens like CDV, CPV, and Canine Influenzavirus, offering valuable insights into the circulating strains.

Conclusions: The turnaround time for obtaining results, from sample extraction to the final report, was impressively short at just three days, making the assay more efficient and cost-effective compared to conventional molecular diagnostic techniques when used separately. Altogether, this assay represents a rapid, sensitive, and cost-effective alternative to current conventional molecular diagnostic methods.

Notes:

3MT01 - Harnessing the power of commensal bacteria in combating infections

Isabel Tobin[1]

[1]Oklahoma State University. isabel.tobin@okstate.edu

Session: 3MT Competition, 2023-01-20, 5:05 - 5:10

Necrotic enteritis (NE), caused by *Clostridium perfringens*, is one of the most economically devastating diseases in poultry, costing the global poultry industry $6 billion each year. However, there are currently no effective preventive or therapeutic measures available. The intestinal microbiota has the ability to resist pathogen colonization and plays a crucial role in maintaining animal health and productivity. We recently screened a library of the cecal bacteria from healthy feral chickens and identified several bacteria, including *Megasphaera stantonii*, that have a strong ability to inhibit *C. perfringens* and also enhance innate immunity by inducing host defense peptide synthesis. Notably, oral administration of *M. stantonii* significantly improved animal survival and alleviated intestinal lesions in a chicken model of NE. These findings provide a timely opportunity to investigate the potential of *M. stantonii* in resisting NE. The outcomes will pave the way for developing a novel antibiotic-free approach to mitigate NE and possibly other enteric diseases in poultry. With rapid emergence of antibiotic-resistant pathogens and an increasing concern over the use of in-feed antibiotics, this line of research will have enormous implications by ensuring animal health, production efficiency, and food safety while reducing antimicrobial resistance.

Notes:

3MT02 - Friend for life: how porcine circovirus (PCV) replicase facilitates the replication of torque-teno viruses (TTV)

Md-Tariqul Islam[1]

[1]North Dakota State University. mdtariqul.islam.1@ndsu.edu

Session: 3MT Competition, 2023-01-20, 5:10 - 5:15

Upon its discovery in 1997 as a possible cause of post-transfusion hepatitis; Torque teno virus (TTV) has been extensively associated with illness in humans, non-human primates, livestock and companion animals. Epidemiological evidence suggesting single-stranded, circular DNA Torque-teno sus virus 1 virus (TTSuV1) have been frequently co-detected in polymicrobial infections with swine influenza virus (SIV), porcine circovirus type 2 (PCV2) in porcine respiratory disease complex. It is suspected to exacerbate clinical signs during co-infections but difficulty in culturing TTV's in cell culture system and a lack of animal models are the roadblocks of understanding TTV's role in infection and co-infections. We and several other investigators observed progressive loss of viable recombinant virus after rescue from transfected cell line and this could be due to inadequacy of TTV viral replicase protein that drives viral genome replication and protein expression. Since the molecular mechanisms involved yet not known, we hypothesized that PCV replicase protein has trans replicase activity for TTSuV1 and thus supports TTSuV1 replication in co-infections. The basis was both TTSuV1s and PCVs have similar genome organization, frequently co-detected in polymicrobial infections, and both replicate by rolling-circle mechanism so replicase protein of both viruses is interchangeable i.e. the PCV1 replicase protein can enhance TTSuV1 replication. To test our hypothesis, the PCV1 replicase (rep) protein was transiently over-expressed in porcine kidney (PK-15), swine testicular (ST), and PCV1 positive PK-15 cell lines, either singly or in combination with a cloned, dimerized TTSuV1 genome. A 1-1.5 $\log_{10}$ significant difference in TTSuV1 viral titers and genome copy numbers was observed between co-transfection of the TTSuV1 genome and PCV1 replicase compared to the single TTSuv1 genome alone. Detection of intracellular TTSuV1 antigen by flow cytometry reveals that presence of PCV1 or PCV1 rep protein enhances TTSuV1 replication by 25%. ST cells supported higher levels of TTSuV1 replication than PK-15 cells across all-treatment conditions. When plasmids encoding the PCV1 rep and a TTSuV1 infectious clone were administrated as co-infection and single infection in two-week old C57/BL6J mice, the presence of PCV1 rep increased the TTSuV1 antigen detection. Similar to in-vitro findings, dually exposed experimental mice had 1-$\log_{10}$ higher mean viral loads in all time points compared to single infection except 30-day post infection (DPI). TTSuV1-specific IgG responses were observed in both treatment group as evidenced by sero-conversion between 0-14 DPI along with qPCR data showed that productive viral replication had occurred. In conclusion, our study provides novel insights on molecular interactions between TTVs and PCVs in co-infections using a helper replicase protein. Therefore, this study is the first to demonstrate trans-replicase activity between different genera of mammalian small DNA viruses and provides a possible explanation for increased co-morbidity between TTV and PCV2.

Notes:

3MT03 - A path to quantifying beef cattle sustainability: Evaluating management decisions and impacts

Taylor McAtee[1]

[1]Kansas State University. taylormcatee@vet.k-state.edu

Session: 3MT Competition, 2023-01-20, 5:15 - 5:20

In recent years, sustainability issues have emerged as crucial for the competitiveness of the beef industry, including stakeholders such as allied industry, beef producers, and government entities. Sustainability is a complex term and involves multiple broad components such as social, economic, and environmental aspects. My research focus is to address critical sustainability issues facing the beef industry by enhancing animal health, animal welfare, and production efficiency in cattle feeding. Understanding that the topic of sustainability is complex and broad with multiple components, my research aims to use an outcomes research approach towards sustainability. This type of approach may potentially provide a framework for comprehensive assessment of animal health and management strategies, while also quantifying these values. Currently there is a lack of practical insights on how sustainability relates to beef cattle, specifically in feedlot cattle. Thus, two examples of my work below highlight the diverseness of sustainability research, and how it is intertwined with beef production.

The primary focus of the first abstract lies in delving into observational data from a commercial feedlot trial to discern factors associated with estimated greenhouse gas emissions. The data includes animal health information, performance metrics, economics, and estimated greenhouse gas emissions. Utilizing various metrics to evaluate sustainability and value for stakeholders will help to generate insights for industry improvement, fill information gaps regarding cattle production, animal health management, and the associated environmental impacts. The overall goal is to quantify the sustainability value and associations of the parameters described above and enable more informed decisions by stakeholders.

The second abstract is a randomized controlled target to evaluate an intervention targeted towards reducing emissions. The intervention is a new feed additive, lubabegron, that is labeled to reduce ammonia emissions per pound of live carcass weight. With my research, we have observed an increase in animal performance when compared to a atypical beta-agonist. This is a more applied approach, by directly evaluating a new feed additive technology and working to reduce emissions. The increased efficiency of lubabegron fed cattle, along with reduction in estimated greenhouse gas emissions, and increase in net returns incorporates the three pillars of sustainability: economic viability, social acceptability, and environmental responsibility.

Both abstracts exhibit the underscore the central theme that my research is established in, sustainability is complex with many interrelated factors. Just as stakeholders within the beef industry have a vested interest in sustainability, so do beef producers. My research encompasses all facets of beef production, including animal health, animal welfare, production efficiency, and greenhouse gas emissions. These are essential for ensuring the competitiveness of the beef industry, but also for advancing sustainability in the industry. By examining the intricate relationships between beef production and sustainability, my work can help to advance and improve both.

Notes:

3MT04 - An evaluation of extending the finishing period of feedlot cattle and frameworks for economic assessment of controlled trial data

Lucas Horton[1]

[1]Kansas State University. lhorton@vet.k-state.edu

Session: 3MT Competition, 2023-01-20, 5:20 - 5:25

In recent years, there has been shift in how feedlot cattle are managed, emphasized by longer feeding resulting in heavier endpoints. For producers and other industry stakeholders, questions on the implications of this practice remain. Outcomes research under the construct of epidemiological research methodology provides confidence in research findings for evidence-based decision-making, which is accentuated by a variety of monetary and non-monetary values. Under this framework, objectives were to evaluate physiological and economic effects of extending feedlot cattle days-on-feed, and to introduce and describe economic methodologies for appropriate assessment of clinical livestock research, striving for more comprehensive evaluations.

Many critical outcomes can be conceived and are frequently reported in animal production research; their relative importance may vary depending on stakeholder views (e.g., producer vs consumer vs policymaker). While research findings often show substantial biological meaning (e.g., environmental sustainability, production characteristics, animal health), it is often challenging for producers to implement new interventions without an economic incentive. It has been reported that economic outcomes are infrequently included along with primary outcomes in animal production trials; when they are, methodologies are often inconsistent and insufficiently described, leading to irreproducible results. This spurred a need to develop and describe reporting recommendations for economic analyses in clinical livestock trials. Alternative approaches exist (e.g., cost-benefit analysis, partial budgeting), and an example of implementing a partial budget with clinical trial data was described, along with recommended methodological reporting guidelines for critical evaluation and reproduction purposes. A gap was also noted, being that there was no reference or resource for estimating revenue from cattle removed from beef production trials for health reasons (i.e., culls, railers). We described feedlot cull populations based on cohort-level characteristics, revenue received, and associations with other US beef markets. Revenue received from feedlot culls was most highly correlated with cull cow prices, which we found could be adjusted (based on animal weight) and used to more confidently estimate revenue.

With a framework in place, economic evaluation feedlot cattle endpoint management could proceed. There are many physiological considerations when managing cattle endpoints, all of which lead to substantial economic implications. When feeding for longer days-on-feed, physiological changes include reduced live feed efficiency and daily gain, while concurrently, increases in live and carcass weight gain, numerical yield grades, and quality grades occur. We then evaluated how a variety of pricing conditions for differing economic variables may influence net returns when feeding cattle for different lengths of time using randomized controlled trial data. These evaluations were performed for both steers and heifers, for which we found differing considerations should be made when targeting optimal harvest endpoints.

To summarize, this thesis targeted a timely and relevant theme of feedlot cattle management. In addition to characterizing key components when extending the finishing period of feedlot cattle, a framework for economic assessments was developed and implemented, providing a tool to aid in producer and stakeholder decision-making ability. This framework may also help guide future researchers with the conduct of economic analyses with clinical livestock trial data.

Notes:

3MT05 - Trans-cinnamaldehyde nanoemulsion: A promising alternative to control *Salmonella* Enteritidis in the poultry industry

Trushenkumar Shah[1]

[1]University of Connecticut. trushenkumar.shah@uconn.edu

Session: 3MT Competition, 2023-01-20, 5:25 - 5:30

Have you ever thought, your favorite chicken wings or chicken burger can make you sick? probably not. However, this can happen due to the consumption of contaminated chicken meat with Salmonella enteritidis. As per CDC, it is estimated that *Salmonella* causes over 1 million illnesses and more than 400 deaths every year, so this situation warrants the development of the robust strategy to control salmonella outbreaks.

Now, the question is why do we have to focus on chickens to control *Salmonella* outbreaks? Though, the chickens are considered as reservoir host for the salmonella therefore chickens can act as potential source for the *Salmonella* transmission from farm to fork. Other than that, the characteristics of *Salmonella* pathogen is, it does not only remain as planktonic cells but it attaches to the surface and is covered by protein, exopolysaccharides, and eDNA and form sanitizer-tolerant Biofilm. By considering all these issues associated with salmonella, it is required to develop novel interventions that should be safe, effective, and user-friendly. In this regard, for my project, I use trans-cinnamaldehyde (TC) oil that is obtained from cinnamon bark. In spite of having strong antimicrobial efficacy of TC, the major challenge with trans-cinnamaldehyde oil is having low solubility in water. So, the question is how we can overcome this challenge? to find the best solution to this issue, we developed TC nanoemulsion (TCNE) by using high energy method of sonication.

There are main two objectives in my thesis. In the first objective, I tested the efficacy of TCNE on matured *Salmonella* biofilm developed on steel and plastic. Whereas in the second objective, we explored the efficacy of TCNE as water supplementation to reduce colonization of *Salmonella in* broiler chickens.

For objective 1, we observed that TCNE reduced *Salmonella* count by 4 logs on steel whereas 3 logs on plastic surface. On confocal microscopy, we observed dead *Salmonella* with TCNE treatment. In addition, we observed that TC significantly downregulated the expression of *Salmonella* genes which are critical for biofilm formation. Moreover, TC nano emulsion does not cause corrosion on steel and degradation on plastic.

In objective 2, I observed that water supplementation of TCNE reduced the colonization of *Salmonella* in broiler chickens by 2 logs and we did not observe reduction in body weight, FCR, or water consumption. In conclusion, the success of this project will help poultry farmer to control *Salmonella* transmission and will provide safe chicken meat for human consumption.

Notes:

3MT06 - MicroRNAs detected in bovine colostrum can be associated with cancer biology

Luciana Mayer Kluppel[1]

[1]Texas Tech University. luciana.kluppel@ttu.edu

Session: 3MT Competition, 2023-01-20, 5:30 - 5:35

Bovine colostrum is a highly nutritious fluid that contains essential biologically active molecules to protect offspring from diseases. One of these molecules is microRNAs, which are small molecules that play a vital role in regulating genes and ensuring the immune system functions properly. These microRNAs can be responsible for regulating up to 60% of human genes. Our group isolated these small molecules from the colostrum of Holstein dairy cows. Some of the isolated microRNAs have been linked to cancer biology in the past. These isolates, which have been associated with both bovine and human colostrum, play an important role in suppressing cancer development. The discovery of high levels of microRNA in colostrum indicates that these bioactive molecules could be potential therapeutic and prognostic markers for a variety of cancers. If this is true, this could lead to early diagnosis of certain cancers in both animals and humans. Our goal is to demonstrate the role of these small molecules isolated from bovine colostrum in cancer development and progression, highlighting the potential of microRNAs as oncogenic markers by which can be transferred from the mother to the offspring via colostrum intake.

Notes:

3MT07 - Harnessing gut microbiota: Advancing poultry health with microbiome-based approaches

Jing Liu[1]

[1]Oklahoma State University. jing.liu12@okstate.edu

Session: 3MT Competition, 2023-01-20, 5:35 - 5:40

Antibiotics have been extensively used in the livestock industry for growth promotion and disease prevention. However, concerns over antimicrobial resistance have led to the phasing out of this practice in many countries, including the U.S. As a result, there is an urgent need for alternatives to antibiotics to maintain animal health and productivity. My research focuses on microbiome-based approaches to enhance feed efficiency, growth performance, and combat necrotic enteritis (NE) in chickens, providing a promising antibiotic-alternative solution.

Through my investigations, I have identified a number of bacteria that are differentially enriched in the intestinal tract of broiler chickens with extremely high or low residual feed intake. I also found that *Anaerobutyricum* and *Subdoligranulum* are differentially enriched in a commercial set of broilers with disparate weight gains. These gut bacteria I identified could be targeted for manipulation to improve the growth rate and production efficiency of broiler chickens.

Currently, I am working on a project to identify the gut microbiota and metabolites that are associated with resistance to NE using different inbred lines of chickens. NE, an inflammatory gut infection in chickens caused by *Clostridum perfringens*, is responsible for a loss of US$6 billion per year in the poultry industry. Conventionally, NE can be effectively controlled by antibiotics. However, due to the increasing prevalence of antibiotic-resistant bacteria, regulatory and voluntary restrictions on using in-feed antibiotics have been set in place. Thus, NE has become a primary concern impairing poultry production in the post-antibiotic era. Identification of NE-associated gut bacteria and bacterial metabolites marks a first step toward developing novel probiotics or postbiotics as antibiotic alternatives for growth promotion and disease prevention. In our recent results, we observed significant differences in NE resistance among highly inbred chicken lines with similar genetic backgrounds. Furthermore, we found that transferring the cecal microbiota of one breed of NE-resistant chickens conferred impressive 100% protection to naïve animals, with either no or mild intestinal lesions, while approximately 40% of chickens died from severe intestinal lesions without microbiota transplantation. These findings highlight the critical role of intestinal microbes in disease prevention.

As my research progresses, I am committed to unraveling the full potential of gut microbiota as an antibiotic-alternative approach. By shedding further light on the intricate interplay between gut microbes and poultry health, we can develop novel probiotics and postbiotics that will redefine the poultry industry and safeguard its productivity in the post-antibiotic era.

Notes:

3MT08 - Feathers, fields, and fowl cholera: Unveiling the environmental role in fowl cholera outbreaks

Lingyu Ouyang[1]

[1]The Ohio State University. ouyang.193@osu.edu

Session: 3MT Competition, 2023-01-20, 5:40 - 5:45

This research initiative explores the nuanced relationship between environmental features, specifically land cover, and the incidence of fowl cholera, a poultry disease caused by *Pasteurella multocida* (PM). The objective is to gain a clear understanding of how varying types of land cover and their proximity to poultry farms impact the transmission risk of PM. We analyzed PM incidence data from a private US poultry production company and National Landcover Database features to obtain a comprehensive picture of this relationship.

Located on the intricate intersection of geography and veterinary medicine, this project underscores the subtle yet important influence that land cover can have on poultry health. By focusing on the Ohio-Indiana border region, an important hub for the US poultry industry, the work unfolds a compelling story of how landscapes can shape and facilitate potential risk zones for disease outbreaks. The knowledge gleaned from this study holds the potential to inform industry best practices, enable preventive measures, and thereby safeguard our poultry supply chain.

Preliminary results suggest a significant association between land cover and fowl cholera outbreaks. Of particular note, woody wetland, pasture/hay, and deciduous forest land cover types appear to play a substantial role in disease transmission. Notably, the effect of these land covers appears to lessen beyond a 5 km radius, suggesting a potential relation to distance.

Notes:

3MT09 - Viral versatility: Unleashing the power of mosaic DNA vaccines against Avian Influenza

Bubacarr JB Touray[1]

[1]University of Wisconsin-Madison. btouray@wisc.edu

Session: 3MT Competition, 2023-01-20, 6:00 - 6:05

Hello, everyone. I'm excited to share a groundbreaking concept with you today—a mosaic DNA vaccine designed to revolutionize our approach to combating avian influenza. Picture a future where a single vaccine can effectively target multiple strains and clades of this notorious virus, providing a more comprehensive and adaptable solution to the ongoing threat. Let's dive in.

Avian influenza remains a global concern, impacting poultry industries and posing a zoonotic risk to humans. Traditional vaccines often struggle to keep up with the virus's rapid mutation, making our strategies less effective over time.

Our innovation is a mosaic DNA vaccine—a game-changer in the world of avian influenza prevention. Instead of relying on a single strain or clade, our vaccine incorporates genetic sequences from multiple strains and clades of the virus.

This approach offers unprecedented versatility and coverage. It essentially creates a composite vaccine that can recognize and neutralize a broader range of avian influenza variants. No more playing catch-up with constantly evolving strains.

Now, let me explain how it works. The mosaic DNA vaccine is administered, and within the host, it instructs cells to produce a mosaic hemagglutinin (mHA) protein—a chimeric protein that incorporates critical elements from various virus strains.

When exposed to mHA, the host's immune system launches a robust defense. It recognizes the shared elements across different influenza strains and clades, effectively training the immune system to respond to a broader spectrum of the virus.

We've conducted promising pre-clinical trials with avian models. Our mosaic DNA vaccine consistently demonstrated higher efficacy compared to traditional single-strain vaccines. It's a significant step towards a more effective and adaptable solution.

Beyond poultry, our mosaic DNA vaccine has exciting potential for human vaccines. In a world threatened by pandemics, having a flexible, multivalent vaccine platform is invaluable.

We're actively seeking collaborations with experts in virology, immunology, and vaccine development to accelerate this breakthrough. Together, we can refine our approach, conduct more extensive trials, and bring this innovation to the forefront of avian influenza prevention.

In conclusion, our mosaic DNA vaccine is poised to reshape how we combat avian influenza. It offers the adaptability and coverage needed to stay ahead of this ever-changing threat. Join us in this mission to transform the future of avian influenza prevention and protect both poultry industries and human populations worldwide.

Thank you for your time. Together, we can make the world safer from avian influenza, one innovative vaccine at a time. Let's connect and explore the endless possibilities that lie ahead.

Notes:

3MT10 - Liver abscesses: Causes and prevention

Daniel Young[1]

[1]West Texas A&M University. jdyoung3@buffs.wtamu.edu

Session: 3MT Competition, 2023-01-20, 6:05 - 6:10

Liver abscesses (LAs) are one of the most important and costly problems of the North American beef industry, as well as one of the most challenging diseases to study. Liver abscesses were first documented in the scientific literature in the 1800s and have been intensively studied for more than 70 years, but we are not better at preventing their occurrence than we were when dietary supplementation with antimicrobial drugs was introduced in the 1950's. This intervention is still the most efficacious method for prevention, but it does not eliminate disease occurrence and will likely become untenable to consumers and public health officials. The FDA is working with the beef industry to identify ways that use of antimicrobial drugs approved for preventing LAs can be used in lesser amounts while maintaining efficacy. In response to this research call, we designed a trial to evaluate different durations of use tylosin phosphate and evaluated liver abscess occurrence and feeding performance. Additionally, we have also engaged in research to better elucidate the causes of liver abscesses which will allow identification of alternative prevention methods. It has long been believed that abscesses are caused by a few species of bacteria that are translocated from the rumen into the portal circulation and then infect the liver. However, recent molecular investigations of LAs have revealed highly polymicrobial abscess communities that do not reflect the rumen microbiome composition. As such, we have investigated the microbial community structures at 10 sites throughout the gastrointestinal tract (GIT). This work identified microbial populations in the other gut locations that reflect microbial communities found in major portions of LAs suggesting that the small intestine and colon may be common sources of bacteria that cause LAs. While the investigations of the gut microbiome suggested additional locations of bacterial translocation, there was still a lack of evidence for how it was occurring. In general, tight junction proteins of the gut are thought to be the regulators of barrier function. But to be able to evaluate the differences between diseased animals and healthy animals, we needed to establish a baseline. Therefore, we sampled gastrointestinal contents and tissues from steers originating from 21 different feedlots across West Texas, to characterize the "normal" gut of feedlot cattle. Using histology, RT-qPCR, immunohistochemistry, *in situ* hybridization, and 16S rRNA gene sequencing, this study evaluated the microbiome, tissue morphology, tight junction gene expression, and tight junction protein expression in one of the most comprehensive and holistic studies of mucosal barrier of GIT in mature cattle. This baseline characterization of normal gut barrier will allow future work to evaluate circumstances when these natural defenses fail. With this information, we will be able to test a broad range of interventions for liver abscesses to reduce our dependence on antibiotic use.

Notes:

3MT11 - Characterization of the microbiome and resistome in young foals and calves

Maggie Murphy[1]

[1]West Texas A&M University. mmmurphy1@buffs.wtamu.edu

Session: 3MT Competition, 2023-01-20, 6:10 - 6:15

Changes in the gut microbiome development and composition can be associated with lasting effects on the established microbiome composition, which can in turn effect the health and performance of animals. Effective characterization of this development from an early age through stabilization could more effectively aid owners and producers in making decisions for health management. Concerning food-production animals, the development of resistance genes is under scrutiny by the public.My dissertation is focused on characterizing the microbiome and resistome of young foals and their dams as well as young dairy calves that have not been exposed to antimicrobial drug administration.

Do young dairy calves that have never been exposed to antimicrobial drugs (AMD) harbor antimicrobial resistance genes (AMR)? In order to answer this question, my research team and I collected fecal samples from young dairy calves that range in ages from 2 d to 14 wks of age to answer this question. Interestingly enough, they have a resistome that is higher in richness and diversity compared to the microbiome at an early age. These calves have never been exposed to AMD yet displayed amounts of AMR genes, which could indicate that certain microbes may possess AMR genes and inhabit the gut early on. To answer a similar question in young foals, fecal collections from 14 dam and foal pairs were analyzed to characterize the microbiome and resistome from d 0 to 120 d of age. In addition to this study, fecal samples were also collected on foals that had varying relatedness to each other at d 14, 30, and 60. These foals ranged in relatedness from half siblings, full siblings, unrelated, and clones (ie. Genetically identical foals). Three of the clones featured in this study originated from tissue in a Przewalski horse that was dead for 40 years. Microbial richness significantly increased as microbial community structures changed over time across the first 4 sampling timepoints, and then stabilized. Interestingly, resistome richness followed an opposite pattern; decreasing significantly between day 28 and 60. The composition of the resistome changed significantly between day 28 and 60, but remained stable afterwards. The microbiomes and resistomes of the dams remained stable throughout the study. Microbiome and resistome composition concerning the relatedness of these horses is still on going.

Notes:

3MT12 - "Connecting the dots" to fight disease spread

Sara Sequeira[1]

[1]The Ohio State University. correiasequeira.1@osu.edu

Session: 3MT Competition, 2023-01-20, 6:15 - 6:20

Let's picture this scenario: There is a new disease outbreak sweeping in the United States with reported human infections that are resistant to antibiotics. What does that mean exactly? It means there will be very limited options or even none to treat those infections. Shockingly, it was estimated that by 2050, resistant infections will be the number one cause of deaths globally and, therefore, this problem should be stopped immediately! But how do resistant bacteria spread in populations?

One of the contributors to this threat are baby calves from dairy farms, which are often overlooked. These young animals often leave the farm within the first days of life, with weak immune systems and frequently carrying high levels of bacteria that are resistant to drugs. These calves travel through a vast network of stakeholders, allowing for resistant bacteria to spread in several ways. Other animals and humans can not only become infected through direct contact with the calves and contaminated environment but also through ingestion of contaminated meat and crops.

Now, going back to the outbreak, if all the "dots" of this animal movement network are well known, disease spread seems easy to stop right? Just like when we know all the stops of a subway route and can point exactly where to stop on the way: if we have all the "dots" for calf movements, we can track where the animals came from and make sure we identify the best places to apply measures to prevent further spread. But what if the current available data is not comprehensive or representative enough?

Preliminary findings from my research, conducted in collaboration with the state of Ohio, reveal a troubling reality: We're likely missing half of the network when relying on "official" movement data. In other words, patterns of the network focused solely on standardized documents, often used in previous work, were largely different from those based on combined, more extensive, document sources. As the system is currently rudimentary and heavily dependent on paper-based documentation, which is often illegible, there is an urgent need for improvement.

My mission is to keep connecting the dots and establish, for the first time in the literature, the extent of calf networks in the US. By "playing detective" and solidifying this network, I plan to create a tool (i.e., a model) that uses this data to benefit animal and human health. If this tool works, we will not only be able to readily identify sources of infection, but also localize potential super-spreaders within this network. This will help prioritize cost-effective strategies to reduce disease spread and control the progression of resistance to antibiotics.

In the end, believe me, "connecting the dots" saves people!

Notes:

3MT13 - An epigenetic approach to develop antibiotic alternatives

Melanie Whitmore[1]

[1]Oklahoma State University. melanie.whitmore@okstate.edu

Session: 3MT Competition, 2023-01-20, 6:20 - 6:25

Our immune system is the armor that protects us from getting sick. When that armor is not strong enough, a doctor can prescribe antibiotics to help combat bacterial infections. Similarly, antibiotics used to be added to the feed of livestock to promote growth and prevent diseases. However, a biological warfare exists between antibiotics and bacteria. When an antibiotic is used long-term, the bacteria may develop a resistance mechanism to avoid being killed. In fact, a majority of bacteria found in hospitals have become resistant to at least one antibiotic. Especially alarming is the rapid rise of so-called “superbugs” that resist the actions of multiple antibiotics and cause infections nearly impossible to treat. The World Health Organization (WHO) has declared that antimicrobial resistance is one of the top 10 global public health threats facing humanity. Because of this, most antibiotics have been banned from livestock production in the U.S. since 2017. So what can we do to keep livestock animals healthy and productive?

This is where my research comes in. I have been developing antibiotic alternatives for livestock by reinforcing the armor of their immune system. More specifically, I want to increase the production of host defense peptides, an important component of animal innate immunity. Not only can these small peptides directly kill bacteria, they also activate other parts of the immune system to help fight off infections.

Our lab developed a high-throughput screening system to test more than 5,000 chemicals. To our surprise we have found scores of epigenetic compounds to be highly efficient in boosting the production of host defense peptides. These epigenetic compounds regulate the amount of a gene product made by changing how the DNA is packaged. The transition from tightly packaged DNA to a more relaxed state increases gene expression. Remarkably, these epigenetic compounds work even better in combination than they do individually. For example, if you combine butyrate and BIX01294, DNA becomes more relaxed and you see a dramatic synergistic increase in host defense peptide expression. I am testing different compound combinations in cell culture to find the best candidates to further evaluate in animals. It will be a dream come true for me if some of these epigenetic chemicals are eventually used in the livestock industry as antibiotic alternatives to prevent and control infections. If they work in animals, I believe they could work in humans, too.

Notes:

3MT14 - Isolation and characterization of bovine coronavirus strains from dairy cows, dairy calves, and beef cattle

Yu Li[1]

[1]The Ohio State University. li.13226@osu.edu

Session: 3MT Competition, 2023-01-20, 6:25 - 6:30

Bovine coronaviruses (BCoV) are prevalent worldwide and cause enteric or/and respiratory diseases in cattle. However, the mechanisms determining the major infection site are unknown. Until now, it has been around half a century since the first BCoV strain was discovered. There are still big knowledge gaps for BCoV pathogenesis. In this study, we isolated BCoVs in HRT-18 cells. The positive rate of BCoV in these farms was 14/89 (15.73%) of dairy calves, 1/39 (2.56%) of beef cattle, and 0/38 (0%) of dairy cows. We successfully isolated 5 BCoV strains in HRT-18 cells. The growth kinetics showed that the BCoV isolate BC7 reached the peak titer of 6 log10 plaque forming unit (PFU)/mL at 48 hpi. According to the phylogenetic tree, the BCoV isolates were clustered in the GIIb group with the North American historical strain DB2. The new isolates shared 98% nucleotide identity with the prototype Mebus strain. We also compared the spike (S) proteins of one nasal sample and one fecal sample from the same farm. There was only one amino acid difference in the S protein (617-T for the respiratory and 617-I for the enteric sample). This study will contribute to the molecular study of BCoV and the relationship between respiratory disease and enteric disease using the BCoV model.

In this 3MT competition, I will show what we found in the genomic difference between the nasal samples and enteric samples to explore the mechanism of respiratory and enteric disease. Why what we do is essential? During the isolation of BCoV, I also observed the BCoV plaque is different from PEDV, which cannot kill the HRT-18 cell. Why will this happen in BCoV? What can we do based on this phenomenon? Maybe the BCoV has the potential to be a polyvalent vaccine vector.

I will talk about all the interesting stories during my research and what I found in this project. Research is not a boring thing, all big achievements come from every small observation and thinking!

Notes:

3MT15 - Early detection of digital dermatitis in dairy cows using computer vision

Srikanth Aravamuthan[1]

[1]University of Wisconsin-Madison. aravamuthan@wisc.edu

Session: 3MT Competition, 2023-01-20, 6:30 - 6:35

Digital dermatitis (DD) is a bovine claw disease responsible for ulcerative lesions on the coronary band of the hoof. DD is associated with massive herd outbreaks of lameness and influences cattle welfare and production. Lost productivity, labor, treatment or preventive measures, and other indirect economic losses attributable to DD cost the US cattle industry hundreds of millions of dollars annually. Early detection of DD can lead to prompt treatment and decrease lameness.

Currently, DD lesions are detected through visual inspection and scored using the M-stage classification system by trained investigators. However, early identification and prompt intervention require extensive training in an industry with a high turnover rate. Moreover, visual inspection is time-consuming, energy-intensive, inaccurate, inconsistent, and lacks the ability to perform early detection. Therefore, computer vision (CV) provides a unique opportunity to improve detection and optimize treatment of DD.

CV can learn the shape, size, and color of the target object in digital images and videos, distinguishing the target object from other objects and the background. Object detection can be used to precisely monitor animal health and accurately diagnose a variety of medical conditions. This technique assists in identifying illnesses and infections, reducing the need for invasive diagnostic tests. By automating visual observation tasks, object detection decreases the time and energy needed for diagnosis using standardized clinical signs that are otherwise difficult to see and may go untreated. Early detection and automatization are essential for underserved and underprivileged areas where veterinary services for food animal production are increasingly scarce.

Consequently, we have designed and developed a workflow to train lightweight CV models for constrained environments and deploy the best CV model on edge devices or as web and mobile applications for the real-time detection of DD in dairy cows. CV models were trained for detection and scoring of DD, compared using performance metrics and inference time, and automated for real-time detection using images and video streams on portable devices. Farms require robust solutions that can be deployed in harsh conditions including dust, debris, humidity, precipitation, and other equipment issues. Portable devices offer advantages in terms of scalability, flexibility, energy efficiency, and privacy. The CV model was able to detect the M-stages of DD on portable devices with high performance with an accuracy of approximately 90% and high speed with an inference time of 40 frames per second.

We have demonstrated that the deployed model can be a low-power and portable solution for real-time detection of DD on dairy farms. This result is a significant step towards applying CV algorithms to veterinary medicine and implementing real-time detection of health outcomes in precision farming. It can help producers, herdsmen, and veterinarians make informed decisions regarding the diagnosis and customize treatment plans according to severity. We hope the proposed tool can be employed in combination with current practice for the prevention of DD in dairy cattle. Ultimately, object detection can help identify high-risk cattle for DD, monitor herds with endemic DD, and improve cattle welfare via on-farm decision-making processes.

Notes:

3MT16 - Wildfires and dairy cattle: A burning issue

Alexandra Pace[1]

[1]University of Idaho. pace4745@vandals.uidaho.edu

Session: 3MT Competition, 2023-01-20, 6:35 - 6:40

Severe wildfires are becoming commonplace as a result of climate change. The dairy industry is challenged with adjusting to extreme environmental conditions arising from climate change, while simultaneously addressing consumer demands for healthy and ethically-produced dairy products. Wildfires produce smoke, which travels to locations far beyond its origination, causing particulate matter air pollution ($PM_{2.5}$). Wildfire-$PM_{2.5}$ inhalation is a health concern for dairy cattle, causing respiratory irritation, alterations in circulating inflammatory markers, and shifts in metabolism in pre-weaned Holstein calves. Yet, the responses within the respiratory tract of calves have yet to be described, which must be evaluated in order to combat health and welfare impacts of calves exposed to wildfire smoke. To evaluate this, 17 Holstein heifers were observed from birth to 90-d of age, which spanned the duration of the region's wildfire season. Weekly thoracic ultrasounds (TUS) were performed, which allowed for lung imaging before (baseline), during, and after wildfire smoke events. With these ultrasound images, severity and extent of lung consolidation were evaluated, which are indications of inflammation. On a subset of 13 heifers, trans-tracheal wash (TTW) fluid was collected once before, during, and after wildfire smoke events, capturing changes in white blood cell populations within the respiratory tract. The behavior of the heifers was monitored continuously using accelerometers. Lastly, blood was collected from the heifers, and white blood cell populations were isolated to evaluate gene expression of inflammatory markers. Environmental data were recorded hourly, including temperature and humidity, which were used to calculate temperature-humidity index (THI), along with $PM_{2.5}$ concentrations. Data were analyzed using generalized linear mixed models (TTW), or mixed models (TUS, behavior), with each individual calf as a random effect, and $PM_{2.5}$ concentrations, THI, and their interaction as fixed effects. The fluctuations in $PM_{2.5}$ concentrations were confirmed to be derived from active wildfires using HYSPLIT modeling. Wildfire-derived $PM_{2.5}$ concentration reached a maximum daily average of up to 113.5µg/m3, which is over 3x higher than the U.S. EPA's threshold for $PM_{2.5}$-exposure related health risk in humans. When calves were exposed to wildfire-derived $PM_{2.5}$, they had a greater proportion of TTW macrophages as compared to baseline (22.9 ± 7.8% vs. 5.7 ± 0.7%; $P<0.001$) and tended to have a reduced proportion of neutrophils as compared to baseline (67.5% ± 6.6 vs. 86.7% ± 1.8; $P=0.08$). Additionally, during wildfire smoke exposure, calves spent more time standing, and thus, less time lying, on a daily basis ($P < 0.05$). Finally, for several days following wildfire smoke exposure, lung consolidation was greater ($P < 0.05$). Alterations in TTW leukocytes and increased lung consolidation indicate an inflammatory response occurring within the respiratory tract. Concomitant behavioral responses may indicate possible implications on calf health and welfare. Understanding these responses is necessary in order to develop interventions for dairy producers whose animals are affected by wildfire smoke. By finding solutions that protect dairy animal health, welfare, and production during wildfire smoke exposure, I aim to contribute to the dairy industry's resilience to extreme environmental conditions caused by climate change.

Notes:

3MT17 - The unexpected ride of rat hepatitis E virus to humans

Kush Kumar Yadav[1]

[1]The Ohio State University. yadav.94@osu.edu

Session: 3MT Competition, 2023-01-20, 6:40 - 6:45

Four decades have passed since the isolation of the hepatitis E virus (HEV). Research programs have described much of the basic viral biology and epidemiology despite difficulty growing this fastidious virus. While a significant volume of knowledge exists regarding Paslahepevirus due to its association with human disease, rat HEV (Rocahepevirus) has now emerged as a new zoonotic threat requiring increased study to fill knowledge gaps.

Rat HEV appears highly endemic in the rat population in US and throughout the world. Rat HEV was not thought to cross species barriers as defined by lack of viremia, viral shedding from experimentally infected non-human primates or pigs. These findings kept rat HEV off the radar for several years until 2018, when rat HEV was reported to cause liver disease in a solid organ transplant patient in Hong Kong and subsequently additional cases of rat HEV were identified in immunocompetent patients demonstrating mild liver dysfunction. Retrospective studies failed to discover the origin of the spread of this newly infectious rat HEV strain.

This is where my research begins. I am a fourth-year doctoral candidate working at the Center for Food Animal Health under Dr. Scott Kenney on HEV spread and pathogenesis. Our goal was to identify if new emerging strains of rat HEV could spill over into agriculturally important species such as pigs or chickens due to their interaction with rats. Our long-term goal is to determine if agricultural species can harbor rat HEV, potentially serving as an intermediate host for transmission to humans.

We achieved our goals via 7 experiments: (a) Establishing an infectious clone of an emerging zoonotic strain of rat HEV. (b) Screening multiple cell lines originating from humans or animals to determine their susceptibility. (c) Testing chicken susceptibility using RNA transcripts derived from the infectious clone. (d) Determining the transmission capacity of viruses from bird to bird via fecal-oral transmission. (e) Testing infection in gnotobiotic pigs by intrahepatic injection of RNA transcripts. (f) Injection of fecal contents from infected pigs intravenously into conventional pigs to study disease pathogenesis. (g) Identifying whether fecal-oral transmission of the virus occurs from inoculated to naive pigs.

We have found that the newly emerged zoonotic strain of rat HEV is infectious to pigs and chickens. The virus was transmitted to naive birds and pigs suggesting a natural fecal oral transmission could occur. We identified mild hepatitis lesions microscopically and identified the presence of virus in the livers of inoculated and naturally infected animals. Our results describe the potential ability of pigs and chickens to act as an intermediate host transmitting rat HEV to humans. Our findings imply that precautions such as screening of pig and poultry products may be recommended to identify transmission sources into the food chain. Our study forms a basis to establish preventative measures for the control of zoonotic rat HEV and models for studying cross-species transmission determinants. I would like to thank the committee for considering my application for a 3MT at CRWAD.

Notes: