Proceedings

Conference of Research Workers in Animal Diseases
January 18 - 21, 2025

Chicago Marriott Downtown Magnificent Mile
Chicago, IL

CRWAD 2025 – Schedule at a Glance

Saturday, January 18, 2025

8:00 am – 10:00 am	**USDA Project Directors Meeting – Chicago F/G - 5th Floor**
10:00 am – 12:00 pm	**ACVM Board of Governors Meeting – Kansas City – 5th Floor**
10:00 am – 4:00 pm	**NC1202 Annual Meeting – Chicago A/B – 5th Floor**
12:00 pm – 4:00 pm	**Speaker Ready Room – Los Angeles – 5th Floor**
12:00 pm – 3:00 pm	**NC 1192 Annual Meeting – Chicago C – 5th Floor**
1:00 pm – 6:00 pm	**Speaker Ready Room – Los Angeles – 5th Floor**
4:00 pm – 6:00 pm	**3MT Student Competition – Salon II – 7th Floor**

Sunday, January 19, 2025

7:00 am – 6:00 pm	**Speaker Ready Room – Los Angeles – 5th Floor**
8:30 am – 12:00 pm	**Set Up Posters – Salon III – 7th Floor**
8:30 am – 10:00 am	**American College of Veterinary Microbiologists Symposium – Salon II – 7th Floor** - **John Ellis, DVM, PHD, DACVP, DACVM, ACVM Distinguished Veterinary Microbiologist,** *Why aren't we all dead? Improving on Mother Nature's plan for immunization* - **Matthew Scott, DVM, PhD,** *Investigating bovine immune-mediated mechanisms influenced by viral respiratory Vaccination through bulk RNA sequencing*
8:30 am – 10:00 am	**CRWAD Research Abstracts – Chicago A-G – 5th Floor**
10:00 am – 10:30 am	**Break**
10:30 am – 12:00 pm	**American College of Veterinary Microbiologists Symposium – Salon II – 7th Floor** - **Scott McVey, DVM, PhD,** *Infection and Immunity - Clinical Outcomes* - **Kyoungjin J. Yoon, DVM,** *Current status of swine viral infections in the US: emerging anre-emerging diseases*
10:30 am - 12:00 pm	**CRWAD Research Abstracts – Chicago A-G – 5th Floor**
12:00 pm – 1:30 pm	**Break (Lunch on own)**
12:00 pm – 6:00 pm	**View Posters – Salon III – 7th Floor**
12:00 pm – 1:30 pm	**CRWAD Fellows Luncheon (Invite only) – Avenue Ballroom – 4th Floor**
1:30 pm – 2:45 pm	**CRWAD Council Keynote – Salon II – 7th Floor** - **Philp Santangelo, PhD, CRWAD Keynote Speaker,** *RNA therapy beyond vaccination: possibilities and challenges*
2:45 pm – 3:30 pm	**CRWAD Special Symposium: Applications of mRNA Technologies in Animal Health Research – Salon II – 7th Floor** - **Jeff Hogan, PhD,** *Development of Countermeasures for High Consequence Zoonotic and Human*
3:30 pm – 4:00 pm	**Break**

Schedule at a Glance – Sunday, January 19 (Continued)

4:00 pm – 6:00 pm **CRWAD Special Symposium: Applications of mRNA Technologies in Animal Health Research– Salon II – 7th Floor**

- **Francois Villinger, DVM, PhD,** *mRNA applications in nonhuman primate models for improved preclinical evaluation*
- **Amelia Woolums, DVM, MVSc, PhD, DACVIM, DACVM,** *Opportunities for RNA therapy in animal health*

Panel Discussion

6:00 pm – 8:00 pm **AVEPM Student Mixer Event– Chicago – 5th Floor**

Monday, January 20, 2025

7:00 am – 6:00 pm **Speaker Ready Room – Los Angeles – 5th Floor**

8:30 am – 6:00 pm **View Posters – Salon III – 7th Floor**

8:30 am – 10:00 am **American Association of Veterinary Immunologists Symposium**

- **Armin Saalmüller, Dr. rer. nat., AAVI Distinguished Veterinary Immunologist,** *Immune response after vaccination - peculiarities of the porcine immune system*
- **Anne DeGroot, MD,** *Potentiating the Application of Immunoinformatics Tools to Animal Health*

8:30 am – 10:00 am **CRWAD Research Abstracts– Chicago A-G – 5th Floor**

10:00 am – 10:30 am **Break**

10:30 am – 12:00 pm **American Association of Veterinary Immunologists Symposium**

- **Colin Parrish, PhD,** *Emergence and spread of viruses in new hosts - the examples of canine parvovirus and H3N8 canine influenza virus*
- **Shayan Sharif, DVM, PhD,** *Perspectives on vaccination as an approach for control of highly pathogenic avian*

10:30 am – 12:00 pm **CRWAD Research Abstracts– Chicago A-G – 5th Floor**

12:00 pm – 2:00 pm **Break (Lunch on own)**

12:00 pm – 2:00 pm **American Association of Veterinary Immunologists Luncheon (Ticketed Event)**

2:00 pm – 3:00 pm **Association for Veterinary Epidemiology and Preventative Medicine Calvin W. Schwabe Symposium**

- **Jan Scarlett, DVM, MPH, PhD, Calvin W. Schwabe Awardee,** *Challenges to conducting epidemiologic research regarding companion animal welfare.*
- **Margaret Slater, DVM, PhD,** *Illuminating the welfare implications of free-roaming unowned cats via epidemiological approaches*

2:00 pm – 3:00 pm **CRWAD Research Abstracts – Chicago A-G – 5th Floor**

3:00 pm – 4:15 pm **CRWAD Business Meeting – Chicago D – 5th Floor**

Schedule at a Glance – Monday, January 20 (Continued)

4:15 pm – 5:45 pm	**Association for Veterinary Epidemiology and Preventative Medicine Calvin W. Schwabe Symposium – Salon II – 7th Floor** - **Alexandra Protopopova, PhD, CAAB,** *The use of mixed methods to explore the complex issue of companion animal relinquishment to and adoption from animal shelters* - **Rachel Kreisler, VMD, MSCE, DACVPM (Epidemiology),** *Epidemiological Insights: Classic Tools for Modern Animal Shelter and Community Animal Health Research* **Panel Discussion**
4:15 pm – 5:45 pm	**CRWAD Research Abstracts – Chicago A-G – 5th Floor**
6:00 pm – 8:00 pm	**CRWAD Poster Reception – Salon III – 7th Floor**
8:15 pm	**Deadline to take down posters – Salon III – 7th Floor**

Tuesday, January 21, 2025

7:00 am – 10:30 am	**Speaker Ready Room – Los Angeles – 5th Floor**
8:30 am – 10:00 am	**Animal Vaccinology Research Symposium – Salon II – 7th Floor** - **Daniel O'Connor, BsC, AFHEA, Msc, DPhil,** *Systems vaccinology: utilising the "-omics" toolkit to elucidate the mechanisms underlying immune responses* - **Gavin Wright. DPhil, FMedSci,** *Genome-led vaccine target discovery for animal African trypanosomiasis*
8:30 am – 10:00 am	**CRWAD Research Abstracts – Chicago A-G – 5th Floor**
10:00 am – 10:30 am	**Break**
10:30 am – 12:00 pm	**Animal Vaccinology Research Network Symposium – Salon II – 7th Floor** - **Laura C. Miller, BSc (Hons), PhD***, Translating omics research into improved swine health* **Yongqun He, DVM, PhD,** *Advancing Systems Vaccinology by Leveraging AI*
10:30 am – 12:00 pm	**CRWAD Research Abstracts – Chicago A-G – 5th Floor**
12:15 pm – 1:30 pm	**Conference Closing and Student Award Presentations – Chicago D – 5th Floor**

CRWAD

2025 CONFERENCE OF RESEARCH WORKERS IN ANIMAL DISEASES

Officers and Administration 2024-2025

President

Rebecca Wilkes, DVM, PhD, DACVM
Purdue University, West Lafayette, IN

Vice-President

Weiping Zhang, PhD
Professor, University of Illinois, Urbana, IL

Council Members

Glenn Zhang, MS, PhD
Oklahoma State University, Stillwater, OK

John Angelos, DVM, PhD, DACVIM
University of California, Davis, CA

Jun Lin, PhD
University of Tennessee, Knoxville, TN

Natalia Cernicchiaro, DVM, PhD
Kansas State University, Manhattan, KS

Immediate Past President

Annette O'Connor, BVSc, MVSc, DVSc, FANCVS
Michigan State University, East Lansing, MI

Executive Director

Paul Morley, DVM, PhD, DACVIM
VERO Program - Texas A&M University, Canyon, TX

CRWAD Administration

Jennifer Stalley, Midwest Solutions
Rachel Morley

CRWAD Program Committee – 2024-2025

Co-Chairs

Brandy Burgess, DVM, MSc, PhD, DACVIM (LAIM), DACVPM
University of Georgia

Angel Abuelo, DVM, MRes, PhD, MSc, DABVP, DECBHM, FHEA, MRCVS
Michigan State University

Program Committee

Renukaradhya Gourapura, DVM, MS, PhD
Ohio State University

Scott McVey, PhD, DVM, DACVM
University of Nebraska – Lincoln

Laura Miller, PhD
Kansas State University

Keri Norman, PhD
Texas A&M University

Laurel Redding, VMD, PhD, DACVPM
University of Pennsylvania

Kathryn Reif, MSPH, PhD
Auburn University

Edan Tulman, PhD
University of Connecticut

Robert Valeris-Chacin, DVM, PhD
VERO Program - Texas A&M University

Carl Yeoman, PhD
Montana State University

Mohamed Abouelkhair, DVM, MS, PhD, DACVM, CABMM.
University of Tennessee

President's Message – CRWAD 2025

January 2025

As this year's Council President, and on behalf of the entire Council, I am honored to welcome you to the 2025 Conference of Research Workers in Animal Diseases (CRWAD). We have an exciting lineup this year, thanks to the hard work of our program committee. Our keynote speaker, Dr. Phil Santangelo from Emory University School of Medicine, will be leading the Special Symposium on "Applications of mRNA Technologies in Animal Health Research."

CRWAD kicks off on Saturday with our Three Minute Thesis (3MT) competition, a dynamic addition introduced last year, where 20 talented students will showcase their research in this ultimate challenge. We hope you'll join us to cheer on your favorites and support the graduate students—one of the hallmarks of this conference.

As a long-time attendee, starting from my days as a graduate student, I can sincerely thank each of you for fostering a welcoming environment for students to present. Your contributions and interactions are essential to CRWAD's success. To the graduate students and post-docs here with us, we're so glad you're part of this community. We hope that as you move into new roles, you'll continue attending and encourage your own students to join as well.

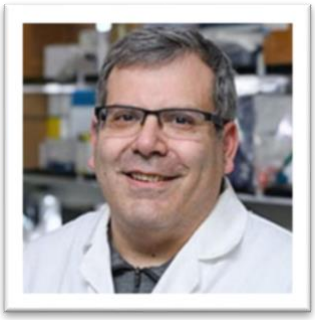
This Conference brings together an exceptional group of animal health researchers who are pushing to improve all aspects of animal health, from prevention to detection to treatment. Building collaborations here allows us to make a collective impact greater than our individual contributions. Take advantage of this opportunity to connect with students and researchers from other institutions.

Thank you all for keeping CRWAD strong for over 100 years. Let's work together to ensure its legacy for the next century. Here's to an exciting meeting ahead!

Sincerely,

Rebecca Wilkes, DVM, PhD, DACVM (virology and bacteriology/mycology)
President, Conference of Research Workers in Animal Diseases

CRWAD 2025 Keynote Speaker



RNA Therapy Beyond Vaccination: Possibilities and Challenges

Phil Santangelo, PhD
Biomedical Engineering at Georgia Tech School of Engineering
Emory University School of Medicine

Sunday, 1/19/2025 1:30 PM

CRWAD Special Symposium

Applications of mRNA Technologies in Animal Health Research

Development of Countermeasures for High Consequence Zoonotic and Human Pathogens

Jeff Hogan, PhD
University of Georgia

Sunday, 1/19/2025, 2:45 PM

mRNA Applications in Non-Human Primate Models for Improved Pre-Clinical Evaluation

Francois Villinger, DVM, PhD
University of Louisiana Lafayette

Sunday, 1/19/2025, 4:00 PM

Opportunities for RNA Therapy in Animal Health

Amelia Woolums, DVM, PhD, DACVIM, DACVM
Mississippi State University

Sunday, 1/19/2025, 4:00 PM

CRWAD 2025 - Distinguished Career Awards

AAVI Distinguished Veterinary Immunologist

Armin Saalmüller, Dr. rer. nat.
University of Veterinary Medicine Vienna

Immune Responses After Vaccination
Peculiarities of the Porcine Immune System
Monday, 1/20/2025, 8:30 AM

ACVM Distinguished Veterinary Microbiologist

John Ellis, DVM, PhD, DACVP, DACVM
University of Saskatchewan

Why Aren't We All Dead?
Improving on Mother Nature's Plan for Immunization
Sunday, 1/19/2025, 8:30 AM

AVEPM Calvin W. Schwabe Award

Jan Scarlett, DVM, MPH, PhD
Cornell University

Challenges to Conducting Epidemiologic Research Regarding Companion Animal Welfare
Monday, 1/20/2025, 2:00 PM

CRWAD 2025 Featured Speakers

Anne DeGroot, MD
University of Georgia

Potentiating the Application of Immunoinformatics Tools to Animal Health

AAVI Featured Speaker
Monday, 1/20/2025 8:30 AM

Yongqun "Oliver" He, DVM, PhD
University of Michigan

Advancing Systems Vaccinology by Leveraging AI

Animal Vaccinology Research Network Featured Speaker
Tuesday, 1/21/2025, 10:30 AM

Rachael Kreisler, VMD, MSCE, DACVPM
Midwestern University

Epidemiological Insights: Classic Tools for Modern Animal Shelter and Community Animal Health Research

AVEPM Featured Speaker
Monday, 1/20/2025 4:15 PM

Scott McVey, DVM, PhD
University of Nebraska

Infection and Immunity – Clinical Outcomes

ACVM Featured Speaker
Sunday, 1/19/2025, 10:30 AM

Laura C. Miller, BSc (Hons), PhD
Kansas State University

Translating Omics Research into Improved Swine Health

Animal Vaccinology Research Network Featured Speaker
Tuesday, 1/21/2025, 10:30 AM

Daniel O'Connor, BSc, AFHEA, MSc, DPhil (OXON)
Oxford Vaccine Group

Systems Vaccinology: Utilizing the '-omics' toolkit to Elucidate the Mechanisms Underlying Immune Responses

Animal Vaccinology Research Network Featured Speaker
Tuesday, 1/21/2025, 8:30 AM

Alexandra (Sasha) Protopopova, PhD, CAAB
University of British Columbia

Using Mixed Methods to Explore the Issue of Companion Animal Relinquishment and Adoption from Animal Shelters

AVEPM Featured Speaker
Monday, 1/20/2025, 4:15 PM

Colin Parrish, PhD
Cornell University

Emergence and Spread of Viruses in New Hosts – the Examples of Canine Parvovirus and H3N8 Canine Influenza Virus

AAVI Featured Speaker
Monday, 1/20/2025 10:30 AM

Matthew Scott, DVM, PhD
Texas A&M University

Investigating Immune-Medicated Mechanisms Influenced by Viral Respiratory Vaccination Through Bulk RNA Sequencing

ACVM Featured Speaker
Sunday, 1/19/2025, 8:30 AM

Shayan Sharif, DVM, PhD
University of Guelph

Perspectives on Vaccination as an Approach for Control of Highly Pathogenic Avian Influenza Viruses

AAVI Featured Speaker
Monday, 1/20/2025 10:30 AM

Margaret Slater, DVM, PhD
ASPCA

Illuminating the Welfare Implications of Free-Roaming Unowned Cats via Epidemiological Approaches

AVEPM Featured Speaker
Monday, 1/20/2025, 2:00 PM

Gavin J. Wright, DPhil, FMedSci
Hull York Medical School

Genome-Led Vaccine Target Discovery for Animal African Trypanosomiasis

Animal Vaccinology Research Network Featured Speaker
Tuesday, 1/21/2025, 8:30 AM

Kyoungjin J. Yoon, DVM, MS, PhD, Diplomate ACVM
Iowa State University

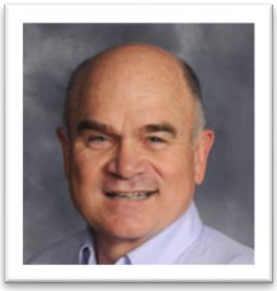
Current Status of Swine Viral Infections in the US: Emerging and Re-Emerging Diseases

ACVM Featured Speaker
Sunday, 1/19/2025, 10:30 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. 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.

2025 CRWAD Fellow Inductees



Frank Blecha, MS, PhD
University Distinguished Professor
College of Veterinary Medicine
Kansas State University

Radhey S. Kaushik, BVSc, MVSc, PhD
Department of Biology and Microbiology
South Dakota State University

Rodney A. Moxley, DVM, PhD, DACVM (Hon.)
Professor of Veterinary Medicine and Biomedical Sciences, University of Nebraska - Lincoln

Fellows of the Conference of Research Workers in Animal Diseases

Cynthia Baldwin, PhD (2021)
David Benfield, MS, PhD (2023)
Yung-Fu Chang, PhD, MS, DVM (2021)
Christopher Chase, DVM, PhD, DACVM (2024)
M.M. Chengappa, BVSC, MVSC, MS, PhD, DACVM (2024)
Norman Cheville, PhD, MS, DVM (2021)
Carol Chitko-McKown, PhD (2023)
Lynette B. Corbeil, PhD, DVM (2021)
Roy Curtiss, III, PhD (2021)
William C Davis, MA, PhD (2021)
Robert Ellis, MS, PhD, CBSP, DACM, DACVM (Hon.) (2023)
Roman Ganta, PhD (2021)
Ian Gardner, BVSc, MPVM, PhD (2021)
Laurel Gershwin, DVM, PhD, DACVM (2023)
Laura Hungerford, DVM, MPH, CPH, PhD, FNAP (2021)
Jun Lin, PhD (2021)
Joan Lunney, PhD (2023)
T.G. Nagaraja, BVSc, MVSc, PhD (2021)
Stuart W.J. Reid, BVMS, PhD, DVM, DipECVPH, FRSB, FRSE, FRCVS (2021)
David Renter, DVM, PhD (2023)
Richard Ross, MS, PhD (2021)
Linda J. Saif, PHD (2021)
Y.M. (Mo) Saif, DVM, PhD (2021)
Janice (Jan) Merrill Sargeant, DVM, MSc, PhD (2021)
Patricia Shewen, BSc, DVM, MSc, PhD (2023)
Lorraine Sordillo-Gandy, BS, MS, PhD (2021)
Subramaniam Srikumaran, BVSc, MS, PhD (2023)
Thomas Wittum, MS, PhD (2023)
Scott McVey, DVM, PhD, DACVM (2024)
James Roth, DVM, MS, PhD, DACVM (2024)
Steve Olsen, DVM, PhD, DACVM (2024)
Amelia Woolums, DVM, PhD, DACVM, DACVIM (2024)

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

Thank You to Our 2025 Conference Sponsors

GOLD SPONSORS

zoetis

SILVER SPONSORS

LONGHORN VACCINES & DIAGNOSTICS LLC

azure biosystems

VERO
VETERINARY EDUCATION, RESEARCH, & OUTREACH

BRONZE SPONSORS

CENTER FOR OUTCOMES RESEARCH & EPIDEMIOLOGY
CORE
KANSAS STATE UNIVERSITY

PeerJ
Life & Environment

CONFERENCE SUPPORT

MIDWEST SOLUTIONS, INC.

preseria

FOURWAVES

1 - Why aren't we all dead? Improving on mother nature's plan for immunization

John Ellis[1]

[1]Department of Veterinary Microbiology, University of Saskatchewan, Canada. maddoge@sasktel.net

Session: ACVM – Featured Speakers, 2025-01-19, 8:30 - 9:15

Vertebrates are born into a dangerous microbial world. Evolution has provided passive and adaptive immunity to increase the chances for survival in that world of endemic infections. Young are primed mucosally in the face of maternal antibodies and then boosted repeatedly to maintain adaptive protective immunity. Heterologous prime-boosting as an approach to iatrogenic vaccination is a way to apply and improve on nature's protective plan. The simple concept is to expose the immune system to different forms of an antigen by different routes of administration to achieve the broadest most durable response. Documenting the efficacy of this approach are a plethora of experimental data, but relatively little applied data deriving from human and veterinary clinical medicine. The objective of this seminar is to briefly review our experience over the past 25 years with the application of commercially available vaccines in heterologous prime-boosting of responses to important veterinary pathogens, with a focus on respiratory syncytial virus in calves and *Bordetella bronchiseptica* in dogs.

Notes:

2 - Investigating bovine immune-mediated mechanisms influenced by viral respiratory vaccination through bulk RNA sequencing

Matthew Scott[1]

[1]Department of Large Animal Clinical Sciences, Texas A&M University. matthewscott@tamu.edu

Session: ACVM – Featured Speakers, 2025-01-19, 9:15 - 10:00

Bovine respiratory disease (BRD) remains the leading cause of morbidity and mortality within North American beef production systems. Vaccines, especially those containing attenuated viruses, are a critical tool for controlling BRD in beef calves, as their use enhances the trained innate and adaptive immune responses against known pathogens. While commercial vaccines undergo rigorous evaluation for purity, safety, potency, and efficacy by the USDA APHIS Center for Veterinary Biologics and the Canadian Centre for Veterinary Biologics, there is growing evidence that the use of these vaccines in commercial production systems is relatively ineffective at controlling the rates of BRD. Moreover, the rigorous efficacy studies performed for vaccine approval often differ significantly from real-world conditions, where vaccines are administered to cattle with diverse health and backgrounds under varying protocols. Such discrepancies may limit the applicability of efficacy data, raising concerns about the consistency of vaccine impact on morbidity rates and performance in field settings. Additionally, traditional metrics used to evaluate adaptive immunity, such as serum antibody titers, have limitations including the need for multiple samples and sufficient time for a mounting immune response.

There is a growing need for adaptable animal health research to assess the influence of commercial vaccines on cattle health, performance, and immune function. Here, the Veterinary Education, Research, and Outreach (VERO) Program, in collaboration with several academic institutes and private partners, aims to identify key immunological impacts and biomarkers of health and performance influenced by vaccination strategies via cutting-edge molecular approaches and bioinformatic analyses. This presentation provides insights into our research, specifically focusing on host transcriptomics, which leverages advancements in biochemistry, computational technologies, and statistical modeling to better understand immunological responses and pathophysiological processes within cattle in relation to vaccine strategies and BRD development.

Notes:

3 - Epidemiology and case fatality associated factors of atypical canine infectious respiratory disease complex

Mahamudul Hasan[1], Gregg A. Hanzlicek[2], Laura Miller[1]

[1]Department of Diagnostic Medicine/Pathobiology, Kansas State University. [2]Veterinary Diagnostic Laboratory, Kansas State University. mahamudul@vet.k-state.edu

Session: Companion animal health 1, 2025-01-19, 8:30 - 8:45

Objective: Recently, an atypical form of Canine Infectious Respiratory Disease Complex (CIRDC), commonly known as Kennel cough, has received attention in the US with multiple states reporting alarming surges of the disease. Traditionally, the primary agents responsible for CIRDC include *Bordetella bronchiseptica*, canine herpesvirus, canine adenovirus type 2, canine parainfluenza virus, canine pneumovirus, respiratory coronavirus, *Streptococcus equi* subspecies *zooepidemicus*, and *Mycoplasma cynos*. The atypical cases exhibited symptoms associated with upper respiratory disease but tested negative for the common respiratory pathogens through PCR; antibiotic treatments were often ineffective, and there were reported deaths. The objective of this study is to understand the extensive epidemiology and case fatality-associated factors responsible for this atypical CIRDC (aCIRDC) outbreak.

Methods: A survey was conducted from November to January 2024. Survey responses were collected from the members of the "2023 Canine Infectious Respiratory Disease Tracking" Facebook group. The group, established on July 12, 2023, aims to monitor cases of aCIRDC that test negative for known causes. Approximately fifty-six thousand members belong to this group, and responses were collected using snowball sampling. A total of four hundred and ninety-seven responses were gathered from pet dog owners. After applying predefined case definitions, inclusion and exclusion criteria, four hundred and thirty complete responses were used for descriptive analysis, as well as univariate and multivariable logistic regression analysis to identify case fatality-associated factors.

Results: The survey found aCIRDC cases were widespread, with the highest responses documented in California, followed by Colorado, Utah, Pennsylvania, Wisconsin, Florida, and Massachusetts. Common exposure locations included dog events, kennels facilities, and dog parks. Most dogs suffered from cough, vomiting, loss of appetite, lethargy, fever, runny nose, and difficult breathing within 5-10 days of exposure, with doxycycline being the most used antibiotic for treatment. 9.1% of dogs were reported to have died, and univariate logistic regression analysis found a higher likelihood of case fatality in dogs treated with IV fluids and oxygen therapy. Similarly, clinical symptoms like lethargy, fever, difficult breathing, and sneezing also showed a strong positive association with death. The study also found that dogs under five years old, with no previous respiratory illness, exposed in boarding facilities or kennels, and vaccinated against both canine influenza and Bordetella had a higher odds ratio of case fatality. In multivariable analysis, only dogs exhibiting lethargy, fever, difficult breathing, and sneezing demonstrated a positive correlation with case fatality.

Conclusions: This survey gives an introductory description of the widespread aCIRDC distribution, highlighting key exposure locations, common treatments, and specific factors associated with mortality. Additionally, this study sets the stage for further metagenomics studies and the identification of causative pathogen(s) with possible treatment or vaccine intervention.

Notes:

4 - Resolution of uncomplicated pneumonia in dogs treated with shorter or longer courses of antimicrobials: a randomized, double-blinded, placebo-controlled study

Laurel Redding[1], Erika Reineke[1]

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

Session: Companion animal health 1, 2025-01-19, 8:45 – 9:00

Objective: Bacterial pneumonia in dogs occurs most commonly due to aspiration or secondary to community-acquired infectious agents. Guidelines for treating pneumonia include recommendations for empiric antibiotic choice; however, definitive recommendations for the duration of antimicrobial treatment were not made due to lack of evidence. Appropriate use of antimicrobials is of particular interest, as the prolonged or inappropriate use of these drugs can lead to antimicrobial resistance, disruption of the gut microbiome, increased or unnecessary treatment costs, and decreased pet owner compliance with administration. The objective of this study is to describe and compare clinical and radiographic outcomes in dogs with uncomplicated pneumonia receiving a shorter duration (2 weeks) versus a longer duration (4 weeks) of antimicrobial therapy.

Methods: Thirty dogs were randomly assigned to either a 2-week course of antimicrobials followed by a 2-week course of placebo (2-week group) or a 4-week course of antimicrobials (4-week group). All study investigators and owners were blinded to treatment group. Dogs were re-evaluated at 12 days +/- 2 days and again at 28 +/- 2 days for a physical examination and thoracic radiography. Standard visit documentation included owner-reported clinical signs, nurse-acquired history, clinician physical exam, number of affected lung lobe segments, and global radiographic severity score. Outcomes investigated included persistence of clinical and radiographic signs of pneumonia, and multivariable regression modeling was used to assess the association between these outcomes and treatment group.

Results: Twenty-eight dogs (93.3%) experienced complete resolution of clinical signs by the first visit, and no dogs experienced relapse of clinical signs at the second visit. Fourteen dogs (46%) and 25 dogs (25/30; 83%) experienced complete resolution of radiographic lesions at the first and second study visit, respectively. There was no difference in the rates of relapse of clinical or radiographic signs of pneumonia between the 2-week and 4-week groups (P=0.99, P=0.67, respectively).

Conclusions: Similar rates of resolution of clinical and radiographic signs were observed in dogs with uncomplicated pneumonia receiving a 2-week course of antimicrobials compared to a 4-week course, although the lack of difference is likely due to the study being underpowered. Clinical signs may be more useful for guiding discontinuation of antibiotic therapy for pneumonia than radiographic signs.

Financial Support: This study was funded by the Companion Animal Research Fund of the University of Pennsylvania School of Veterinary Medicine.

Notes:

5 - Home-prepared diets for companion dogs are made from a diverse group of food ingredients and few are balanced

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

[1]Virginia-Maryland College of Veterinary Medicine, Virginia Tech University. janiceobrien@vt.edu

Session: Companion animal health 1, 2025-01-19, 9:00 – 9:15

Objective: To describe dog diets that are prepared entirely at home, the typical food items included, and to determine the overall dietary completeness of these diets in accordance with the Association of American Feed Control Officials (AAFCO) standards.

Methods: 1765 free-text responses provided by dog owners from 1/1/2023-12/31/2023 were evaluated by two independent coders to describe dietary ingredients. The ingredients listed by owners were input into a website which is designed to assist owners in creating a balanced diet (Balance.it) for their pet. Diets with 10 or more nutrient imbalances (deficiencies or excesses) were categorized as unbalanced, diets with 1-10 nutrient deficiency imbalances were classified as partially balanced, and those with no nutrient imbalances were categorized as completely balanced.

Results: The ingredients in this group of home-prepared diets were: meat (contained in 89% of diets), vegetables (64%), organs/offal (26%), fruit (25%), non-meat proteins (22.6%), fats/oils (14%), and nuts/seeds (6%). Of the meat and offal categories, the most common animal sources reported by owners were: chicken, beef, turkey, fish, and pork. In addition to these most common sources, owners also reported feeding: goose, quail, camel, alpaca, moose, kangaroo, ostrich, antelope, pheasant, emu, guinea hen, and beaver. 410 owners reported feeding raw meat diets, while 672 reported cooking the meat before feeding. 71 owners reported feeding a mixture of raw and cooked animal ingredients (e.g., raw beef but cooked chicken or cooked muscle meat but raw liver). Non-meat-based proteins reported by owners were: eggs, beans, lentils, chickpeas, and tofu. Of the 391 owners that fed eggs, 47 (12%) reported also feeding the shell. Bones and bone products reported by owners were: whole bone, bone broth, and bone meal/ground bone. The most common starch sources mentioned by owners were sweet potato, rice, brown rice, squash, oats, potato, quinoa, noodles, barley, and wild rice. The most common vegetables reported by owners were carrots, green beans, broccoli, leafy greens, peas, squash, cauliflower, celery, kelp, cabbage, pepper, corn, beets, asparagus, mushrooms, and avocado. Common fruits reported by owners were apples, blueberries, bananas, cranberries, strawberries, melons, and pears. The most frequently reported dairy products were kefir/yogurt, cheese, cottage cheese, and raw milk. Feeding non-dog origin colostrum was reported by a few owners. Some owners added animal-based fats like butter and rendered fat, but the most common oils added were fish and plant oils. Nonfood items that were added to some diets include clay and diatomaceous earth. Due to insufficient information, approximately 7% of diets could not be categorized according to balance. 10% of diets were classified as complete, 43% were partially balanced, and 40% were unbalanced. These percentages may change as coding is completed; however, they are not expected to be much different from the final numbers.

Conclusions: The ingredients that owners choose to feed their pet dogs in home-prepared diets are diverse. Uncommon meat sources like venison and ostrich were reported, and many owners added eggshells to increase the calcium in their pet's diet. Unfortunately, only 10% met AAFCO standards for proper nutrient balance.

Notes:

6 - Marked neurotropism and potential adaptation of H5N1 clade 2.3.4.4.b virus in naturally infected domestic cats

Surabhi Srinivas[1], Shubhada Krishna Chothe[1], Sougat Misra[1], Noel Nalipogu[1], Elizabeth Gilbride[1], Lindsey LaBella[1], Swastidipa Mukherjee[1], Christian H Gauthier[2], Heidi Pecoraro[3], Brett T. Webb[3], James M. Pipas[2], Santhamani Ramasamy[1], Suresh V. Kuchipudi[1]

[1]Department of Infectious Diseases and Microbiology, University of Pittsburgh, [2]Department of Biological Sciences, University of Pittsburgh, [3]Veterinary Diagnostic Laboratory, North Dakota State University. sus197@pitt.edu

Session: Companion animal health 1, 2025-01-19, 9:15 – 9:30

Objective: The Highly Pathogenic Avian Influenza (HPAI) A H5N1 clade 2.3.4.4b viruses have caused widespread outbreaks across both avian and mammalian species worldwide, resulting in a notable increase in interspecies transmission events. We investigated an outbreak of HPAI H5N1 clade 2.3.4.4b leading to death of 10 cats in rural South Dakota. The study focused on histopathological lesions and the virus's tissue tropism in the affected cats. In addition, we conducted a comprehensive analysis of Sialic Acid (SA) receptor distribution in both healthy and infected feline tissues to determine whether receptor distribution played a role in the tissue-specific tropism of H5N1 clade 2.3.4.4b. We also recovered viral sequences from the infected cats and analyzed them to identify any host-specific adaptations.

Methods: The infected cat tissues were examined for histopathological lesions and influenza viral nucleoprotein using immunohistochemical staining. Lectin histochemistry was performed to analyze the distribution of SA α-2,3-Gal and SA α-2,6-Gal receptors in healthy cat tissues. Virus-receptor binding assays were performed on healthy cat lungs, cerebral cortex, and gastrointestinal tissues (stomach, ileum, jejunum, and duodenum) using pseudoviruses expressing H5 from H5N1 clade 2.2 or H5N1 clade 2.3.4.4b generated in our laboratory.

Results: Immunohistochemistry of infected tissues showed a significantly higher expression of nucleoprotein in the brain compared to the lungs. The H5N1 clade 2.3.4.4b isolate from the cats revealed unique mutations in the Haemagglutinin, Neuraminidase, and Polymerase Acidic protein. There was widespread co-expression of avian influenza receptor SA α-2,3-Gal and mammalian influenza SA α-2,6-Gal receptors in the brain and lungs, with predominant expression of SA α-2,6-Gal receptors in the intestines. Virus-receptor binding assays showed no significant differences in binding between pseudoviruses expressing H5 from clades 2.2 and 2.3.4.4b across the various cat tissues.

Conclusions: We found that H5N1 clade 2.3.4.4b infection in cats exhibited marked neurotropism, similar to infections in foxes, in contrast to earlier H5N1 clades where higher viral loads were typically observed in the lungs. This increased viral presence in the brain was not associated with differences in Sialic Acid (SA) receptor distribution, as no significant variations in receptor binding were noted between clade 2.2 and 2.3.4.4b H5 pseudoviruses. Additionally, evidence of potential viral adaptation in cats was observed. Given the widespread distribution of both avian and mammalian SA receptors in feline tissues, cats may play a crucial role in the ecology of H5N1 and could potentially facilitate viral reassortment, highlighting the need for ongoing surveillance in the context of avian influenza.

Financial Support: This study is partially funded by Chair startup funds from the School of Public Health, University of Pittsburgh (SVK), Pennsylvania Department of Agriculture, contract number # 4400026513, and by the American Rescue Plan Act through USDA APHIS (APHIS/NIFA Collaborative Award# 2023-70432-41395).

Notes:

7 - Re-purposing arbidol as a potential antiviral agent against canine parvovirus type-2

Ayoung Son[1], Jong-Woo Lim[1], Minjoo Yeom[1], Daesub Song[1]

[1]Department of Virology, Seoul National University. aaa022@snu.ac.kr

Session: Companion animal health 1, 2025-01-19, 9:30 – 9:45

Objective: Canine Parvovirus type-2(CPV-2) is a fatal infectious disease that causes gastrointestinal symptoms in dogs, especially in puppies. While no specific antiviral therapy for CPV-2 infection exists, the only treatment option for the infection is supportive therapy based on symptoms. Given the decreasing vaccination rates and the lack of effective treatment, the development of targeted antiviral drugs for CPV-2 is essential. Drug repurposing is the process of changing the target of a well-known drug to produce a new effect. The advantage of using an approved drug is that it can lower clinical risk, reduce costs, and shorten drug development time. This study aims to repurpose arbidol, which is an antiviral drug for influenza virus, to suggest it as a potential antiviral agent for CPV-2.

Methods: Molecular docking simulation was performed using Autodock Vina and imaged by PyMOL. Molecular structures were edited from original PDB: CPV-2 capsid(2cas), spike protein of SARS-CoV-2(7df3), Hemagglutinin(HA) of IAV(1ru7). The in vitro effect of arbidol on CPV-2 infection was tested using the canine fibrosarcoma (A72) cell line. CPV-2 were propagated in A72 cells in DMEM containing 2% FBS. Viral titer was calculated by qPCR and HA test.

Results: Using the molecular docking software, we confirmed that arbidol also binds to the capsid of CPV-2 with low affinity values(-5.9~6.1kcal/mol). Based on structural similarity between a specific region of the CPV-2 capsid and influenza HA, arbidol can bind to the CPV-2 capsid that lacks HA. The region where arbidol binds to the capsid of CPV-2 maps to the receptor-binding domain(RBD). Further, we determined the 50% inhibition dose (EC50), 50% cytotoxicity dose (CC50) and selectivity index (SI: CC50/EC50) for arbidol on A72 cell. At all concentrations (12.5-100μM), arbidol shows >70% viral inhibition. At the highest concentration(100μM), it shows 86.9% inhibition. By sub-dividing the arbidol treatment period, it is showed that arbidol inhibits the receptor binding process of CPV-2, which influences the early stage of virus infection.

Conclusions: With this study, we suggest that arbidol is a potential antiviral drug for CPV-2. Arbidol, known as an antiviral drug for influenza, binds to the CPV-2 RBD and inhibits the entry of the virus into the cell. The re-purposing of arbidol provides the basis for a more efficient and reliable treatment system for CPV-2..

Financial Support: This study was partially supported by the South Korea National Institute of Wildlife Disease Control and Prevention as part of “Specialized Graduate School Support Project for Wildlife Disease Specialists” Program.

Notes:

8 - ORF6 contributes to SARS-CoV-2 virulence and pathogenicity in the naturally susceptible feline species

Mohammed Nooruzzaman[1], Salman L. Butt[1], Nathaniel Jackson[2], Chengjin Ye[2], Luis Martinez-Sobrido[2], Ying Fang[3], Diego G. Diel[1]

[1]Department of Population Medicine and Diagnostic Sciences, Cornell University. [2]Texas Biomedical Research Institute. [3]Department of Pathobiology, University of Illinois at Urbana-Champaign. mn496@cornell.edu

Session: Companion animal health 1, 2025-01-19, 9:45 – 10:00

Objective: We performed *in vitro* and *in vivo* characterization to evaluate the role of ORF6 protein in SARS-CoV-2 pathogenicity and host transcriptional profile in a naturally susceptible feline model of SARS-CoV-2 infection.

Methods: We generated a recombinant virus containing a deletion of ORF6 on the backbone of highly virulent SARS-CoV-2 WA1 virus (rWA1ΔORF6). The growth kinetics, plaque size phenotype and innate immune suppressing effects of the rWA1ΔORF6 was evaluated and compared to recombinant wild-type WA1 (rWA1) *in vitro*. The infection dynamics, replication and pathogenicity of the rWA1ΔORF6 was also investigated and compared to the parental rWA1 virus using a feline model of SARS-CoV-2 infection.

Results: While both rWA1 and rWA1ΔORF6 replicated efficiently in cell culture *in vitro*, rWA1ΔORF6 produced smaller plaques, suggesting reduced cell-to-cell spread. Luciferase reporter assays showed marked immune suppressing effects of ORF6 on innate interferon and nuclear factor kappa B (NF-κB) signaling pathways. Pathogenesis assessment in a naturally susceptible feline model of SARS-CoV-2 infection revealed that animals inoculated with rWA1 were lethargic and presented with fever on days 2 and 4 post-infection (pi), whereas rWA1ΔORF6-inoculated animals developed subclinical infection. Additionally, animals inoculated with rWA1ΔORF6 presented reduced infectious virus shedding in nasal and oral secretions and broncho-alveolar lavage fluid when compared with rWA1 inoculated cats. Similarly, rWA1ΔORF6-inoculated cats presented reduced virus replication in the respiratory tract as evidenced by lower viral loads and reduced lung inflammation on days 3 and 5 pi when compared to rWA1-inoculated animals. Host gene transcriptomic analysis revealed marked differences in differentially expressed genes in the nasal turbinate of animals infected with rWA1 when compared to rWA1ΔORF6. Importantly, type I interferon signaling was significantly upregulated in rWA1ΔORF6-infected cats when compared to rWA1-inoculated animals which corroborates the reduced virus in tissues of rWA1ΔORF6 in tissues of inoculated animals.

Conclusions: Our study demonstrates that ORF6 contributes to the host range of SARS-CoV-2 and is an important virulence determinant contributing to modulation of antiviral immune responses in cats, a naturally susceptible animal species.

Financial Support: This work was funded by the National Institute of Health (NIH) and the National Institute of Allergy and Infectious Diseases (NIAID) (grant no. R01AI166791-01).

Notes:

9 - Understanding antimicrobial action and resistance of *Edwardsiella ictaluri* in response to trans-cinnamaldehyde

Ali Dawood[1], Hossam Abdelhamed[1]

[1]College of Veterinary Medicine, Mississippi State University. asd324@msstate.edu

Session: Aquaculture, 2025-01-19, 8:30 - 8:45

Objective: Channel catfish is one of the most cost-effective farm-raised food sources, particularly in the southern United States. *Edwardsiella ictaluri* poses a significant challenge to catfish farmers, causing enteric septicemia of catfish (ESC). Trans-cinnamaldehyde (TC), a key compound found in the essential oil of cinnamon, has gained significant attention due to its antimicrobial activity against various bacteria. Our previous studies indicated that TC exhibits strong antibacterial activity against *E. ictaluri*. This study aims to perform quantitative proteomics analysis to explore the response of *E. ictaluri* exposed to TC, unraveling the mode of action and drug targets of TC against *E. ictaluri*.

Methods: The minimum inhibitory concentration (MIC) of TC against *E. ictaluri* was determined using the microdilution method. To investigate the mode of action of TC, *E. ictaluri* strain 93-146 was incubated with two doses of TC (0.09 µl/ml equivalent to 3/4 MIC and 0.06 µl/ml equivalent to 2/4 MIC) for 18 hours followed by proteomics analysis. A concentration of 0.016 µl/ml, which allowed normal growth of *E. ictaluri*, was selected for daily passage over 30 days, generating the D30-adapted strain. D30-adapted strain was used for MIC determination and proteomics analysis. Data-independent acquisition (DIA)-based quantitative proteomics was applied to analyze differentially expressed proteins (DEPs) between the treated and control groups. To investigate TC resistance in the adapted strain, Dunn test analysis was used to compare the inhibition zone of D30-adapted strain with the parent strain (non-adapted) using the disc diffusion assay. Specific pathogen-free fingerlings were used to evaluate the virulence of the D30-adapted strain using an immersion challenge model.

Results: Statistical analysis indicated no significant variations in the inhibition zone of TC against D30-adapted strain compared to wild-type strain. Proteomic analysis revealed 124 upregulated and 141 downregulated proteins in the E. ictaluri D30-adapted strain compared to wild-type strain. Notably, downregulation of proteins associated with the type III secretion system (T3SS) (13 proteins) and type VI secretion system (T6SS) (8 proteins) were observed in E. ictaluri D30-adapted strain relative to the wild-type strain. The efflux pump-related proteins were significantly upregulated, while pathways related to purine and pyrimidine metabolism were downregulated after exposure of E. ictaluri to 3/4 and 2/4 MIC of TC. No mortality was observed in catfish infected with D30-adapted strain compared to ≈88% for those infected with *E. ictaluri* wildtype.

Conclusions: Our study demonstrated that D30-adapted strain is safe and highly effective for protecting catfish against *E. ictaluri* 93-146. The downregulation of T3SS and T6SS likely contributes to the decreased pathogenicity of the *E. ictaluri* D30-TC strain. The upregulation of efflux pump-related proteins might contribute to the bacteria's drug resistance by facilitating the removal of drugs or toxic substances from the bacterial cell.

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:

10 - Application of trans-cinnamaldehyde in catfish aquaculture

Ali Dawood[1], Hossam Abdelhamed[1], Fernando Yamamoto[2], Matt Griffin[2]

[1]College of Veterinary Medicine, Mississippi State University, [2]Department of Wildlife, Fisheries and Aquaculture, Mississippi State University. asd324@msstate.edu

Session: Aquaculture, 2025-01-19, 8:45 - 9:00

Objective: Aquaculture is the fastest-growing animal food-producing sector, with catfish production being the largest aquaculture industry in the United States. Trans-cinnamaldehyde (TC) has the potential for application in the aquaculture field to reduce the mortalities associated with *Edwardsiella ictaluri*, a devastating bacterial pathogen causing enteric septicemia of catfish (ESC). This study aims to optimize the dietary TC feeding regime, investigate the ability of pectin, quercetin, phytic acid, and different fish oils to potentiate the antibacterial effects of TC, and explore the in vitro antibacterial effects of quercetin and phytic acid against *E. ictaluri.*

Methods: The in vitro antibacterial activities of quercetin and phytic acid were assessed using disc diffusion method. Challenge experiments were performed to determine the optimum TC feeding regime by evaluating the survival of catfish fed different concentrations of TC (10, 15, 20, 25, 30, and 35 mL/kg feed). The effectiveness of various fish oils (cod liver oil, menhaden oil, and catfish oil) in protecting catfish against *E. ictaluri* were also evaluated. Dietary TC (15 mL/kg) was supplemented with quercetin (2, 10, and 20 mL/kg) and phytic acid (5 and 15 mL/kg) to assess potential synergistic effects with TC. Pectin was used for coating TC medicated diets.

Results: Our results indicate that the optimum TC feeding regime is 15 mL/kg feed twice daily with 3% of body weight. This regime results in a significant ($P < 0.05$) reduction in fish mortality (35.8%) compared to control (85%). Using this regime, significant improvement was achieved using a combination of TC (15 mL/kg) with cod liver oil (15 and 25 mL/kg) with ≈26.14% and ≈26.67% fish mortalities. TC-coated with pectin resulted in a non-significant ($P > 0.05$) reduction in catfish mortalities. In vitro disc diffusion test of quercetin and phytic acid demonstrated weak antibacterial activities. A significant ($P < 0.05$) reduction in mortality was observed for catfish received TC (15 mL/kg) in combination with quercetin (2 ml/kg) compared to control group (30.5% vs 83.33%). Meanwhile, the catfish mortalities were 15% and 20% in the groups received TC (15 mL/kg) in combination with phytic acid (5 and 15 mL/kg), respectively, compared to 83.33% in catfish received regular diet and 93.18% in catfish group received phytic acid.

Conclusions: Quercetin and phytic acid exhibited limited antibacterial activities. TC coated with pectin did not improve the survival of catfish following E. ictaluri infection compared to TC alone. However, the combination of cod liver oil, quercetin, and phytic acid with TC for the treatment of ESC was beneficial and resulted in a significant improvement in catfish survival.

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:

11 - Glutamate effects on reactive oxygen species in intestinal mucosal leukocytes of juvenile hybrid striped bass

Karina L. Hissen[1], Wenliang He[2], Guoyao Wu[2], Michael F. Criscitiello[1, 3]

[1]Department of Veterinary Pathobiology, Texas A&M University, [2]Department of Animal Science, Texas A&M University, [3]Department of Microbial Pathogenesis and Immunology, Texas A&M Health Science Center.
klhissen@tamu.edu

Session: Aquaculture, 2025-01-19, 9:00 - 9:15

Objective: The aquaculture industry is under increasing pressure to improve fish health and growth, driven by the need for more sustainable farming practices. Major challenges include high-density production environments that increase disease risk, and the push for carnivorous species like hybrid striped bass (HSB; *Morone chrysops* × *Morone saxatilis*) to adopt plant-based diets. Glutamate is a major metabolic fuel essential for synthesizing glutathione (a potent antioxidant) in the intestine of HSB. However, little is known about dietary glutamate's effects on these animals' intestinal innate immunity. During preliminary studies, a fortuitous aquatic system failure led to an unexpected finding: HSB fed diets containing 1-5% glutamate showed 52-54% survival rates under the conditions of decreased dissolved oxygen, while all HSB fed a glutamate-free diet died. In addition, all HSB fed a glutamate-free diet died within 10 min after being placed in College Station's regular water with high chlorine concentrations (> 0.5 ppm), further indicating a critical role for dietary glutamate in fish survival. The present study investigated the effects of dietary supplementation with 0% or 5% glutamate to HSB on intestinal mucosal leukocytes. We hypothesize that such AA supplementation can optimize growth and modulate immune response within the intestinal mucosa, offering significant benefits for aquaculture production.

Methods: Juvenile HSBs with an initial weight of ~10 g were housed in a recirculating aquaculture system for an 8-week period, during which they were fed purified diets supplemented with 0% or 5% L-glutamate. The content of L-glutamate in the basal diet was 3%. At the end of the 8-week feeding, fish in each tank were divided randomly into groups to receive the intraperitoneal administration of 0.1 ml of RPMI medium containing 0 (sham) or 100 μg of trinitrophenyl-lipopolysaccharide (LPS). Fish continued to be fed their respective diets. On Day 7 after LPS administration, fish were weighed, and the gut mucosa was obtained to the isolation of leukocytes. The cells were used to measure the release of superoxide anion and hydrogen peroxide.

Results: A two-way ANOVA assessed the effects of diet and LPS stimulation, including interaction terms. We observed that glutamate supplementation increased glutamate concentrations in the intestinal lumen and the plasma by 133% and 109%, respectively. On days 7 and 8 after the intraperitoneal administration of LPS, there was no change ($P > 0.05$) in mRNA levels for interleukin-1β, tumor necrosis factor-α, or immunoglobulin T. However, glutamate supplementation enhanced ($P < 0.05$) the whole-body growth of fish post LPS challenge, and the net release of O2_ plus H2O2 in intestinal mucosal leukocytes.

Conclusions: Glutamate may influence reactive oxygen species balance, particularly through modulating O2- and H2O2 production. Although the exact mechanism of glutamate's action remains unclear, there is evidence linking its effects to oxidant-mediated killing of pathogens. Further investigation is required to elucidate how glutamate alters mucosal leukocyte function through redox changes. Our findings could offer a non-invasive strategy to enhance the health of HSB. Such an approach would contribute to sustainable aquaculture practices, supporting global food security.

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:

12 - Glycine is a nutritionally essential amino acid in juvenile hybrid-striped bass (*Morone chrysops* x *Morone saxatilis*)

Erin D. Connolly[1], Blaine A. Suehs[2], Karina L. Hissen[3], Michael F. Criscitiello[3], Delbert M. Gatlin[2], Guoyao Wu[1]

[1]Department of Animal Sciences, Texas A&M University, [2]Department of Ecology and Conservation, Texas A&M University, [3]Department of Veterinary Pathobiology, Texas A&M University. econnolly19@tamu.edu

Session: Aquaculture, 2025-01-19, 9:15 - 9:30

Objective: Glycine plays an important role in immune responses in animals including fish. This study evaluated the effects of glycine intake on glycine and serine availabilities in the intestine and plasma of juvenile hybrid striped bass (HSB), as well as their growth.

Methods: HSB with a mean initial body weight of 15 g were randomly assigned to one of three treatment groups, representing dietary levels of 0%, 2% and 4% glycine. The basal diet contained a crystalline glycine-free mixture of amino acids (at levels found in a typical diet containing 60% fishmeal), 11% fish oil, 1% soy oil, 20% dextrinized starch, 14% cellulose, 3% carboxymethyl cellulose, 0.1% vitamin premix, 7.7% mineral premix, and 1.1% other substances (containing choline chloride, betaine; inosine 5-monophosphate, creatine, and carnitine to match their content in the 60%-fishmeal diet). Appropriate amounts of L-alanine were added to the 0% and 2% glycine diets as isonitrogenous control. The HSB were maintained in a recirculating aquaculture system and were fed a pre-weighed amount of feed twice per day. Fish in all tanks were weighed every other week and fed at 5% of their BW in weeks 1-2, 4% in weeks 3-4, and 3% in weeks 5-8. Both the 0% and 4% glycine treatment groups had four tanks, but due to unforeseen illness unrelated to the experiment, there were only 3 tanks for the 2% glycine diet. At the end of 8 weeks, blood samples were taken from two HSB from each tank to measure glycine and serine levels in plasma by high-performance liquid chromatography (HPLC).

Results: Increasing the dietary glycine level from 0% to 4% increased ($P < 0.05$) the growth of HSB in a dose-dependent manner. Results of regression analysis showed an R2 value of 0.75 between average weight gain and dietary glycine level (P value = 0.004). Concentrations of glycine in plasma (means ± SEM, n = 6-8 fish/group) were 157 ± 15, 484 ± 33, and 1407 ± 157 nmol/ml in the 0%, 2%, and 4% glycine groups, respectively ($P < 0.001$; different from each other). Concentrations of serine in plasma (means ± SEM, n = 6-8 fish/group) were 82 ± 7.1, 152 ± 11, and 530 ± 51 nmol/ml in the 0%, 2%, and 4% glycine groups, respectively ($P < 0.001$; different from each other).

Conclusions: Glycine is a nutritionally essential amino acid for the growth and health of juvenile HSB.

Financial Support: Supported by USDA/NIFA grant No. 2022-67015-36200

Notes:

13 - Evaluation of genomic selection for acute hepatopancreatic necrosis disease resistance in white pacific shrimp: Genomic prediction

Breno Fragomeni[1], Issabelle Ampofo[1], Hung Mai[2], Daniel grevers[3], Monique Luna[3], Harris Wright[3], Arun Dhar[2]

[1]University of Connecticut, [2]University of Arizona, [3]Shrimp Improvement Systems. breno.fragomeni@uconn.edu

Session: Aquaculture, 2025-01-19, 9:30 - 9:45

Objective: The economic impact of infectious diseases in shrimp aquaculture sums up to two billion dollars annually. Acute hepatopancreatic necrosis disease (AHPND), caused by *Vibrio parahaemolyticus* carrying plasmid-borne binary toxin gene, is one of the leading causes of losses in shrimp aquaculture. Disease resistance is of paramount importance in crustacean aquaculture because of the limited adaptive immunity in such individuals and the lack of commercially available therapeutics for most diseases. The objective of this study is to investigate the genetic mechanisms underlying disease resistance in the white pacific shrimp (*Penaeus vannamei*).

Methods: Phenotypes were collected on 4,800 Specific Pathogen Free individuals after two independent 3-day immersion challenges using *V. parahaemolyticus* at 4.95x107 CFU/ml. In each challenge, 40 family lines were divided into 20 fast-growth and 20 slow-growth lines. Each family line had three replicate tanks and ten animals/tank (20 L size tank). Genotypes were available for 1900 individuals from the first challenge using a 50k SNP chip. Genomic predictions were calculated using the single sGBLUP approach and were compared to pedigree predictions using Henderson Mixed Model equations, using an animal threshold model. Additionally, animals from each family were kept in a pathogen-free growth test to evaluate the trade-offs between disease resistance and challenge. Finally, animals from every family were sampled for histopathology and PCR pathogen quantification.

Results: Heritabilities (SE) calculated with complete data were 0.38(0.08). Within-line heritabilities were estimated at 0.16 (0.07) for the slow growth line and 0.14 (0.06) for the fast growth line. The difference between the heritabilities of the two traits is negligible due to the high SE. Those values indicate that a mass selection program would increase disease resistance in this population. The correlation between breeding values and family survival was always above 0.85, demonstrating that the breeding values could identify the superior families. Those correlations increase to above 0.90 when using genomic data. Differences in correlations were significantly different ($p<0.05$). Finally, we calculated breeding values for genotyped individuals that were not in the disease challenge, demonstrating the ability to implement a genomic selection program for disease resistance for AHPND in *L. vannamei.*

Conclusions: The two lines of *P. vannamei*, a fast growth line and a slow growth line used in this study, can be improved for AHPND resistance by implementing a selection program following a disease challenge. Genomic prediction is more accurate than mass selection, and it is a feasible approach for this type of trait. More data are needed to validate the best genomic evaluation approach, identify biological markers, and understand the genetics underlying AHPND resistance.

Financial Support: Supported by USDA/NIFA grant No. 2022-67015-36200

Notes:

14 - Underwater vaccination using biopolymeric microneedles

Meng Li[1], Gregory Wiens[2], Jad Raad[1], Gael Kurath[3], Benedetto Marelli[1]

[1]Dept. of Civil and Environmental Eng., MIT, [2]National Center for Cool and Cold Water Aquaculture, USDA, [3]USGS Western Fisheries Research Center. mengli1024@gmail.com

Session: Aquaculture, 2025-01-19, 9:45 - 10:00

Objective: Aquaculture has been increasing for the past four decades to meet the increasing food supply demand and nutritional demands from consumers. Disease spread remains the number one factor causing fish loss in aquaculture. While current vaccination methods for juvenile fish include oral, immersion, and injection, injection remains the most efficient method. However, the challenge of handling juvenile fish, the need to bring fish out of the water and sedate them, the safety hazard for farm workers of using hypodermic needles, and the generation of needle waste pose a practical challenge to vaccinating juvenile fish in hatcheries. We want to overcome these challenges using biomaterial-based microneedle technology that can ultimately be automated for underwater fish vaccination.

Methods: We use food-graded biomaterials to fabricate microneedles that can maintain their mechanical strength to penetrate fish skin in aquatic environments. After penetration, the microneedles will release the pre-loaded bacteria/DNA vaccine to fish tissue to introduce immunity. Pathogen challenge and antibody titer will be used to validate immunity.

Results: A pilot study of group size 60 juvenile rainbow trout (~2.5g) shows microneedle delivering Yersinia ruckeri (Yr) vaccine has a mortality of 65%, statistically different from the negative control (PBS) groups' >80% mortality and positive control groups' (intramuscular injection) 10% mortality in Y. ruckeri challenge test.

Conclusions: Though microneedle administration shows protection, optimization needed to reach higher protection for farm efficacy.

Financial Support: This project was supported by Agriculture and Food Research Initiative Competitive Grant no. 2024-67012-42411 from the USDA National Institute of Food and Agriculture and by the MIT Abdul Latif Jameel Water and Food Systems Lab (J-WAFS).

Notes:

15 - Effects of varying temperatures on host-pathogen coevolution and disease transmission

Bret Elderd[1], Kale Rougeau[1], Lucy Detweiler[1], Lucy Aucoin[1]

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

Session: Epidemiology 1, 2025-01-19, 8:30 - 8:45

Objective: As the global climate warms, we are seeing a variety of ecological 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 effects of climate change on host-pathogen coevolution and, in turn, how this impacts disease transmission.

Methods: Using an easily manipulated insect host-pathogen system -- the fall armyworm (Spodoptera frugiperda), an agricultural pest, and its species-specific 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. Fall armyworm larvae become infected while feeding on leaf tissue that has been contaminated with the virus. To examine the eco-evolutionary dynamics of disease transmission, we exposed hosts that had either been coevolved with the virus or who had never been exposed to the virus to varying amounts of a coevolved or non-coevolved virus while the host fed on leaf tissue. These data were used to examine how eco-evolutionary processes affect disease transmission across different temperature regimes.

Results: Our transmission experiments show how transmission dynamics vary across multiple generations according to temperature. These changes arise due to two different factors related to disease transmission - the first factor being the mean transmission rate and the second factor being the variability about that rate. The degree of variability measures population-level hetereogeniety in disease susceptibility with some individuals being more susceptible to the disease and others less susceptible.

Conclusions: Changes in temperature as the climate warms will have a considerable impact on disease transmission due to both ecological and evolutionary processes as measured by changing transmission rates and the variability about those rates. While we focus on a single agricultural pest, the conclusions drawn are applicable to numerous other silvicultural and agricultural systems whose pest species are susceptible to insect pathogens. Thus, this research will, in turn, improve our ability to determine how best to use these pathogens as bioinsecticides under a warmer climate as both host and pathogen coevolve.

Notes:

16 - Seroprevalence of SARS-CoV-2 Delta and Omicron variants in cats and dogs in the USA

Subarna Barua[1], Nneka V. Iduu[1], Daniel F.B. Murillo[1], Asfiha Tarannum[1], Hill Dimino[2], Suchita Barua[2], Yue Shu[2], Calvin Johnson[3], Megan R. Miller[4], Kelly Chenoweth[1], Peter Christopherson[1], Laura Huber[1], Theresa Wood[1], Kelley Turner[1], Chengming Wang[1]

[1]Department of Pathobiology, Auburn University, [2]Department of Biological Sciences, Auburn University, [3]Veterinary Medicine Administration, Auburn University, [4]Center for Veterinary Medicine, U.S. Food and Drug Administration. szb0116@auburn.edu

Session: Epidemiology 1, 2025-01-19, 8:45 - 9:00

Objective: Understanding SARS-CoV-2 epidemiology in companion animals is critical for evaluating their role in viral transmission and their potential as sentinels for human infections. Epidemiological data on SARS-CoV-2 infections in companion animals have been extensively studied, but information on antibodies against SARS-CoV-2 variants in these animals is still limited. To address these knowledge gaps and investigate the potential of companion animals as sentinels for SARS-CoV-2 surveillance, we aimed to determine the prevalence of antibodies against SARS-CoV-2 variants, including the Delta variant and five Omicron sublineages. We also sought to identify potential risk factors, such as sex, age, and breed.

Methods: This large-scale serosurvey analyzed serum samples from 706 cats and 2,396 dogs collected across the USA in 2023 using a standard and variant-specific surrogate virus neutralization test (sVNT) to detect antibodies for SARS-CoV-2 variants. All data were analyzed using the STATISTICA 7.1 software (Statsoft, Tulsa, Oklahoma). Chi-square tests were employed to analyze the significance of the relationships between sex, age, breed, and the presence of seroprevalence of SARS-CoV-2.

Results: Overall, 5.7% of cats and 4.7% of dogs tested positive for antibodies, with younger animals (under 12 months) exhibiting significantly lower seropositivity rates (p=0.0048). No significant differences were found based on sex or breed. Furthermore, we analyzed 153 positive samples for variant-specific antibody responses using six sVNT kits that target the Delta variant and five Omicron sublineages. Among cats, 67.5% showed antibodies to Delta, with positivity rates for Omicron sublineages as follows: BA.1 (62.5%), BA.2 (42.5%), BA.4/BA.5 (77.5%), XBB (52.5%), and XBB.1.5 (45.0%). In dogs, 55.8% were positive for Delta, and Omicron sublineage rates were BA.1 (46.0%), BA.4/BA.5 (37.2%), XBB (58.4%), BA.2 (13.3%), and XBB.1.5 (9.7%).

Conclusions: Considering the close interactions between companion animals and humans, along with the persistence of antibodies against various SARS-CoV-2 variants and sublineages, our findings suggest that cats and dogs could be valuable sentinels for monitoring COVID-19 epidemiology.

Financial Support: Our study was supported by the Food and Drug Administration (FDA) of the U.S. Department of Health and Human Services (HHS) as part of a financial assistance award 1U18FD008031-01 funded by FDA/HHS.

Notes:

17 - Evaluating sources of uncertainty in predictions of epidemic spread

Eli Horner[1], Ross Meentemeyer[1], Christopher Mundt[2], Chris Jones[1]

[1]North Carolina State University, [2]Oregon State University. chris.mundt@oregonstate.edu

Session: Epidemiology 1, 2025-01-19, 9:00 - 9:15

Objective: Spatially explicit models of epidemics are useful for predicting future spread, assessing impacts, and designing management strategies. Robust predictions of pathogen transmission require understanding the effects and sources of uncertainty, however. Uncertainty in forecasts is primarily driven by initial disease conditions (e.g., observation error, cryptic infection, incomplete sampling), parameter estimates (e.g., parameter distributions), data drivers (e.g., weather variables and host distributions), and process error (e.g., model structure, choice of dispersal kernel, and stochastic processes). When real-time forecasts of these data variables are used in disease forecasts, the uncertainty is propagated through the model to affect the final predictions. Not all uncertainty derives from changing or inaccurate input data; unknown or non-measurable variables often influence the predictions as well. Our objectives were to model disease spread for cases in which variation among different sources of uncertainty can be estimated from real world data and determine how these sources of uncertainty combine to give uncertainty around predicted disease levels in time and space.

Methods: We used the Sobol global sensitivity analysis method to quantify and partition uncertainty spatially to understand where and why predictive uncertainty is high. We quantified the effect of different sources of uncertainty on model predictions to highlight areas that would provide the greatest impact on predictive ability, and how these sources of variation combine. Sudden oak death (SOD) was modeled as an example of a disease with significant long-distance dispersal, as data for all sources of variation were available for this disease. The Oomycete causal agent of SOD, *Phytophthora ramorum*, has a diverse host range with over 100 host plant species reported and is currently a significant disease in western U.S. forests and horticultural nurseries. We conducted 100,000 model runs for each forecast using the spatially explicit simulation model PoPS. Uncertainty was quantified from forecast data drivers (e.g., weather) by using ensemble forecasts of data drivers where each simulation uses a different ensemble member. We drew a parameter set for each simulation from a calibrated parameter distribution. Stochastic variables were incorporated into the models based on biological evidence and effective calibration methods.

Results: Different sources of uncertainty had different degrees of importance to variation in predicted epidemic spread, and the relative importance of uncertainty sources varied spatially. Our results showed that uncertainty in initial pathogen population size and host availability were the largest contributors to overall uncertainty in the leading edge of the outbreak. By contrast, random processes tended to dominate in the core outbreak area.

Conclusions: Incorporating and evaluating sources of uncertainty can improve predictions of the spatiotemporal spread of disease and highlight areas for future data collection. 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 was derived over varying spatial scales. Similar analyses will be conducted for other diseases for which adequate uncertainty data are available.

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:

18 - ICVI exceptions in U.S. States: A risk to cattle disease traceability and movement networks

Katherine P. Phillips[1], Sara C. Sequeira[1], Gregory Habing[1], Andréia G. Arruda[1]

[1]Department of Veterinary Preventive Medicine, The Ohio State University. sequeira.23@osu.edu

Session: Epidemiology 1, 2025-01-19, 9:15 - 9:30

Objective: Interstate Certificates of Veterinary Inspection (ICVIs) are widely used in studies to describe animal movements across the United States (US) and subsequently, explore the potential for disease spread. However, unique state-level exceptions to ICVI documentation could undermine their essential role in traceability. This project aimed to describe these exceptions in US state regulations and investigate the association between state-level predictors - such as cattle in shipments; number of beef and dairy cattle; value of exports (in US dollars) of milk and beef; number of cattle establishments; and, number of slaughter operations - and the number of ICVI exceptions per state.

Methods: ICVI exceptions were systematically recorded for all 50 states by accessing state regulations using official sources. These included exceptions for cattle 1) moving directly to slaughter, 2) moving to a livestock market, 3) using other forms of animal identification, 4) using commuter permits, 5) using grazing permits, 6) moving directly to veterinary care, 7) moving to a feedlot, 8) moving to an approved tagging site, 9) moving to their origin farm, 10) being held in quarantine, and 11) under a certain age. Then, a multivariable Poisson regression model was used to investigate the association between the number (count) of exceptions and the cattle-related characteristics by state mentioned below. Region was also included in the models. Statistical significance was determined at a p-value of < 0.05.

Results: Results revealed that 86% of states had at least one exception to ICVI usage, with a mean 2.5 exceptions per state. Notably, 82% of states included exceptions for slaughter movements, and half of the states allowed alternative forms of animal identification. The final multivariable model showed that states with 17 to 33 slaughter operations had a significantly higher rate of exceptions (IRR = 2.13; P= 0.034) compared to those with fewer than 17 slaughtering facilities. Furthermore, there was a numerical increase in incidence rate ratio (IRR) for cattle in shipments as the numbers of exceptions increased (IRR = 2.25, P = 0.08). There was no significant association between US region and the number of exceptions.

Conclusions: Findings from this study showed that basing cattle movement networks solely on ICVI documentation may negatively impact disease traceability in cattle. Additionally, it highlighted the fact that US states that could highly impact disease transmission, such as those receiving large numbers of cattle and/ or receiving cattle for slaughter, had higher number of exceptions to ICVI usages. This indicates critical vulnerabilities in the broader network of cattle movement, highlighting the need for more comprehensive approaches to disease traceability and prevention.

Financial Support: This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Notes:

19 - Optimizing environmental surveillance

Anwesha Chakravarty[1], Bo Li[1], Rebecca Smith[1]

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

Session: Epidemiology 1, 2025-01-19, 9:30- 9:45

Objective: Environmental surveillance requires collection of samples meant to represent a generalized risk in a specific area. For example, mosquito traps are used to test for the presence of West Nile virus (WNV) in mosquito populations, playing a crucial role in monitoring risk and informing response. But how do you decide where to take a sample for environmental surveillance? What makes a good sampling site?

Methods: We present a multi-step statistical approach for using longitudinal environmental sampling data to determine the value, or score, of a location in predicting an outcome of interest. This score is then used to understand what landscape, infrastructure, demographic and socioeconomic factors are associated with predictive ability. As a practical example, we apply this method to mosquito trapping in the Chicago metropolitan area and its suburbs and its ability to predict human cases of WNV.

Results: We find a minimum threshold for human population in the vicinity of the trap is necessary for overall prediction, with a weighting towards prioritizing sensitivity due to the low number of cases and severity of disease. However, different landscape factors become important for maximizing either sensitivity or specificity of the prediction, indicating that the optimal sampling location may vary based on the relative importance of each of these values.

Conclusions: This approach enables resource-limited environmental surveillance programs to identify better locations for their sample collection, which may help in reducing the number of samples needed while increasing their individual efficiency.

Financial Support: Support from #U01CK000651, the Centers for Disease Control and Prevention. Contents solely the responsibility of the authors and don't necessarily represent the official views of the Centers for Disease Control and Prevention or the Department of Health and Human Services.

Notes:

20 - Combining machine learning with H3 influenza A virus whole genome sequence data for host prediction

Hoc Tran[1], Olaf Berke[1], Nicole Ricker[2], Zvonimir Poljak[1]

[1]Department of Population Medicine, University of Guelph, [2]Department of Pathobiology, University of Guelph. htran10@uoguelph.ca

Session: Epidemiology 1, 2025-01-19, 9:45 - 10:00

Objective: Influenza A viruses (IAV) are major contributors to seasonal influenza and have caused several pandemics to date. IAVs can be classified into 18 hemagglutinin (H) and 11 neuraminidase (N) subtypes based on their antigenic properties. More severe influenza seasons are often attributed to H3 IAVs and the rapid evolution of H3 IAVs has given them the potential to cause pandemics through crossing the species barrier. Consequently, between-species transmission of H3 IAVs is a global concern due to their ability to infect a very wide range of mammalian and avian species that includes humans, swine, canines, equines, and poultry. Identification of hosts for H3 IAVs that have high potential for between-species transmission is therefore a necessity to limit spread and in preventing the emergence of a new H3 IAV pandemic. Past infections have also resulted in large pools of sequence data becoming publicly available, allowing for opportunities to make use of machine learning. Machine learning methods have been widely explored for host prediction of IAV using genomic data, however, this is often limited to data from only one of the eight segments of IAV or using all subtypes to predict broad categories of hosts. Thus, the objective of this research was to combine machine learning algorithms with H3 IAV whole genome sequence data to train models for distinct host prediction and identification of variants with high potential for between-species transmission.

Methods: Whole genome sequence sets were retrieved from public repositories and k-mers and amino acid properties were extracted from the sequence data to use as features for model training. Models were trained using these features alongside machine learning algorithms that include random forest and extreme gradient boosting machines for each of the 8 IAV segments. Trained models were then validated on a testing dataset and subsequently used to investigate patterns of between-species transmission within case study datasets involving canine H3N8 and swine H3N2 2010.2 through analytics of model predicted probabilities.

Results: Models demonstrated strong performance in host prediction across all 8 segments on the testing dataset, with overall accuracies and kappa values ranging from 0.995-0.997, 0.984-0.990, respectively. Between-species transmission patterns within case study model predicted probabilities were also identified to be consistent with what is known in the literature.

Conclusions: These models allow for rapid and accurate host prediction of H3 IAV datasets from any of the eight IAV segments and provide a solid framework that allows for identification of variants with higher than typical between-species transmission potential through model predicted probabilities.

Financial Support: This research was funded by an Ontario Veterinary College Scholarship and Ontario Agrifood Innovation Alliance and NSERC Alliance grants.

Notes:

21 - Organ-dependent intestinal barrier restitution is impaired by prebiotics

Courtney Deck[1], Madison Caldwell[1], Elizabeth Rose[1], Jack Odle[1], Laurianne Van Landeghem[1], Anthony Blikslager[1], Amanda Ziegler[1]

[1]North Carolina State University. amanda_ziegler@ncsu.edu

Session: General health and physiology, 2025-01-19, 8:30 - 8:45

Objective: Ischemic injury damages the intestinal epithelium leading to sepsis and death unless the mucosal barrier is restored. Devastating diseases featuring intestinal ischemia such as necrotizing enterocolitis1 or small intestinal volvulus are associated with higher mortality rates in younger patients, and the cause of this is not well described. In our porcine model, acute repair of barrier function in the ischemia-injured intestine of juvenile pigs (6-8-weeks) by rapid epithelial restitution is strikingly absent in younger neonatal pigs (2-weeks) but can be rescued by direct application of homogenized ischemia-injured juvenile mucosa. Perinatal microbiota colonization is known to contribute to postnatal maturation of gut physiology and the colon possesses a larger and more complex microbiota earlier in perinatal development than the jejunum. However, age-dependent colonic restitution responses have not been examined, and we have shown that microbiota colonization can be modulated by dietary oligosaccharide (prebiotic) supplementation. Therefore, we hypothesized that colonic restitution responses are similarly underdeveloped in neonates, and that prebiotic supplementation will accelerate postnatal microbial colonization jejunum and colon, thus enhancing restitution after ischemic injury.

Methods: Piglets 2- and 6-weeks-of-age were subjected to 0-, 30- or 60-minutes of jejunal and colonic ischemic injury and recovered ex vivo to assess barrier restitution, and restituting enterocytes were collected for RNA sequencing. In a separate study, after 24-hours of colostrum, piglets were fed control or oligosaccharide-supplemented formula (control-fed or prebiotic-fed) for 21 days and colonic and jejunal swabs were sequenced for 16S rDNA. Surgically ischemia-injured jejunal and colonic mucosal samples from select 2-week-old pigs were recovered ex vivo.

Results: Surprisingly, unlike the jejunum in which TEER values remained low throughout the recovery period, the colon values increased to control levels indicating the restoration of barrier function ($P<0.05$). Histological analysis which demonstrated restitution of the intestinal epithelium in the colon ($P<0.05$) but not the jejunum, indicating that restitution is both age- and organ-dependent in the perinatal period. Corresponding to this observation, transcriptional pathways critical for restitution were enriched in the colonic enterocytes, as were many pathways associated with microbial sensing and immune development. A significant decrease in Shannon alpha diversity ($P\leq0.05$, $n=12$) at 14-days-of-age was observed in the jejunal, but not colonic, microbiota, suggesting that decreased microbial sensing activity in the jejunum may be attributable to changes in the microbial diversity at that time of development. Inverse to our expectations, prebiotics had no effect on epithelial restitution in the jejunum and in fact impaired restitution in the colon ($P=0.016$). Indeed, prebiotic feeding induced a shift in the microbial beta diversity (PCoA) at 14-days-of-age and shifted numerous microbial pathways in the colon but not the jejunum, correlating with the lost capacity for intestinal restitution in that organ.

Conclusions: Ongoing work to understand diet-microbiome-epithelial interactions during postnatal development may inform novel approaches to preventative and clinical practices to improve intestinal health in vulnerable neonates.

Financial Support: NIH P30DK034987, K01OD028207, R01HD095876, U01TR002953; USDA NIFA 2019-67017-29372, 2022-67015-37125

Notes:

22 - Characterizing tight junction protein expression in 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 Ellis[4], Kendall Langsten[2], John T. Richeson[1], Paul S. Morley[2]

[1]West Texas A&M Univeristy, [2]VERO, [3]Texas A&M University, [4]University of Saskatchewan. pmorley@tamu.edu

Session: General health and physiology, 2025-01-19, 8:45 - 9:00

Objective: The gut barrier is a complex system comprised of microbes, microbial metabolites, a mucus layer, 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 models has associated changes in gastrointestinal barrier function with a growing litany of health conditions. While much remains to be learned about maintaining optimal gastrointestinal barrier function, tight junction proteins (TJPs) have been identified as key modulators of this process. Various complex TJPs have been identified in humans, but less is known in cattle about their abundance and composition throughout the gastrointestinal tract, despite their assumed influence on health. Therefore, the objective of this study was to characterize gene and protein expression of key TJPs 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). Tissue samples were collected from the rumen, jejunum, and spiral colon in standardized locations immediately post-evisceration. Tissue samples were fixed and prepared for histology to evaluate tissue morphology and immunohistochemistry (IHC) to evaluate protein expression of TJPs (Claudin 1, Claudin 2, Occludin, E-cadherin, Zona Occludin 1). Additionally, RT-qPCR for mRNA was used to quantify the same TJPs. Expression was characterized by GIT segment, and comparisons were made between organs using non-parametric methods in R (version 4.3.2). Differences were considered significant at $P \leq 0.05$.

Results: This study represents one of the broadest investigations of GIT barrier function in feedlot steers. Major differences in TJP composition exist between regions of the GIT. Overall, minor differences exist between qPCR and IHC results, and both data sets support the conclusion that each organ exhibits unique expression of TJPs. In general, Occludin was not different between sample types ($P = 0.08$). But Claudin 1, Claudin 2, and E-cadherin were less expressed ($P \leq 0.01$) in the rumen compared to the small intestine. However, the small intestine exhibited less Zonal Occludin 1 expression compared to the large intestine ($P = 0.03$).

Conclusions: This is one of the first studies to evaluate both gene and protein expression of TJP in healthy cattle. The data suggests high variability among different portions of the GIT, implying that interventions for improved GIT health may need to target a specific portion of the GIT that is affecting health outcomes. These findings provide valuable baseline information for future research, offering a new paradigm for factors affecting health and disease in cattle.

Financial Support: This research was funded by Texas A&M University.

Notes:

23 - Oleic acid promotes lipogenesis and improves mitochondrial function in bovine adipocytes: role of PPARα signaling

Ursula Abou-Rjeileh[1], G. Andres Contreras[1], Adam L. Lock[1]

[1]Michigan State University. abourje2@msu.edu

Session: General health and physiology, 2025-01-19, 9:00 - 9:15

Objective: In periparturient cows, oleic acid (OA) limits lipolysis and improves adipose tissue (AT) insulin sensitivity. In rodent models, OA enhances mitochondrial biogenesis and function. However, mechanisms behind OA effects are unknown. Our objective was to determine the effect of oleic acid (OA) on lipid accumulation, mitochondrial function, and its mechanisms of action in bovine adipocytes. Additionally, evaluate how OA impacts inflammation, lipolysis, and oxidative stress to support both metabolic and immune health in periparturient dairy cows.

Methods: Pre-adipocytes were isolated from subcutaneous AT (n=6, non-lactating, non-gestating Holstein cows) and induced to differentiate into adipocytes in standard differentiation media (CON) supplemented with palmitic acid (PA), OA, or 60-40 mix of PA and OA (60-40) at 300 µM with or without PPARα antagonist (GW6471; 10 µM) for 7 d. PPARα expression was determined by capillary electrophoresis (d 4). Triglyceride levels were quantified using Triglyceride-Glo Assay. Gene networks expression was evaluated using RT-qPCR. Data was analyzed using ANOVA, with cow included as a random effect.

Results: OA and PA increased PPARα protein expression compared with CON ($P < 0.02$). Compared with CON, supplementing fatty acids enhanced lipid accumulation ($P < 0.01$). However, PPARα inhibition decreased OA and 60-40 triglyceride content ($P < 0.05$) but not for PA ($P = 0.26$). Within the mitochondria, PA tended to decrease expression of fatty acid transport protein system (CAC, CPT1, CPT2), complex I protein (NDUFS1), SIRT1, and PGC1α compared with CON and OA ($P = 0.06$).

Conclusions: Our results show that OA and PA enhance lipid accumulation in adipocytes. OA improves mitochondrial biogenesis, restores oxidative phosphorylation, and potentially mitigates inflammation and oxidative stress by regulating mitochondrial function. These findings provide mechanistic evidence for the use of OA, along with PA, in dairy cow diets during the periparturient period to limit lipolysis by enhancing AT lipid accumulation. Enhancing metabolic and immune health may minimize health disorders and improve production of early postpartum cows.

Financial Support: Research supported by U.S. Department of Agriculture's National Institute of Food and Agriculture competitive grants 2019-67015-29443 and 2021-67015-33386, Agriculture and Food Research Initiative Competitive Grants Program Education and Workforce Development Predoctoral Fellowship (2024-67011-42894), and Michigan Alliance for Animal Agriculture (AA18-028).

Notes:

24 - A genome-wide association study of measures of stress response in young healthy pigs and in grow-to-finish pigs exposed to a natural polymicrobial disease challenge

Fazhir Kayondo[1], Hayder Al-Shanoon[2], Yolande M. Seddon[2], David Janz[2], Dylan Carette[2], Carmen Cole[2], Frederic Fortin[3], John C.S. Harding[2], Michael K. Dyck[4], Graham Plastow[4], PigGen Canada[5], Jack C.M. Dekkers[1]

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

Session: General health and physiology, 2025-01-19, 9:15 - 9:30

Objective: Identify the genetic basis of responses of growing pigs to infectious and non-infectious stressors.

Methods: This study measured stress response in Landrace x Yorkshire barrows using responses to a backtest at 27 days of age (n=899), including the number and intensity of vocalizations (VN, VI) and struggles (SN, SI), and levels of cortisol, cortisone, DHEA, and DHEA-S in hair (Hair 1) collected from 863 pigs at ~40 days of age, before their entry into a natural polymicrobial disease challenge, and hair regrowth (Hair 2) during the challenge, at ~82 days of age. Previous research indicated that these traits are heritable and genetically correlated with disease resilience. Pigs were genotyped with a 50K SNP panel and imputed to 650K SNPs. A genome-wide association study was performed using Bayesian methods.

Results: 1-Mb windows that explained >1% of the estimated genetic variance were identified on SSC3 for DHEA-S in Hair 1 (3.0% of genetic variance), on SSC12 for cortisone in Hair 2 (1.7% of genetic variance) on SSC14 for cortisol in Hair 2 (1.4% of), and on SSC2 for cortisol in both Hair 1 (45.3% of genetic variance) and Hair 2 (37.8% of). For the latter, the lead SNP, rs341258564, neighbors the glucocorticoid receptor gene (NR3C1) and explained all the for this major quantitative trait locus. An extra copy of the minor allele at this SNP (frequency = 9%) significantly ($p<0.001$) reduced cortisol levels by 30±6% in Hair 1 and by 30±4% in Hair 2, and cortisone levels by 19±4% in Hair 1 and by 23±6% in Hair 2, and increased VN by 5±2%. With borderline significance ($p<0.10$), it increased average daily feed intake under challenge by 3±2 kg/day and reduced loin depth by 1.0+0.5 and mortality in the finisher from ~17% for homozygotes for the major allele to ~14% for heterozygotes.

Conclusions: These findings suggest the genomic location of a potential causal variant affecting glucocorticoid levels in hair of pigs and offer insight into the genetic basis of stress response and disease resilience.

Financial Support: This research was supported by Genome Canada, PigGen Canada, RDAR, and USDA-NIFA grant #2021-67015-34562

Notes:

25 - Novel bovine reference gene candidates for RT-qPCR identified via publicly available tissue RNA-seq datasets

Cassandra Barber[1], Matthew Scott[2], Amelia Woolums[1]

[1]Mississippi State University, [2]Texas A&M University. cdb835@msstate.edu

Session: General health and physiology, 2025-01-19, 9:30 - 9:45

Objective: Reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) is commonly used to characterize gene expression for various research and diagnostic applications. However, relative quantitation of gene expression requires the use of reference genes to normalize differential gene expression. Current recommendations for best practices in RT-qPCR indicate that some previously used reference genes lack stable expression under certain experimental conditions. Thus, there is a need to identify new reference genes with stable expression across different tissues and experimental conditions. While there are a plethora of new studies identifying stable reference genes for humans, mice, and rats, there are few studies confirming stably expressed bovine reference genes. Next-generation RNA sequencing, or RNA-seq, can be used to identify the entire repertoire of genes expressed in a tissue. Transcriptomes generated by RNA-seq allow the identification of stably expressed reference genes. The objective of this study was to identify candidate bovine reference genes using publicly available transcriptomes.

Methods: Forty-one transcriptomes were acquired from the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO). Datasets were acquired from projects having sampled various organ systems in apparently healthy Bos taurus cattle; data from 11 different tissue samples were accessed, including bone marrow, bronchial lymph node, mesenteric lymph node, kidney, liver, lung, nasal epithelium, nasopharyngeal lymph node, spleen, thymus, and trachea. Raw sequenced read files for each dataset were quality assessed with FastQC v0.11.9 and trimmed with Trimmomatic v0.39. Trimmed read fragments were mapped to the bovine reference assembly ARS-UCD2.0 via STAR v2.7.11a. Raw gene counts generated for each transcriptome dataset were processed and analyzed in R v4.0.2. To adjust raw gene counts for uncontrolled variance, or batch effects, across each BioProject submission, the “ComBat_seq” empirical Bayesian function from the Bioconductor package sva v3.46.0 was utilized. Library normalization was performed with the Relative Log Expression (RLE) method, using default settings. The NormFinder algorithm was then applied to the complete dataset, retaining 2000 candidate reference genes ranked by dataset expression stability values.

Results: The top two thousand most stable genes were returned with stability values ranging from 0.175 to 1.291. The top twenty identified candidate reference genes are as follows with the respective stability value: CRNKL1 (0.175), SPOP (0.202), NMT1 (0.216), PIGU (0.217), YKT6 (0.232), GZF1 (0.251), ARIH2 (0.257), MTERF4 (0.272), CERS5 (0.277), SNUPN (0.280), RANBP9 (0.281), C11H9orf78 (0.281), POP4 (0.284), TMEM183A (0.286), DDX23 (0.288), UBE2Q1(0.291), GNL2 (0.292), UBAP1 (0.294), FBXW11 (0.300), and TTC4 (0.300). Of these genes, UBE2Q1 is the only gene that has been reported to be used as a reference gene for bovine samples, while ARIH2 and POP4 have been used for human samples. The stability of NMT1 was unexpected as previous research described upregulation of expression during bovine Mannheimiosis.

Conclusions: Notably, these candidate reference genes are stably expressed genes across all the tissue types based on RNA-seq experimentation. This analysis of the transcriptomes serves as a foundation for RT-qPCR analysis and the genes identified as candidate reference genes will be validated for stability in future research.

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

Notes:

26 - Intrauterine wildfire-PM2.5 exposure affects calf growth, health, and metabolic and inflammatory markers

Alexandra Pace[1], Patricia Villamediana[2], Maristela Rovai[2], Gabriella Ponce[3], Natalie Ellis[1], Angela Stegeman[1], Mallery Larson[1], Denise Konetchy[1], Pedram Rezamand[4], Amy Skibiel[1]

[1]University of Idaho, [2]South Dakota State University, [3]University of Tennesee, [4]University of Connecticut. pace4745@vandals.uidaho.edu

Session: General health and physiology, 2025-01-19, 9:45 - 10:00

Objective: Dairy cattle health and production can be affected by the intrauterine environment through developmental programming. Recent research has shown that exposure to wildfire particulates in-utero is associated with intrauterine growth restriction in human neonates. Fine particulates ($PM_{2.5}$) in wildfire smoke affect pre-weaned calf health, inflammatory markers, and metabolism. It is unknown, however, whether intrauterine wildfire-$PM_{2.5}$ exposure has consequences on post-natal calf development.

Methods: Hourly PM2.5 concentrations were recorded. Animals were considered exposed to wildfire-$PM_{2.5}$ if daily average $PM_{2.5}$ was greater than 35 µg/m3 and traced back to active wildfires using HYSPLIT modeling. Calves were born to cows that were either exposed (WFS, n=17, five days of exposure to natural wildfire-$PM_{2.5}$ that reached 113.5 µg/m3 during mid-gestation) or unexposed [(CON, n=26, not exposed to elevated $PM_{2.5}$ concentrations during gestation ($PM_{2.5}$ < 32 µg/m^3)]. Management and nutritional protocols were matched between both groups. Blood samples, growth measurements, vital signs, and health scores were taken 2X per month from birth (prior to colostrum feeding) to 6 months of age. Thoracic ultrasonography was performed 1X per month. Colostrum and an additional blood sample was collected at 48 h after birth to assess passive immune transfer and apparent efficiency of IgG absorption (AEA). Circulating white blood cell counts (WBC) and concentrations of glucose, β-hydroxybutyrate (BHB), non-esterified fatty acids (NEFA), serum amyloid A (SAA), serum albumin (SA), and haptoglobin (Hp) were measured. Data were analyzed using t-tests or linear mixed models.

Results: WFS calves were born smaller than CON calves (36.87 ± 1.02 vs. 40.81 ± 1.02 kg; P < 0.002) but had greater average daily gains (ADG) through the pre-weaning period (0.81 ± 0.04 vs. 0.69 ± 0.03 kg/day; P = 0.01). After weaning, ADG of WFS was lower than CON (0.65 ± 0.03 vs. 0.76 ± 0.02 kg/day; P < 0.003). Glucose was greater and BHB was lower in WFS calves at several timepoints within the pre-weaning period, while NEFA was greater during the pre-weaning and post-weaning periods compared with those for CON (P < 0.03). Hp was lower in WFS at 12 wk of age (0.54 ± 0.07 vs. 1.13 ± 0.11g/L; P < 0.0001). SAA and WBC was lower while SA was greater in WFS than those for CON throughout the study (P < 0.003). All calves received adequate passive immune transfer and there was no difference in AEA between the groups. CON had greater odds of having lower enteric symptoms relative to WFS (Odds Ratio: 7.41; 95% confidence interval: 2.45, 22.54; P = 0.0004), whereas CON calves had lower odds of having respiratory pathologies relative to WFS (Odds Ratio: 0.07; 95% confidence interval 0.02, 0.21; P < 0.0001).

Conclusions: Although WFS calves were born smaller, they grew faster than CON calves, suggesting that wildfire-PM2.5 affect intrauterine growth restriction. This can impact tissue and organ ontogeny and growth, and cause altered metabolic processing and inflammatory responses. Our results suggest that intrauterine to wildfire-$PM_{2.5}$ affects postnatal growth, metabolism, inflammatory status, and health, which could have significant implications on future production.

Financial Support: This project was supported by Agriculture and Food Research Initiative Competitive Grant no. 2022-68016-38665 from the USDA National Institute of Food and Agriculture, and by NIH Grant no. P20 GM103408 from the National Institute of General Medical Sciences.

Notes:

27 - Integration of laboratory testing for bovine respiratory disease: Perceptions of Canadian feedlot veterinarians

Olufunto O. Adewusi[1], Candace I.J. Nykiforuk[1], Cheryl L. Waldner[2], Sherly P. Gow[2], Nathan Erickson[2], Simon J.G. Otto[1]

[1]School of Public Health, University of Alberta, [2]Department of Large Animal Clinical Sciences, University of Saskatchewan. olufunto@ualberta.ca

Session: Diagnostic testing - Cattle, 2025-01-19, 8:30 - 8:45

Objective: Timely, accurate, and reliable laboratory tools are important for antimicrobial stewardship (AMS). In Canadian feedlots, traditional laboratory methods have not informed individual animal bovine respiratory disease (BRD) antimicrobial treatment decisions due to sample-to-result times of up to seven days. The objectives of this study were to: 1) identify factors that influence respiratory sample collection for laboratory testing, and 2) describe the potential for integration of laboratory testing into an AMS strategy for BRD management in Canadian feedlots.

Methods: Utilizing focused ethnography, virtual key informant interviews were conducted with eight feedlot veterinarians from the Canadian provinces of Alberta, Ontario, and Saskatchewan, representing five practices responsible for greater than 70% of Canadian-fed cattle. Thematic analysis revealed four themes: 1) Lived experience of feedlot veterinarians with laboratory testing for BRD pathogens and AMR, 2) Evidence-informed BRD management that integrates multiple data sources and their components, 3) Organizational factors that affect the uptake of laboratory tests, and 4) The role of laboratory testing to support AMS in BRD management under future regulatory requirements.

Results: Participants identified substantial experience in respiratory sample collection among feedlot veterinarians and staff from BRD and AMR research and surveillance. They highlighted that while these activities provide retrospective data and evidence used in their decisions about BRD management, pen-level sampling strategies and testing of live animals are currently not part of routine BRD management. Feedlot veterinarians are willing to incorporate pen-level sampling and laboratory testing, including at new times early in the feeding period (e.g., 10-14 days on feed), if there is demonstrable benefit of reduced BRD morbidity and mortality. This time point shortly after arrival allows BRD pathogens to circulate in groups of high-risk animals. Also, the arrival period follows changes in AMR due to selection pressure after metaphylaxis. Any sampling and laboratory testing strategy must prioritize efficiency and provide tangible benefits to the feedlot client's operation regarding animal welfare and cost for uptake to be considered. Such a strategy could support AMS in BRD management in feedlots and address a need for laboratory testing should this become a regulatory or market requirement. The demonstrated benefits of any strategy must be communicated clearly within the feedlot organizational structure to foster uptake. Animal health, welfare, and economic benefits relative to feedlot workflow must be relayed to managers and staff to inform and balance competing priorities. Together, these findings highlight the complex decision-making process feedlot veterinarians encounter when considering laboratory testing as part of a BRD management strategy. These decisions must not only align with the needs of their clients but also consider their professional ethical and medical responsibilities.

Conclusions: By gaining insights into the lived experience of Canadian feedlot veterinarians, this study contributes to the broader conversation about the utilization and optimization of laboratory testing as an AMS strategy in food animal production. This understanding of the perspectives of Canadian feedlot veterinarians is crucial for the future implementation of laboratory testing strategies to detect BRD pathogens and AMR to support AMS.

Financial Support: This research project is funded by Genome Canada with support from Genome Prairie, Genome Alberta, and the Saskatchewan Agriculture Development Fund, as well as support from, the University of Saskatchewan and the University of Alberta.

Notes:

28 - Associations of gross and histopathological cardiac and hepatic findings in feedyard mortalities

Katie A. Long[1, 2], Brad J. White[1, 2], Brandon L. Plattner[1, 3], Eduarda M. Bortoluzzi[1, 4], Laura Carpenter[1, 2], Robert L. Larson[1, 2], Brian V. Lubbers[1, 2]

[1]Kansas State University, [2]Beef Cattle Institute, [3]Department of Diagnostic Medicine & Pathobiology, [4]Department of Anatomy and Physiology. kal023@vet.k-state.edu

Session: Diagnostic testing - Cattle, 2025-01-19, 8:45 - 9:00

Objective: Bovine Congestive Heart Failure (CHF) is increasingly concerning for veterinarians and producers. The objective of this case-control study was to evaluate potential associations between grossly diagnosed CHF and cardiac and hepatic histologic lesions.

Methods: Cases (CHFCASE) and controls (CONT) were identified as a subset from a larger population of feedyard mortalities. Criteria for a gross CHF diagnosis included grossly misshapen heart deemed to be non-infectious, and at least one of the following ancillary signs: chronic passive congestion of the liver, pleural effusion, ascites, or brisket edema. Following identification of a gross CHF diagnosis, the next animal without CHF at the same feedyard was sampled as a control. All hearts were scored, and severe heart changes were considered CHFCASE, while normal/mild heart changes were CONT. Heart and liver histologic necrosis and fibrosis were scored as severe or non-severe. Multivariable logistic regression was used to assess potential relationship between histologic scores and the outcome of interest (CHFCASE/CONT).

Results: Of 87 animals enrolled in the study, 34 (39%) were CHFCASE. Myocardial necrosis or fibrosis, and liver fibrosis, were not significantly associated with CHFCASE. Liver necrosis was higher ($p<0.05$) in CHFCASE (62%) compared to CONT (33%).

Conclusions: Cardiac histopathology was not associated with gross CHF diagnosis, yet liver necrosis was higher in CHFCASE compared to CONT. These findings indicate injury to the liver would be more consistent than injury to the heart in CHF.

Financial Support: The authors would like to acknowledge support from the American Angus Foundation, Foundation for Food and Agriculture Resources ICASA (grant number ICASA-0000000017), Innovative Livestock Services, and Kansas State University's Beef Cattle Institute.

Notes:

29 - Comparing 0.9% sterile saline to phosphate buffered saline as a transport media for *Tritrichomonas foetus* RT-qPCR testing

T. M. Jumper[1], M. Thoresen[1], E. H. King[1], D. Loy[2], J. D. Loy[2], D.R. Smith[1]

[1]Department of Pathobiology and Population Medicine, Mississippi State University, [2]Nebraska Veterinary Diagnostic Center, University of Nebraska-Lincoln. tyler.jumper@msstate.edu

Session: Diagnostic testing - Cattle, 2025-01-19, 9:00 - 9:15

Objective: The limit of detection for *T. foetus* RT-qPCR is 1 organism/extraction in phosphate buffered saline (PBS) without prior culture. The objective of this study was to determine if 0.9% sterile saline (SAL), a readily available media, was not inferior to PBS as a transport media for *T. foetus* RT-qPCR testing at that concentration.

Methods: Smegma was collected via 10 weekly preputial washings from known *T. foetus* negative bulls. Each week, 1.3 mL of PBS with smegma (n=60) and 1.3 mL of SAL with smegma (n=60) were prepared. For each media, 30 samples were inoculated with *T. foetus* to a concentration of 1 organism/100 µL to evaluate sensitivity (Sn), 30 were left uninoculated to evaluate specificity (Sp). A total of 1200 (SnPBS=300, SnSAL=300, SpPBS=300, SpSAL=300) samples were tested by RT-qPCR. Differences in Sn and Sp between SAL and PBS were tested in logistic regression models, with week as a random effect. The Farrington-Manning test for noninferiority was used to evaluate the difference between diagnostic parameters with Δ=2.5%.

Results: For PBS, Sn =70.7% (95%CI: 65.2-75.8%), and Sp=99.7% (95%CI: 98.2-100%); for SAL, the Sn=73.3% (95%CI:68.0-78.3%), and the Sp=100% (95%CI: 98.8-100%). No statistical differences between SAL and PBS were detected for Sn or Sp. The SpSAL was not inferior to SpPBS (p=0.008), however, noninferiority of SnSAL to SnPBS was inconclusive (p=0.08).

Conclusions: The Sn for samples with very low concentration of *T. foetus* in SAL was similar to, and Sp was as good as PBS, suggesting either would be acceptable as transport media.

Financial Support: Supported by funds from the Mississippi State University College of Veterinary Medicine Office of Research and Graduate Studies House Officer Program, as well as the United States Department of Agriculture (USDA) Agriculture Research Service (ARS) fund #58-0200-0-002

Notes:

30 - Likelihood ratios of *T. foetus* RT-qPCR around limit of detection

T. M. Jumper[1], M. Thoresen[1], E. H. King[1], D. Loy[2], J. D. Loy[2], D.R. Smith[1]

[1]Department of Pathobiology and Population Medicine, Mississippi State University, [2]Nebraska Veterinary Diagnostic Center, University of Nebraska-Lincoln. tyler.jumper@msstate.edu

Session: Diagnostic testing - Cattle, 2025-01-19, 9:15 - 9:30

Objective: *Tritrichomonas foetus* is a venereally transmitted pathogen that can cause reproductive losses in cattle. A recent *T. foetus* RT-qPCR has reported a higher sensitivity and specificity, and a lower limit of detection (LOD) at ~1 organism/100 µL. The interpretation of results can have major economic consequences if misclassification occurs. The objective of this study was to determine if likelihood ratios would be more informative than a single cutoff point of ≤35.00 Ct for clinical interpretation.

Methods: 600 samples inoculated with *T. foetus* at approximately the LOD, and 600 samples left uninoculated, all in either saline or PBS transport media (n total=1200) were submitted to a diagnostic laboratory for *T. foetus* RT-qPCR testing. Descriptive statistics were performed in spreadsheet software. Category specific likelihood ratios (LRcat) were calculated for Ct results ≤ 35.00, 35.01-39.99, and 40.00+.

Results: The median Ct of inoculated samples was 32.93 (min=22.39, max = 40.00) and the median of uninoculated samples was 40.00 (min=32.81, max=40.00). For Ct ≤ 35.00, LRcat =432 (95% CI: 60.91-3063.85); Ct=35.01-39.99, LRcat =149 (95% CI: 20.92-1061.3); and Ct=40.00+, LRcat = 0.03 (95% CI: 0.02-0.05).

Conclusions: The clinical decisions should be based off of posttest probability, calculated from the pretest probability and LRcat . The high LR for categories Ct ≤35.00 and Ct=35.01-39.99 show that any amplification in these categories have a high ratio of true positive test results to false positive test results. Therefore, results in Ct=35.01-39.99 category could be true positives, and misinterpreted with the cutoff point of Ct≤35.00.

Financial Support: Supported by funds from the Mississippi State University College of Veterinary Medicine Office of Research and Graduate Studies House Officer Program, as well as the United States Department of Agriculture (USDA) Agriculture Research Service (ARS) fund #58-0200-0-002

Notes:

31 - Optimizing *Salmonella* Dublin detection in clinically ill dairy calves

M.J. Craig[1], K.J. Cummings[1], L.B. Goodman[1], E. Frye[2], J.D. Siler[1], R.J. Franklin-Guild[3]

[1]Department of Public and Ecosystem Health, Cornell University, [2]Department of Population Medicine and Diagnostic Sciences, Cornell University, [3]Department of Population Medicine and Diagnostic Sciences, Animal Health Diagnostic Center. mjc437@cornell.edu

Session: Diagnostic testing - Cattle, 2025-01-19, 9:30 - 9:45

Objective: Enhancing *Salmonella* Dublin detection in clinically ill cattle is critical for treatment purposes and mitigating the risk of spreading disease. *Salmonella* Dublin causes signs of pneumonia in calves, suggesting that the organism harbors in the respiratory tract and thus presenting an opportunity to refine diagnostic protocols. The objective of this study was to investigate the use of readily accessible clinical samples for optimizing *Salmonella* Dublin detection in clinically ill dairy calves.

Methods: Herds with endemic *Salmonella* Dublin infection were recruited for sampling. Upon the onset of clinical disease, participating veterinarians were asked to collect nasal swab, saliva, and fecal samples on the first three days following disease onset, as well as blood culture samples on the first two days. Clinical disease was defined as signs of pneumonia and fever of ≥104°F or a fever of ≥104°F in combination with depression with or without respiratory signs. Calves under 4 months of age with clinical signs were sampled, and all samples were sent to the Cornell University Animal Health Diagnostic Center for *Salmonella* PCR, with subsequent culturing and serogrouping for PCR-positive samples. A mixed-effects logistic regression model was used to evaluate associations between PCR results, day of sampling, and sample type for calves with confirmed *Salmonella* Dublin infection.

Results: A total of 407 samples were collected from 43 calves representing 17 dairy herds in the northeastern United States. Twenty-six of 43 calves (60%) were identified as blood culture-positive for *Salmonella* Dublin. *Salmonella* Dublin was isolated from blood, feces, and nasal swab samples on days 1-3 following disease onset. Nasal swab (OR = 0.14, p = 0.02) and saliva samples (OR = 0.14, p = 0.02) were associated with a decreased odds of *Salmonella* PCR positivity in comparison to fecal samples. However, nasal samples collected on day 3 (OR = 2.33, p = 0.42) of disease onset were associated with an increased odds of PCR positivity in comparison to fecal samples collected on day 1 of disease onset.

Conclusions: *Salmonella* Dublin can be isolated from blood, nasal swab, and fecal samples from clinically ill calves. However, PCR detection of *Salmonella* is most likely to occur using blood or fecal samples collected on the first day of disease onset or nasal samples on day 3 of disease onset. The described diagnostic criteria can be used to identify suspect calves that may require sampling for *Salmonella* Dublin diagnosis. This project is ongoing.

Financial Support: This project is funded by a USDA NIFA AFRI grant titled “Novel diagnostic approaches to comprehensively define *Salmonella* Dublin transmission dynamics and improve disease control in dairy cattle”

Notes:

32 - Evaluating the accuracy of brix and serum total protein as proxies for IgG in assessing the transfer of passive immunity in calves

Christian Bernal-Córdoba[1], Jéssica Pereira[1], Fernanda Ferreria[1], Noelia Silva-del-Rio[1]

[1]University of Califonia, Davis. crbernalcordoba@ucdavis.edu

Session: Diagnostic testing - Cattle, 2025-01-19, 9:45 - 10:00

Objective: This study aimed to evaluate the accuracy of Brix refractometry (Brix) and serum total protein (STP) in assessing the transfer of passive immunity (TPI) in calves, using serum IgG concentration (radial immunodiffusion) as a reference on days 2 and 7 of age.

Methods: A total of 198 newborn dairy-beef crossbred calves were enrolled upon arrival at a calf-raising facility in California. Blood samples were collected to measure IgG, STP, and Brix on days 2 and 7. TPI was categorized as follows: IgG (g/L): Excellent ≥ 25, Good 18-24.9, Fair 10-17.9, Poor < 10; STP (g/dL): Excellent ≥ 6.2, Good 5.8-6.1, Fair 5.1-5.7, Poor < 5.1; Brix (%): Excellent ≥ 9.4, Good 8.9-9.3, Fair 8.1-8.8, Poor < 8.1. Statistics included Cohen's Kappa to assess the agreement between TPI categories, Bowker's Test to evaluate changes in TPI classifications over time, Spearman's rank correlation to examine associations between variables, and linear regression models to assess the effectiveness of STP and Brix as IgG proxies.

Results: Median (IQR) values on day 2: STP = 5.4 g/dL (4.7-6.0), Brix = 8.4% (7.7-9.2), IgG = 20.1 g/L (11.2-26.7). On day 7: STP = 5.2 g/dL (4.7-5.5), Brix = 8.2% (7.7-8.7), IgG = 15.7 g/L (8.1-22.3). The distribution of TPI categories varied across variables. On day 2, STP: 40.4% poor, 30.3% fair, 13.6% good, and 15.7% excellent; Brix: 39.4% poor, 27.8% fair, 16.7% good, and 16.2% excellent; IgG: 23.7% poor, 17.7% fair, 28.3% good, and 30.3% excellent. A similar pattern was observed on day 7. The distribution of TPI categories within the same variable (STP, Brix, and IgG) changed between day 2 and day 7. Cohen's Kappa indicated moderate agreement on day 2 between STP and IgG ($\kappa = 0.496$) and Brix and IgG ($\kappa = 0.508$), with fair agreement on day 7 between STP and IgG ($\kappa = 0.376$) and Brix and IgG ($\kappa = 0.368$). Agreement between STP and Brix was almost perfect on days 2 ($\kappa = 0.963$) and 7 ($\kappa = 0.943$). Bowker's Test showed significant changes in TPI classification from day 2 to day 7 for STP ($\chi^2 = 28.55$), Brix ($\chi^2 = 30.23$), and IgG ($\chi^2 = 40.75$; $p < 0.001$ for all). Strong correlations were observed between Brix and IgG on days 2 ($\rho = 0.86$) and 7 ($\rho = 0.83$), and between STP and IgG on days 2 ($\rho = 0.86$) and 7 ($\rho = 0.83$; $p < 0.001$ for all). STP significantly predicted IgG on days 2 (Estimate = 11.22, $R^2 = 0.78$) and 7 (Estimate = 12.59, $R^2 = 0.76$), and Brix significantly predicted IgG on days 2 (Estimate = 9.50, $R^2 = 0.78$) and 7 (Estimate = 10.65, $R^2 = 0.76$).

Conclusions: While STP and Brix were strongly correlated, their cut-off values led to different TPI classifications. Significant changes in TPI classification occurred over time, which reduced the agreement between variables on day 7. Further studies are needed to explore alternative cut-off values for Brix and STP and to investigate other factors that may influence the assessment of TPI in calves.

Notes:

33 - Infection and immunity – Clinical outcomes

D. Scott McVey[1]

[1]University of Nebraska-Lincoln. dmcvey2@unl.edu

Session: ACVM - Featured Speakers, 2025-01-19, 10:30 - 11:15

The bovine respiratory disease complex continues to be problematic, and a significant amount of morbidity and mortality continues in cattle. There are similar problems in the swine industry with continued persistence of respiratory disease. The pathogens that cause these diseases are numerous and the mechanisms by which they initiate tissue injury are numerous. Veterinary science has recognized that the host response to these pathogens is also partially responsible for the degree of tissue damage and therefore, the associated costs. The infectious agents and the subsequent inflammation mediate direct damage to the respiratory tissues. This is associated with high morbidity in many cases and often high mortality. In addition, recovery is often incomplete. There are opportunities through continued research to improve clinical recovery associated with these respiratory infections. It is recognized that the veterinary biologicals industry has vastly improved the quality of products in the last few decades, and these continue to work reasonably well in many cases. Also, the industry has continued to improve the efficacy testing approaches associated with biological development. Many veterinarians have observed recovery processes in these animals for many years and we have opportunities to define the mechanisms of resolution of infection and inflammation. Specific evaluation and characterization of biological processes such as endothelial/epithelial regeneration, pro-inflammatory signaling and receptors, myeloid derived suppressor cells, as well as other resolution factors may identify opportunities to prevent interstitial damage and allow endothelial and epithelial recovery and repair. This discussion will include possible approaches to evaluate these factors, especially as associated with clinical recovery. Possible outcomes of this research would include enhanced assessment of vaccine efficacy, reductions in clinical cost of treatment along with reductions in losses due to incomplete recovery and mortality.

Notes:

34 - Current status of swine viral infections in the US: emerging and re-emerging diseases

Kyoungjin Yoon[1]

[1] Dept. of Veterinary Diagnostic and Production Animal Medicine, Iowa State University. kyoon@iastate.edu

Session: ACVM - Featured Speakers, 2025-01-19, 11:15 - 12:00

Viral infectious diseases are a significant challenge to pig health and swine production worldwide. Compared to many pig-producing countries, the US swine industry is fortunate to be free of several high-impact viral diseases, such as African swine fever, classical swine fever, foot-and-mouth disease, and pseudorabies (Aujeszky's disease), yet. Nonetheless, US domestic swine populations have experienced the emergence of previously unrecognized diseases/viruses (e.g., porcine reproductive and respiratory syndrome, porcine circovirus-associated diseases, swine hepatitis E, atypical porcine pestivirus, influenza D virus, porcine parainfluenzavirus) or foreign animal diseases (e.g., porcine epidemic diarrhea, porcine deltacoronavirus) in last 30-40 years. It has also experienced the re-emergence of 'old' foes with high economic significance from time to time, such as porcine astrovirus, porcine enterovirus, porcine rotavirus, Seneca Valley virus, influenza A virus, porcine sapovirus. In addition, there have been reports of newly identified viruses in the literature, mostly due to advances in diagnostic technology. This talk will review emerging and re-emerging swine viral infections in the US and abroad based on diagnostic data and literature, present the current status of prevention and control measures (both success and failure), and discuss knowledge gaps and future research needs.

Notes:

35 - Screening novel biocides for rapid killing of *Salmonella* isolates of concern

Maya P.N. Encinosa1, Lucas Thompson[1], William M. Hart-Cooper[2], Bruno E. Quintanilla Florian[2], Roberto D.J. Avena-Bustillos[2], Keith Meeks[3], Torey Looft[1], Shawn M.D. Bearson[1]

[1]USDA-ARS, National Animal Disease Center, [2]USDA-ARS, Western Regional Research Center, [3]iPura, Global Food Technologies. Maya.encinosa@usda.gov

Session: Diagnostic testing 1, 2025-01-19, 10:30- 10:45

Objective: *Salmonella* is a human foodborne pathogen that frequently colonizes food animals sub-clinically, resulting in unknowing contamination of food products such as chicken, eggs, turkey, pork and beef. The USDA Food Safety and Inspection Service recently proposed *Salmonella* as an adulterant in specific poultry products and selected serovars I 4,[5],12:i:-, Enteritidis, Typhimurium, Hadar, and Muenchen as key performance indicators (KPIs) to monitor progress towards the reduction of *Salmonella* in food products. To decrease *Salmonella* contamination of processing facilities and poultry products, disinfectants and sanitizers are employed such as Peroxyacetic acid (PAA), an organic peroxide-based compound with a low pH and broad-spectrum bactericidal properties. PAA has a recommended use of 50-2000 ppm depending on product application and has a risk of eye and respiratory irritation at concentrations as low as 5 ppm. Other available disinfectants used in processing facilities include Wexcide, Virkon, and Virocid with recommended dilutions of 1:128 (502 ppm), 1:100 (2291 ppm), and 1:170 (2093 ppm), respectively. With the goal of identifying alternative sanitizers, this study screened and evaluated novel biocides for their ability to rapidly kill *Salmonella* strains of concern.

Methods: Following an initial screening of 44 new biocide compounds, two biocides (A6 and A16) were selected for further evaluation (along with PAA, Wexcide, Virkon, and Virocid) for their efficacy against five serovars of *Salmonella enterica* representing serogroup B (Typhimurium and I 4,[5],12:i:-), serogroup C (Infantis and Hadar), and serogroup D (Enteritidis). Overnight cultures of *Salmonella* were diluted to ~ 1 x 10^5 CFU/ml and added (equal volumes) to serial two-fold dilutions of each biocide in tap water. After 30 seconds of exposure, reactions were serially diluted (10-fold to 1 X 10-8), plated on LB (Lennox) agar, and grown overnight to determine CFUs present after biocide exposure compared to water-only control samples. CFU counts of *Salmonella* from biocide treated wells were compared to water control wells using an unpaired T-test to determine the significance of killing at each biocide concentration ($p \leq 0.05$).

Results: A6 (≥ 25 ppm), A16 (≥ 25 ppm), PAA (≥ 2 ppm), Wexcide (≥ 80 ppm), Virkon (≥ 286 ppm), and Virocid (≥ 111 ppm) significantly reduced CFU counts for all strains when compared to non-treated controls. At 50-100 ppm, the 30 second exposure to A6 and A16 resulted in 100% killing of the five serovars (I 4,[5],12:i:-, Typhimurium, Infantis, Hadar, Enteritidis). Complete killing of the five *Salmonella* serovars was observed at 2 ppm of PAA, 161-322 ppm of Wexcide, 286-573 ppm of Virkon, and 111 ppm of Virocid.

Conclusions: The efficacy of novel biocides A6 and A16 at low ppm concentrations suggests promising alternative compounds for disinfection of food products, sanitation of food processing surfaces, and potentially improved worker safety.

Notes:

36 - Rapid serotype-independent differential detection of biofilm-positive and biofilm-negative *Salmonella* using FTIR biotyping

Asmita Shrestha[1], Smriti Shringi[1], Devendra H Shah[1]

[1]Texas Tech University School of Veterinary Medicine. asmshres@ttu.edu

Session: Diagnostic testing 1, 2025-01-19, 10:45- 11

Objective: Foodborne illnesses caused by *Salmonella* represent a significant public health threat globally, with biofilm-forming strains exhibiting enhanced food safety risks due to their ability to persist due to resistance to antimicrobial agents, disinfectants, and environmental stresses. While conventional *Salmonella* detection methods are effective for identification and source tracing, they often overlook the biofilm-forming capacity of isolates, limiting their predictive value for food safety risks. This study assessed Fourier Transform Infrared (FTIR) biotyping system, for its ability to rapidly differentiate biofilm-positive (BFP) from biofilm-negative (BFN) *Salmonella* strains, independent of serotype.

Methods: A total of 270 *Salmonella* strains were classified using three conventional biofilm assays (CRCBB agar, calcofluor test, and tube test) into true BFP (n=80), true BFN (n=64), and uncertain (n=59) biofilm producers. Biofilm production for each group was confirmed with a microtiter plate assay. FTIR biotyping was then applied to a subset of 115 strains (61 BFP, 54 BFN) representing 12 common serotypes. Using spectral windows of 1180-1050 cm^{-1} and 1400-1200 cm^{-1}, FTIR biotyping accurately differentiated BFP from BFN strains. Additionally, validation was performed using six sets each of 9-10 different challenge strains and 30 training strains (same 15 BFN and 15 BFP), and six dendrograms were created. All the dendrograms were developed using correlation and the unweighted pair group method with arithmetic mean.

Results: Hierarchical clustering for the differential distinction of 115 BFP and BFN, achieved 93.4 % (57/61) sensitivity, 83.3 % (45/54) specificity, and 88.7 % (102/115) overall accuracy. Furthermore, FTIR biotyping differentiated 59 strains with uncertain biofilm status into BFN (n=45) and BFP (n=14) with a likelihood of 0.92 (57/62) for RDAR (Red, Dry and Rough) strains to be clustered as BFP, and 0.81(47/58) for SAW (Smooth and White) strains to be clustered as BFN strains.

Conclusions: FTIR biotyping provides a rapid, objective, and cost-effective method for distinguishing biofilm-forming *Salmonella* strains, addressing a critical gap in current surveillance methods. Its application could enhance food safety risk assessments and improve outbreak prevention by incorporating testing of biofilm production into pathogen detection.

Financial Support: This study was funded by the Texas Tech University School of Veterinary Medicine.

Notes:

37 - Comparative analysis of PRRSV RT-qPCR Ct value distributions across various sample types

Rabsa Naseer[1], Michael A. Zeller[2], Phillip C. Gauger[1], Giovani Trevisan[1]

[1]Department of Veterinary Diagnostic & Production Animal Medicine, Iowa State University, [2]Veterinary Diagnostic Laboratory, Iowa State University. rnaseer@iastate.edu

Session: Diagnostic testing 1, 2025-01-19, 11:00 - 11:15

Objective: Accurate and timely diagnosis of PRRSV is crucial for effective management and control of the disease. One of the key diagnostic tools used for detection is reverse transcription quantitative polymerase chain reaction (RT-qPCR), which measures the cycle threshold (Ct) value, and is a standard benchmark for determining positive detection of the virus. This study aims to analyze the distribution of Ct values across different sample types, PRRSV ORF5 lineages, and year, providing insights into their diagnostic utility in detection.

Methods: Data was sourced from the Iowa State University Veterinary Diagnostic Laboratory (ISU VDL), encompassing years of PRRSV testing records. This repository offers access to historical and current PRRSV diagnostic results, including specimen type, RT-qPCR Ct value, collection date, ORF5 sequence lineage, and associated metadata. Analyses focused on available cases with both positive Ct values, defined by a cutoff of < 38, and ORF5 sequences. The dataset includes over 31,000 sequences received from 2018 to 2023. The data was divided by sample type, with an emphasis on lung, serum, oral fluid, and processing fluid. Analyses were completed in R to compare Ct value distributions and their correlation regarding specimen type, year, and/or lineage.

Results: Lung samples consistently exhibited lower Ct values compared to other specimen types. Lung samples had a median Ct value of 18.7, whereas serum, processing fluids, and oral fluids had median values of 24.9, 27.5, and 29.3, respectively. Comparatively, median Ct values remained relatively stable over the years for most lineages and specimen types. However, Lineage 1C Ct values showed some variability, with median Ct values ranging from 16.7 and 19.6 for lung samples over time. Looking at the Lineage 1C.2 and Lineage 1C.3 variants, we see variability in the lung samples as well, with median Ct values ranging from 16.7 to 21.4 and 16.5 to 19.5, respectively.

Conclusions: This analysis of Ct value distributions based on sample type reveals key trends in viral detection and load, contingent on the underlying diagnostic objectives. The findings emphasize the significance of specimen type and viral lineage in the observed Ct values, offering insights into how sampling strategies may affect testing rationale, the need for additional diagnostics, and further molecular characterization. These insights contribute to optimizing sampling strategies for downstream testing and developing effective biosecurity measures. While preliminary, the study provides valuable guidance for improving diagnostic protocols and ensuring accurate, timely PRRSV detection.

Financial Support: We would like to acknowledge the Iowa State University Veterinary Diagnostic Laboratory for the funding of this research.

Notes:

38 - Evaluation of aggregate oral fluids for African swine fever real-time PCR diagnostics using samples collected on Romanian farms with an active outbreak

Chungwon Chung[1], Marta Remmenga[1], Sarah Mielke[1], Matthew Branan[1], Andrei Mihalca[2], Oana-Maria Balmos[2], David Oglan[3], Alexandru Supeanu[4], Attila Farkas[5]

[1]USDA, [2]University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Romania, [4]National Sanitary Veterinary and Food Safety Authority, Romania, [5]Carthage Veterinary Service. chungwon.chung@usda.gov

Session: Diagnostic testing 1, 2025-01-19, 11:15 - 11:30

Objective: African swine fever (ASF), caused by African swine fever virus (ASFv), is a highly contagious disease of domestic and wild pigs with a mortality rate that can reach 100%. Continuous spread of the virus into ASF-free regions, including the Caribbean Island of Hispaniola in 2021, is causing socio-economic burdens and presents a threat to food security. Pork producing countries, including the United States and Canada, are urgently looking for efficient tools for early detection to reduce spread of the virus in the event of an outbreak. In this study, real-time PCR with porcine oral fluids was further evaluated to better understand diagnostic performance using samples from three Romanian farms with an ongoing ASF outbreak.

Methods: Three farms reported with ASF clinical signs were used for sample collection and clinical observation. The collection of individual blood from live animals and lymphoid tissues from dead animals was performed. Aggregate oral fluid (OF) samples were collected using cotton ropes (TEGO® Swine Oral Fluid kit, ITL Biomedical, Reston, USA) in each pen of the three farms. Individual blood and OF samples were collected from a subset of pens on each of the three farms. Individual blood samples were collected in serum collection tubes (BD Vacutainer SST Tubes, 8.5mL) and stored at -80℃ until used for viral DNA extraction. Clinical observation of all animals in each pen was carried out by veterinarians and recorded as the number of healthy, sick, and dead pigs in each pen.Using a Bayesian latent class model, statistical difference in diagnostic sensitivity was compared between the real-time PCR using aggregate oral fluids and the process of determining pen disease status by testing individual blood samples collected from a subset of pigs from the same pen.

Results: In this limited dataset, no statistical difference in diagnostic sensitivity was found between the real-time PCR using aggregate oral fluids and the process of determining pen disease status by testing individual blood samples collected from a subset of pigs from the same pen when analyzed using a Bayesian latent class model. Known negative aggregate oral fluid samples from pigs in the United States had no occurrences of false positives, suggesting reliable diagnostic specificity of the sample matrix used for this study. Until results are produced from further studies with sufficient sample size, aggregate oral fluid testing using real-time PCR could cautiously be used as a supplementary sample type for ASF diagnosis alongside currently approved sample types, including blood and lymphoid tissues.

Conclusions: The analyses of data from three different farms showed no significant difference between the diagnostic sensitivities for real-time PCR using aggregate OF and pen-level determination using real-time PCR of individual blood samples when pens were sampled at varying levels. Although only a subset of pigs per pen were blood tested with varying subsets, the estimated within-pen prevalence values varied, and pen sizes were small, this study presents the first field evaluation on aggregate OF samples for ASFv real-time PCR diagnosis using farms with a current, naturally occurring ASF outbreak.

Financial Support: The study conducted at the Romania farms was supported in part by the National Pork Board NPB #20-177. The studies conducted at the PIADC were supported by the intramural funds from the United States Department of Agriculture.

Notes:

39 - Automated 384-well RT-qPCR assay advances high-volume testing for Porcine Reproductive and Respiratory Syndrome Virus

B. Reddi[1], A. Saxena[1], LG. Giménez-Lirola[1], PC. Gauger[1], RG. Main[1], J. Zhang[1], RK. Nelli[1]

[1]Veterinary Diagnostic and Production Animal Medicine, Iowa State University. mounika9@iastate.edu

Session: Diagnostic testing 1, 2025-01-19, 11:30 - 11:45

Objective: Porcine reproductive and respiratory syndrome virus (PRRSV) remains a major threat to the swine industry, leading to a substantial estimated economic loss of $1.2 billion annually. Early detection of PRRSV is crucial for implementing biosecurity measures that can limit the spread of the disease. Quantitative Reverse Transcription Polymerase Chain Reaction (RT-qPCR) is the widely utilized diagnostic tool for PRRSV detection. In 2023, the Iowa State University Veterinary Diagnostic Laboratory (ISU-VDL) tested nearly 400,000 samples for PRRSV, underscoring the need for high-throughput testing platforms to efficiently handle large volumes. This study aims to evaluate the performance of an automated 384-well RT-qPCR assay using the QuantStudio 7 Pro (QS7Pro; ThermoFisher) compared to the conventional 96-well format on the ABI Fast 7500 (ABI; ThermoFisher).

Methods: Viral RNA was extracted from two PRRSV isolates (MN184, V1 and 1-7-4 L1A, V2) using the MagMAX Pathogen RNA/DNA kit on the KingFisher Apex system (ThermoFisher). RT-qPCR assays were conducted with VetMAX™ PRRSV 3.0 reagents (PRRSV 3.0; Thermo Fisher) with a total reaction volume of 20 µL for 96-well assays (12 µL reaction mix + 8 µL sample) and 10µL for 384-well assays (6 µL reaction mix + 4 µL sample) following the recommended thermal cycling conditions. The Automated Liquid Handler Instrument (ALHI; Bravo, Agilent Technologies) was used to dispense reaction mixtures and samples onto 384-well plates. The plates were heat-sealed, centrifuged, and transferred to a 4°C incubator (Cytomat-2-C-LiN; iCL; ThermoFisher). The 384-well plates were sequentially transferred from the incubator to the QS7pro by Orbitor-RS2 microplate mover (ORM; ThermoFisher) using plate scheduling Momentum software (ThermoFisher). After the RT-qPCR run, the ORM unloads the 384-well plates onto the reserve stacking nests, loads the next plate in the queue, and repeats until all RT-qPCR plates are run in iCL.

Results: The PRRSV 3.0 assay demonstrated comparable performance on both the 96-well ABI and 384-well QS7Pro platforms, with a consistent Cq cutoff of 37. Intra- and inter-assay variability on the 384-well platform were minimal, with coefficients of variation (CV) of 1.4% and 0.4%, respectively. The variability between the 96-well and 384-well assays ranged from 2.2% to 2.6% across multiple runs. Positive controls on the 384-well plates consistently showed Cq values of 28.5 ± 0.5 across eight plates in three separate high-throughput runs. All plates stacked in the iCL were reliably loaded onto the QS7Pro by the ORM without lag or delay during the time periods tested.

Conclusions: The automated 384-well RT-qPCR workflow using the QS7Pro platform offers comparable analytical sensitivity to the conventional 96-well ABI platform, with the potential to process ~4000 RT-qPCR assays overnight. Future studies will expand testing to include a broader range of PRRSV strains, both vaccine and wild-type, to evaluate assay repeatability across various sample matrices. A cost-benefit analysis will also be conducted to evaluate the feasibility of routine PRRSV surveillance using this high-throughput system.

Notes:

40 - Leveraging automation and 384-well RT-qPCR assays for rapid detection of SARS-CoV-2 in animal species

A Saxena[1], B Reddi[1], L.G. Giménez-Lirola[1], P.C. Gauger[1], R.G. Main[1], P.G. Halbur[1], R.K. Nelli[1]

[1]Department of Veterinary Diagnostic and Production Animal Medicine, Iowa State University. asaxena@iastate.edu

Session: Diagnostic testing 1, 2025-01-19, 11:45 - 12:00

Objective: The COVID-19 pandemic highlighted the importance of early detection and rapid response to the outbreaks. SARS-CoV-2 has been identified in ~50 animal species, with predictions suggesting potential infections in ~500 species. Although the severity of the disease in animals is comparatively less than in humans, these species could act as reservoirs for viral mutations and transmission. A lack of cost-effective surveillance tools for rapidly detecting the SARS-CoV-2 virus was one of the several contributors to the pandemic. This study uses laboratory automation to establish a high-throughput (HT) 384-well RT-qPCR assay in various animal sample types to enhance workflow efficiency while maintaining PCR accuracy.

Methods: For the initial evaluation, viral RNA was extracted using the MagMAX Viral/Pathogen Nucleic Acid Isolation Kit with the automated magnetic bead-based nucleic acid extraction system (KingFisher Apex). A total of 552 serum samples from white-tailed deer, collected between October 2021 and April 2023 and submitted to the Iowa State University Veterinary Diagnostic Laboratory (ISU-VDL) were tested. Singleplex RT-qPCR assays from CDC targeting nucleocapsid regions specific SARS-CoV-2 (N1, N2 genes) and subgenus Sarbecovirus (N3) were included in a 10 µL reactions (6 µL assay mix + 4 µL sample) format on a 384-well plate, and compared against a standard 20 µL reactions on a 96-well plate. Reactions included an internal positive control (IPC). An automated Liquid Handler (ALHI) dispensed the reaction mix and samples into the plates, which were then heat-sealed, centrifuged, and placed in the automated Cytomat-2-C-LiN (iCL) incubator at 4°C. The plates were subsequently transferred sequentially from the iCL to the QS7pro thermocycler using the Orbitor-RS2 microplate mover (ORM) and Momentum scheduling software. After each RT-qPCR run, the ORM unloads the 384-well plates onto the reserve stacking nests, loads the next plate in the queue, and repeats this process until all RT-qPCR plates in the iCL are run.

Results: The optimized HT workflow effectively facilitated overnight runs, with six plates transferred from the iCL to the QS7Pro and subsequently to stacking nests. The Cq values of the positive amplification control (PAC) demonstrated consistency, with averages of 28.4 ± 0.1 (N1), 28.7 ± 0.03 (N2), and 28.8 ± 0.1 (N3) across six plates in a single high-volume automation run, yielding a coefficient of variation (%CV) < 0.5. The Cq values for the IPC across the six 384-well plates also remained stable, averaging 29.5±0.4. A 10-fold serial dilution series of known positive samples showed comparable PCR efficiencies between the 96-well and 384-well formats. None of the tested deer serum samples were positive for SARS-CoV-2 or the Sarbecovirus subgenus. Future studies will expand the investigation to different sample types including spiked deer serum samples, as well as samples from various animal species such as bison, bats, foxes, and rabbits.

Conclusions: The established HT workflow facilitates the testing of 3,800 RT-qPCR reactions overnight, i.e., 7,600/20 hours, paving the way for innovative veterinary molecular diagnostics. This method may also be adapted for detecting other emergent pathogens, such as Highly Pathogenic Avian Influenza (HPAI) H5N1.

Notes:

41 - A survey to assess risk factors associated with tick and mosquito exposure in Illinois

Holly E. Black[1], Sulagna Chakraborty[2], Rebecca L. Smith[1]

[1]Department of Pathobiology, University of Illinois, [2]Department of Veterinary Clinical Medicine, University of Illinois. hollyb2@illinois.edu

Session: Tick-borne diseases, 2025-01-19, 10:30 - 10:45

Objective: Tick and mosquito borne illnesses continue to threaten the health of people and their pets in Illinois. The purpose of this study is to determine risk factors associated with exposure to ticks, mosquitoes, and vector-borne disease in adults, with a focus on dog owners. We hypothesize that there is a relationship between human exposure to ticks and dog ownership, and a relationship between dog exposure to ticks and time spent outdoors, activity level, and home environment type.

Methods: We created an online survey to collect information about tick and mosquito exposure, time spent outside, dog ownership, and vector-borne disease prevention use. Survey participants were recruited from online social media posts, emails, and in-person outreach events. Outreach events included festivals, markets, and educational events at nature centers throughout different regions of Illinois. Data analysis and visualization was conducted in R to identify risk factors associated with exposure to ticks or mosquitoes.

Results: Survey recruitment and analysis are ongoing, but 487 surveys have been completed, representing 69 counties in Illinois. Preliminary results show that 57% of survey participants have observed ticks and 93% have observed mosquito bites on themselves or a family member in the past year. 62% of participants report owning a dog and 46% report observing ticks on their dog in the last year. Risk factors related to dog ownership, tick prevention use, home environment type, time spent outdoors, outdoor activities with and without a dog present, and tick encounter frequency are undergoing analysis.

Conclusions: The incidence of vector-borne diseases has been increasing rapidly in Illinois, and we believe that this study will help to provide a systematic and scientifically sound assessment of the risks and potentially effective prevention and communication methods, benefiting both the health of people and their pets.

Financial Support: Research support from Cooperative Agreement Number U01CK000651 from the Centers for Disease Control and Prevention. Student support from the Midwest Center of Excellence for Vector-Borne Disease and Office of the Director, NIH, T35 OD011145.

Notes:

42 - Invasive plant species as risk factors for tickborne disease in humans and livestock

YJ Johnson-Walker[1], TL Steckler[2], CM Stone[3], HC Tuten[3], CW Evans[2], MS Myint[1], Erica Jackson[1]

[1]University of Illinois Center for One Health, [2]University of Illinois Cooperative Extension Service, [3]Illinois Natural History Survey. yjjohn38@illinois.edu

Session: Tick-borne diseases, 2025-01-19, 10:45 - 11:00

Objective: The objective of this research is to investigate the relationships between infestations of different species of invasive plants with tick abundance and pathogen carriage in southern Illinois.

Methods: Ten research plots were selected at Dixon Springs Agricultural Center (University of Illinois). Three plots each were classified as invaded by *Lonicera maackii (*Amur honeysuckle), *Microstegium vimineum* (Japanese stiltgrass), and uninvaded. One plot was classified as invaded by *Alliaria petiolata* (garlic mustard). Standard tick dragging methodology was conducted from April - December in 2021 and 2022 at three-week intervals. Temperature and humidity data was recorded at each plot. Ticks were transferred to Illinois Natural History Survey Medical Entomology Laboratory for identification and PCR analysis for pathogens. Kruskal-Wallis ANOVA was used to compare tick abundance by season and plant species. T-tests were used to compare tick abundance by collection year and life-stage. Multivariable linear regression models were used to assess the relationship between tick abundance and plant species while controlling for potential confounders.

Results: A total of 751 (year 1 N=511, year 2 N=240) adult and nymph ticks were collected. The most prevalent tick species was *Amblyomma americanum* (Lone Star tick) (621 total, year 1 N = 436, year 2 N = 185). *Dermacenter variabili* (American dog tick) was the second most common species (117 total, year 1 N= 73, year 2 N = 44). There were 11 *Ixodes scapularis* (Black legged ticks) (year 1 N= 1, year 2 N= 10) and 2 *Amblyomma maculatum* (Gulf Coast ticks) collected during the study period (1 each year 1 and 2). The greatest mean number of ticks per plot (mean = 103) was in the *A. petiolata* plots. *L. maackii* plots had a mean of 83.3 ticks per plot. *M. vimineum* plots had a mean of 69.3 ticks per plot. Uninvaded plots had the fewest ticks (mean = 63.3 ticks per plot).There was a statistically significant difference in the median number of *A. americanum* nymphs by plant species (p= 0.0014). The final reduced linear regression model (F = 22.32, p = 0.0000) of the relationship between plant species and Lone Star tick nymph abundance indicated that when controlled for season, plots invaded by *A. petiolata* garlic (p=0.011) and *L. maackii* (p=0.0097) were associated with increased *A. americanum* nymph abundance compared to uninvaded plots. Approximately 1.3% (N= 8 positive; N= 621 total) of the *A. americanum* ticks collected, tested positive for Ehrlichia spp. (E. ewingii (N=3) and E. chaffeensis N=5). Of the eight pathogen positive ticks, seven were collected from plots with invasive plants.

Conclusions: The Lone Star Tick is abundant in the study region and 1.3% harbor *Ehrlichia* spp. Bites from the Lone Star Tick can also result in the development of Alpha-gal syndrome -- a potentially life-threatening allergic condition. These findings are consistent with studies in other regions and tick species. Amur honeysuckle and garlic mustard are invasive plant species associated with increased Lone Star tick nymph abundance. Understanding the interaction between micro-climate, plants, animals, and arthropods may lead to sustainable interventions to prevent tickborne disease.

Financial Support: University of Illinois College of Agricultural, Consumer and Environmental Sciences Dudley Smith Initiative DSynergy Grants Program

Notes:

43 - Identification of tick antigens after vaccination with tick extracellular vesicles in white-tailed deer

Adela Oliva Chavez[1], Julia Gonzalez[2], Cristina Harvey[3], Carita de Souza Ribeiro-Silva[4], Brenda Leal-Galvan[3], Kelly Persinger[5], Tammi Johnson[5]

[1]Department of Entomology, University of Wisconsin, Madison, [2]Veterinary Integrative Biosciences, Texas A&M University, [3]Department of Entomology, Texas A&M University, [4]Departamento de Biociências e Tecnologia, Instituto de Patologia Tropical e Saúde Pública, Universidade Federal de Goiás, Brazil, [5]Texas A&M AgriLife Research. olivachvez@wisc.edu

Session: Tick-borne diseases, 2025-01-19, 11:00 - 11:15

Objective: Current tick control measures are focused on the use of synthetic acaricides. Nevertheless, the emergence of acaricide resistance and the wildlife aided movement of ticks to new geographic regions has precluded the efficient management of ticks. This project aims to evaluate extracellular vesicles (EVs) from *Amblyomma americanum*, commonly known as the Lone star tick, as vaccine candidates and identify antigenic proteins recognized by white-tailed deer (*Odocoileus virginianus*; WTD) immune responses.

Methods: Female *A. americanum* were fed on 1-year-old WTD for 5 days during three different infestations. Salivary glands (SG) and midguts (MG) from 5-day fed ticks were removed and cultured ex vivo in vesicle free-tick media and EVs were isolated by ultracentrifugation. For vaccination, three 1.5-year-old female WTD were vaccinated with 200 ug MG and 200 ug SG EVs at day 0 and boosted at 28 and 49 days. Two control deer were injected with adjuvant and PBS only. Serum samples were recovered pre-vaccination and then every seven days from day 7 after vaccination until day 57. To assess serum reactivity after tick infestation and long-term memory, serum was also taken the final day of tick drop-off (post-infestation) and one year (Y1) and 1-year and 1-month (Y1M1) since last-boosting. Serum reactivity was evaluated by ELISA. At 58 days, WTD were infested with 100 *A. americanum* nymphs, 50 females, and 50 males that were allowed to feed to repletion. On-host and off-host mortality, tick engorgement weight, nymph molting, time to oviposition, and egg hatchability were evaluated. Parameters were compared for statistical significance.

Results: Transmission Electron Microscopy (TEM) and Nanoparticle tracking analysis (NTA) of EVs recovered after ex vivo culture showed a mixture of exosome and microvesicles. Vaccination resulted in seroconversion and significant increases in IgG levels starting at day 7 for anti-MG EV IgG levels and at day 35 for anti-SG EV IgG levels. ELISAs at Y1 and Y1M1 showed significantly higher levels of IgG when compared to pre-vaccination levels and controls. No negative effects were observed in nymphs, but on-host mortality of female *A. americanum* was significantly higher in vaccinated animals than control. No effects were observed on reproductive parameters. Proteomic analysis of immunoprecipitated proteins identified 7 antigenic proteins in SG EVs and one in MG EVs that were statistically significant and unique to vaccinated serum/animals. Additionally, 2 antigenic proteins in MG EVs and 24 SG EV proteins show antigenic potential.

Conclusions: These results indicate that proteins within female tick vesicles are not good candidates for vaccine design against nymphs; however, the on-host adult mortality suggests that tick EVs harbor protective antigens against female *A. americanum* tick infestation. Further, these results show that EVs from tick salivary and midgut ex vivo cultures harbor antigenic proteins that confer long lasting antibodies levels, which could be exploited in the design of anti-tick vaccines. Antigenic proteins identifying during proteomic analysis can be used to design nanovaccines against ticks.

Financial Support: This project was funded by the USDA NIFA award #2022-67015-42166 and start-up funds to AOC; an ORISE fellowship from the USDA and Knippling-Bushland-Swahrf fellowship to BLG; and a CAPES fellowship to CSR.

Notes:

44 - Monitoring of various tick-borne viruses in ticks living in the Republic of Korea in 2024

Junho Yoon[1], Minjoo Yeom[1], Jong-Woo Lim[1], Daesub Song[1]

[1]Department of Virology, Seoul National University. mathlover92@snu.ac.kr

Session: Tick-borne diseases, 2025-01-19, 11:15 - 11:30

Objective: Ticks act as carriers to spread various pathogens to humans and animals. In particular, it is important to monitor tick-borne infectious diseases as they can transmit dangerous viruses such as Severe fever with thrombocytopenia syndrome virus (SFTSV) and tick-borne encephalitis virus to humans. Currently, various tick-borne infectious diseases are monitored in the Republic of Korea, but they are concentrated on SFTS viruses. The objective of this study is to identify neglected viral infections among ticks residing in the Republic of Korea.

Methods: From April to October 2024, ticks were collected from the vegetation in the Republic of Korea by using the flag. Tick species were classified morphologically using electron microscopy. Depending on the species, sex, stage of development and collection site 1 adult, 1-6 nymphs and 1-30 larvae were pooled into collection tubes. The nucleic acids were extracted from ticks using RNA extraction kit. One-Step Reverse transcription PCR assays with target gene-specific primers were used to detect viral families (Phenuiviridae and Flaviviridae) in ticks. The target band identified in gel electrophoresis was analyzed through sanger sequencing.

Results: In this study, 530 ticks were collected, of which 398 ticks were *Haemaphysalis longicornis* (362 nymphs and 36 adults), 14 ticks were *Haemaphysalis flava* (12 nymphs and two adults), four ticks were *Ixodes nipponensis* (one nymph, three adults) and 114 ticks were larvae of *Haemaphysalis* spp. Phenuiviridae virus was detected at a minimum infection rate of 2.83% (15 positive pools/530 ticks). The 15 nucleotide sequences of the each fragments (509bp) exhibited identity with *Uukuvirus dabieshanense*. Meanwhile, there were no positive samples for Flaviviridae virus in this study.

Conclusions: Molecular diagnostic methods using viral family specific universal primers can be used to detect unknown tick-borne viruses. Considering that ticks might be infected with the other viruses than SFTS virus in the Republic of Korea, extensive surveillance of tick viruses will be necessary to prevent tick-borne diseases.

Financial Support: This work was supported by Korea Institute of Planning and Evaluation for Technology in Food, Agriculture and Forestry through High-Risk Animal infectious Disease Control Technology Development Program, funded by Ministry of Agriculture, Food and Rural Affairs(No. RS-2024-00399940)

Notes:

45 - Identification of *A. marginale* surface-protein binding receptors on *Dermacentor andersoni* tick cells.

Sammuel Shahzad[1], Susan Noh[1]

[1]USDA-ARS. sammuel.shahzad@wsu.edu

Session: Tick-borne diseases, 2025-01-19, 11:30 - 11:45

Objective: *Anaplasma marginale* is a tick-borne, obligate, intracellular rickettsial pathogen that causes bovine anaplasmosis, characterized by acute anemia, and production losses in cattle worldwide. Entry and replication in the tick midgut and salivary gland cells are essential for transmission. Thus, the goal of this work is to identify the tick proteins that are used by *A. marginale* for entry as these proteins may serve as vaccine targets for blocking tick transmission.

Methods: Major surface protein 1b (Msp1b) is one of several *A. marginale* proteins that mediate binding to tick cells. To identify the Msp1b binding partner on tick cells, GST- tagged or His-tagged Msp1b was immobilized on magnetic beads to pulldown interacting proteins from lysates of cultured *Dermacentor andersoni* (DAE100) cells or midguts from adult male *D. andersoni* ticks. The empty vector which includes thioredoxin and GST-tag or 6x-His tag was used as the negative control. Following pulldown, tick proteins eluted from the recombinant Msp1b, and control proteins were subjected to analysis by label-free bottom-up liquid chromatography-mass spectrometry in three independent replicates. Proteome Discoverer v. 2.2 (Thermo), software was used to calculate the abundance ratios and p- values. The GST or His-tagged Msp1b pull-down were statistically compared to the respective tag-only assays by using ANOVA followed by the Benjamini-Hochberg procedure. To identify *D. andersoni* proteins that may play a role in cell surface adhesion and binding to Msp1b, human orthologs for *D. andersoni* proteins significantly ($p<0.05$) more abundant in the Msp1b pulldown assays as compared to the tag-only assays were used in GO enrichment analysis for cellular components. The proteins in the relevant enriched pathways were then mapped back to *D. andersoni.*

Results: There were 93 and 59 proteins more abundant in the elutions from Msp1b as compared to the control in GST and His-tag pulldowns, respectively. Additionally, using tick midguts, mass-spectrometry identified 97 proteins more abundant in elutions from Msp1b as compared to the GST-only control. GO enrichment analysis identified focal adhesion proteins in all samples. Next, adhesin proteins common to all samples will be identified. Additionally, enriched proteins with conserved domains that could play a role in pathogen binding to host cells or predicted to be surface exposed were considered as candidates, including tenascin, erlin, laminin, and papilin-like protein.

Conclusions: Using a combination of approaches and *D. andersoni* cultured cells and midguts, we have identified several surface proteins that may interact with *A. marginale* Msp1b, and thus play a role in pathogen entry. Next, functional assays will be done to confirm the molecular interactions of these candidates and Msp1b. Ultimately, we will identify the binding partners for the other *A. marginale* adhesins and determine if antibody against these *D. andersoni* surface proteins will prevent *A. marginale* tick transmission.

Financial Support: U.S. Department of Agriculture

Notes:

46 - Inactivated whole cell antigen vaccine confers protection against heterologous *Rickettsia rickettsii* strains in the canine host.

Perle Latré de Laté[1], Ian Stoll[1], Jonathan Ferm[1], Francy Liliana Crosby[1], Jodi McGill[2], Kim D. Yong[3], Dominica Ferm[1], Deepika Chauhan[1], Debika Choudhury[1], Huitao Liu[1], Suhasini Ganta[1], Roman R. Ganta[1]

[1]Department of Veterinary Pathobiology, University of Missouri, [2]Department of Veterinary Microbiology and Preventive Medicine, Iowa State University, [3]Department of Veterinary Pathology, Missouri Veterinary Diagnostic Laboratory. plh4n@missouri.edu

Session: Tick-borne diseases, 2025-01-19, 11:45 - 12:00

Objective: Rocky mountain spotted fever (RMSF) is a deadly tick-borne infectious disease in dogs and people caused by *Rickettsia rickettsii*, an obligate, intracellular bacterial pathogen. If untreated, the disease can quickly progress to life-threatening illness. Doxycycline is the most recommended treatment against RMSF. In recent years, the severe form of the disease is frequently reported in parts of the USA and Mexico. Currently, there is no vaccine to prevent RMSF in either dogs or people. We previously demonstrated that the whole cell inactivated antigen vaccine (WCAV) offers complete protection against virulent *R. rickettsii* infection in dogs when tested with homologous virulent strain (i.e. the same strain used for the preparation of the vaccine). The objective of the current study is to determine if the WCAV protects against heterologous strain infection (i.e. against different strains of Rickettsia rickettsii).

Method: Five groups of dogs were vaccinated with three different WCAV prepared from two different virulent strains (Sheila Smith and Morgan) and an avirulent strain (Iowa strain). Adjuvant only preparations were administered to all non-vaccinated and infection control group animals. Dogs received primary and booster vaccination and then challenged with the two virulent *R. rickettsii* strain infections by I.V. Infection progression was monitored for 30 days to assess differences in clinical illness as well as to measure changes in the hematology, the presence or absence of immunological response and bacterial presence in blood and tissues.

Results: All dogs vaccinated with WCA did not develop the clinical RMSF, whereas nonvaccinated *R. rickettsii*-infected dogs developed the disease. Independent of the WCA vaccine used, all vaccinated dogs, including the vaccine prepared with the avirulent strain, were protected from the disease and systemic and tissue bacterial infection. All control animals receiving avirulent strain infection developed very mild or no clinical disease with no detectible bacteremia. Only non-vaccinated virulent strain infected dogs tested positive for the culture recovery. All vaccinated dogs also remained healthy and developed vaccine-specific IgG responses following the primary vaccination, which was enhanced following the booster vaccination. Although the IgG response varied among different vaccinated groups, it did not impact the vaccine protection. Predominantly, only vaccinated dogs had a detectable cellular immune response.

Conclusions: This study provides the first evidence of the protective ability of WCAV against RMSF in dogs resulting from different virulent strains, including with the vaccine prepared using an avirulent pathogen strain.

Financial Support: This work is supporting by the grant # R01AI152417 from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, USA.

Notes:

47 - Associations between estimated greenhouse gas emissions in feedlot cattle cohorts and their health and performance

Taylor B. McAtee[1], David G. Renter[1], Nick B. Betts[2], Natalia Cernicchiaro[1]

[1]Center for Outcomes Research and Epidemiology, Kansas State University, [2]Elanco Animal Health. taylormcatee@vet.k-state.edu

Session: Epidemiology 2, 2025-01-19, 10:30 - 10:45

Objective: With a growing emphasis on sustainability, beef producers are increasingly considering environmental impacts of production practices. However, gaps remain in clearly defining how cattle health and performance metrics align with both sustainability goals and the need to meet global protein demands. Our objective was to quantify associations between cattle health and performance metrics in cohorts (lots) of beef-breed feedlot steer and heifers with cohort-level estimates of greenhouse gas (GHG) emissions per unit of production.

Methods: We used retrospective data from 9,436 cattle cohorts in nine U.S. commercial feedlots (2017-2021) and fitted mixed effects multivariable models to assess associations between cohort-level characteristics and estimated emissions. Four separate models, each based on a priori causal diagrams and directed acyclic graphs, focused on the primary explanatory factors of interest: average daily body weight gain (ADG), feed efficiency as a ratio of body weight gain to weight of feed (G:F), total medicine costs per initial cohort size (n) as a proxy for disease burden, and cumulative all-cause mortality. Sex, season, days on feed, and arrival weight category were considered as potential confounders. The primary outcome was estimated carbon dioxide equivalent (CO_2e) emissions per kg of final body weight (FBW), derived from a proprietary life cycle assessment system, Uplook 1.0 (Elanco Animal Health).

Results: We identified significant associations between key cattle health and performance indicators and estimated lifetime emissions (kg CO_2e/kg FBW) across four separate models focused on different explanatory factors. In the ADG and G:F models, increased ADG and G:F were associated with lower emissions per kg of FBW. Conversely, higher cumulative mortality and disease burden (based on medicine costs) were associated with increased emissions. However, there were significant interaction terms in all models. The ADG and G:F effects were modified by sex and season, with significant interactions observed between ADG and sex ($P < 0.01$), ADG and season ($P < 0.01$), G:F and sex ($P < 0.01$), and G:F and season ($P < 0.01$). Improved ADG and G:F were consistently associated with lower emissions, but the magnitude of these associations differed between steers and heifers, and varied among seasons. The disease burden and cumulative mortality models showed that these effects varied by both season and arrival body weight, with significant three-way interactions (P values < 0.01) in both models. Poorer health was consistently associated with higher emissions in feedlot cohorts per unit of FBW, but the magnitude of these negative associations was more pronounced in cohorts with lighter weight categories in certain seasons.

Conclusions: Our findings provide insight into the crucial role of health in sustainability, demonstrating that cohorts of healthier animals with better performance had reduced GHG emissions per unit of production. While macro-level environmental concerns often focus on total emissions produced during beef production, this study highlights that improving health, G:F, and ADG can significantly reduce GHG emissions per unit of beef produced. Rather than considering total emissions without regard for protein production, focusing on specific health and performance indicators offers beef producers clear information on opportunities for reducing emissions while maintaining production efficiency.

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:

48 - Cross-sectional study estimating seroprevalence of *Anaplasma marginale* in Mississippi beef cow-calf herds

W. Isaac Jumper[1], Brandi Karisch[2], Carla L. Huston[1], David R. Smith[1]

[1]Mississippi State University College of Veterinary Medicine, [2]Mississippi State University Department of Animal and Dairy Sciences. isaac.jumper@msstate.edu

Session: Epidemiology 2, 2025-01-19, 10:45 - 11:00

Objective: The objective of this study was to estimate the within and between herd seroprevalence of Anaplasma marginale in Mississippi cow-calf herds, as well as determine factors related to herd seroprevalence and animal infection status.

Methods: Serum was collected from all females ≥ 2 years of age (n=2,126) in 40 herds across 23 counties in Mississippi. Serum was tested via competitive enzyme-linked immunosorbent assay (cELISA) to detect *A. marginale* antibodies. Herd demographic information, biosecurity procedures, parasite control measures, and other herd health practices were collected from participating herds. Logistic regression mixed models were used to test individual animal factors for association with *A. marginale* infection status, and herd-level factors for association with herd seroprevalence. Herd was considered a random variable in all models, and statistical significance was defined a priori at alpha=0.05.

Results: The average size of participating herds was 54 cows ≥ 2 years of age. Overall, 512 of 2,126 (24%) serum samples tested positive for *A. marginale*. Thirty-six of 40 (90%) herds had at least one animal seropositive for *A. marginale*. Of those infected herds, the average proportion of the herd infected was 31.7%. Increasing herd seroprevalence of *A. marginale* was associated with feeding chlortetracycline-medicated mineral (OR=5.3, 95%C.I.=1.7,17.0). Individual animal infection with *A. marginale* was associated with bovine leukemia virus infection (OR=1.6, 95%C.I.=1.2-2.2).

Conclusions: Within-herd seroprevalence of *A. marginale* differed greatly among participant herds, and naïve herds exist. Feeding chlortetracycline-medicated mineral and comorbidities such as BLV infection may influence herd seroprevalence and individual animal infection status, respectively.

Financial Support: Supported by funds from the Mississippi State University College of Veterinary Medicine Office of Research and Graduate Studies Internal Grant Program, as well as the United States Department of Agriculture (USDA) Agriculture Research Service (ARS) fund #58-0200-0-002.

Notes:

49 - Cross-sectional study to determine factors related to gastrointestinal parasite prevalence in Mississippi beef cow-calf herds

Caroline L. Howell[1], David R. Smith[1], W. Isaac Jumper[1]

[1]Mississippi State University College of Veterinary Medicine. clh1045@msstate.edu

Session: Epidemiology 2, 2025-01-19, 11:00 - 11:15

Objective: The objective of this study was to test individual and herd-level factors for association with prevalence and concentration of gastrointestinal (GI) parasites in Mississippi cow-calf herds.

Methods: Fecal samples were collected from 1,666 beef cows across 33 herds in 18 Mississippi counties. Participant surveys were used to collect individual and herd-level risk factor data. The modified McMaster's technique was used to estimate strongyle-type fecal eggs per gram (FEPG). Linear regression mixed models were employed to test the effect of age on FEPG. A logistic regression generalized linear mixed model was used to test herd-level factors for association with GI parasite prevalence. Herd was considered a random variable in all models. Multivariable models were assembled by manual forward variable selection.

Results: No eggs were detected in 74% of samples. Age was related to individual animal FEPG (≤3yrs: -1.96, 95% C.I.= -25.5, 21.6; 4-5yr: -6.63, 95% C.I.= -29.2, 15.9; 6-8yr: -24.4, 95% C.I.= -44.9, -3.84; compared to ≥9yr). Factors associated with proportion of cows infected with GI parasites include oral dewormer use (OR= 0.6, 95% C.I.= 0.5-0.9), summer calving (OR= 0.4, 95% C.I.= 0.3-0.6), observing lethargy/weakness (OR= 2.4, 95% C.I.= 1.7-3.6), season of sample collection (Spring: OR= 0.5, 95% C.I.= 0.3-0.8; Summer: OR= 0.4, 95% C.I.= 0.2-0.7; Fall: OR= 0.6, 95% C.I.= 0.4-0.9; compared to Winter), purchased herd bull (OR= 0.5, 95% C.I.= 0.4-0.8), and injectable dewormer use (OR= 0.5, 95% C.I.= 0.3-0.8).

Conclusions: Gastrointestinal parasite infections were rare in this study and their presence was associated with deworming practices, season, husbandry practices, and age.

Financial Support: Research Grant: United States Department of Agriculture- Agriculture Research Service NACA Sponsor Number 58-0200-0-002 and Mississippi State University College of Veterinary Medicine Office of Research and Graduate Studies Internal Grant Program. Student Support: National Institutes of Health (NIH)

Notes:

50 - Evaluating beef cattle producers' disease prevention knowledge, attitude, and practices in Illinois, USA

Csaba Varga[1]

[1]University of Illinois. cvarga@illinois.edu

Session: Epidemiology 2, 2025-01-19, 11:15 - 11:30

Objective: Assessing the biosecurity knowledge and practices of beef cattle farms is crucial to understanding their level of disease prevention preparedness. Identifying their biosecurity knowledge and practices will benefit producers by increasing their involvement in implementing effective biosecurity programs on their farms. The main objective of this study was to identify differences among beef cattle production types (cow-calf, feedlot, backgrounder, and "whole cycle") regarding their biosecurity practices. Also, we aimed to assess farmers' attitudes and perceptions of disease prevention.

Methods: A stratified random survey of beef cattle producers in Illinois was conducted between June and August 2022. A mail survey was sent out to 3000 producers. Nonresponders were contacted, and if they agreed, the survey was administered via phone. A multinomial logistic regression model assessed the associations between the outcome variable representing the four production types (cow-calf, feedlot, backgrounder, and "whole cycle") and the predictor variables representing various on-farm biosecurity practices. Ordinal logistic regression models evaluated associations between beef cattle farmers' disease risk perception (5-scale outcome) and having a biosecurity evaluation on a beef cattle farm (predictor).

Results: A total of 537 (18%) producers (505 via mail and 32 via phone) responded to the survey (18%). Compared to the cow-calf operations (base category) the probability of providing boot wash for visitors was higher in feedlots (RRR= 2.28; p=0.04). The odds of having a designated parking for visitors was higher in backgrounder operations (RRR=1.93; p= 0.06) and "whole-cycle" beef production systems (RRR= 1.92; p=0.02) compared to cow-calf operations. Having a biosecurity evaluation on beef cattle farms positively impacted beef farmers' perceptions of the importance of disease prevention measures to limit the introduction of pathogens onto farms (OR=1.83; p=0.001), the importance of a biosecurity plan to be used during a foreign animal disease outbreak (OR= 2.86; p<0.001), and the importance of education materials on foreign animal diseases (OR= 1.94; p<0.001).

Conclusions: Differences in biosecurity practices between different beef production types were detected. Beef producers had a positive attitude towards disease prevention and control practices. The study results can aid animal health authorities, beef cattle farmers, and other stakeholders in developing effective disease prevention programs.

Financial Support: This research was funded by Farm Bill funding through the USDA Animal and Plant Health Inspection Service (APHIS) Veterinary Services (VS)' National Animal Disease Preparedness and Response Program (NADPRP) 2020 (Award number: AP21VSSP0000C037).

Notes:

51 - Prevalence of bovine coronavirus in nasal samples collected from beef calves during a controlled commingling event

Joanna Urbaniec[1], Peers Davies[1], John Ellis[2], Federico Hoffmann[3], Florencia Meyer[3], Paul S. Morley[4], Matt Scott[4], Amelia Woolums[3], Noelle Noyes[5], Joseph Neary[1]

[1]University of Liverpool, [2]University of Saskatchewan, [3]Mississippi State University, [4]Texas A&M University, [5]University of Minnesota. nnoyes@umn.edu

Session: Epidemiology 2, 2025-01-19, 11:30 - 11:45

Objective: The overall objective of this multi-year, multi-country study is to model bovine coronavirus (BCV) transmission dynamics during commingling events, accounting for differences in individual immune and microbiome responses. An initial study was completed, with the objective of describing the prevalence of BCV in nasal samples collected from calves during a controlled commingling event.

Methods: Forty Angus x Holstein calves (19 heifers and 21 bulls) born within a 2-week period across 5 dairy farms in March 2024 were enrolled. Calves were weaned by 8-10 weeks old, sampled, and transported to a small-pen research facility. Calves were quarantined according to source farm for 12-14 days and then randomized, within the strata of Farm and Sex, into one of four commingling pens (Day 0): no commingling (single-source pen), low-level commingling (pen with 2 calves from one source farm and 8 calves from a second source farm), moderate commingling (pen with 5 calves from one source farm and 5 calves from a second source farm), and intensive commingling (pen with 2 calves from each of the 5 source farms). There were 10 calves per pen (5 x 3 m with solid walls) and at least 3 m air-gap between groups. Individual nasal swabs from all calves were collected on source farms at collection, then on Day 0 (day of commingling), 3, 7, and 21 post-commingling. Nasal swabs were subjected to quantitative PCR for BCV, targeting the conserved region of the membrane (M) protein. Furthermore, serum samples were collected on source farms at collection, and then day of commingling and D21 post-commingling and those were tested for the presence of anti-BCV IgG antibodies.

Results: All calves were seropositive for BCV antibodies at collection, and their antibody levels remained high throughout the study period. Crude prevalence of BCV prior to and on the day of commingling was 10% and 5%, respectively. After commingling, BCV prevalence remained relatively low and steady until Day 21, when 53% of samples were positive. Highest prevalence (100% on D21 post-commingling) was observed in the pen with animals from a single source farm (no-commingling), with prevalence decreasing with commingling intensity: 12.5% in low and moderate commingling pens and 7.5% in the intensive-commingling pen. Additionally on Day 21, environmental samples collected near the pens were positive for BCV. BCV shedding from positive animals was relatively low until D21 (average 104, 105, 104, 105, and 107 viral copy number/µL for each consecutive sampling point).

Conclusions: Prevalence of BCV in nasal samples increased after commingling across all pens, showing a large increase in Day 21 samples. It is unknown whether this widespread increase was due to cross-pen airborne transmission, or concomitant increases in within-pen shedding. Additional analysis of BCV variant(s) within each pen is ongoing, and a second trial with new calves is currently being conducted, with additional environmental sampling for BCV. Results from these analyses should help disentangle the source of increased BCV on Day 21 post-commingling.

Financial Support: Funding was provided by the joint NIFA-NSF-NIH Ecology and Evolution of Infectious Disease award 2023-67015-40862 and Biotechnology and Biological Sciences Research Council (BBSRC) and Medical Research Council (MRC) award BB/Y006887/1.

Notes:

52 - Exploring subpopulation dynamics of *Salmonella* enterica in harvest-ready beef cattle and feedlot environment

Yesica Botero[1, 2], Kasi Schneid[3], Kendall L. Samuelson[3], John T. Richeson[3], Ty E. Lawrence[3], Gizem Levent[1, 2]

[1]Department of Veterinary Integrative Biosciences, Texas A&M University, [2]Texas Tech University School of Veterinary Medicine, [3]Department of Agricultural Sciences, West Texas A&M University. ybotero@tamu.edu

Session: Epidemiology 2, 2025-01-19, 11:45 - 12:00

Objective: *Salmonella* enterica, a foodborne pathogen found in cattle and contaminated beef products, poses a significant public health risk due to its potential to cause outbreaks. Multidrug-resistant *Salmonella* are considered a serious threat, yet the drivers of cattle-origin antibiotic-resistant *Salmonella* remain unclear. While the emergence of AMR *Salmonella* in food-producing animals is often linked to specific serotypes or clonal lineages and can be driven by antibiotic use in cattle, the presence of the resistant population in the background microbiota is crucial for such selection. Our study that initially aimed to investigate the long-term effects of dietary and management changes (high-starch vs. low-starch diet and regular vs. erratic feeding) on *Salmonella* populations in cattle gave us a unique opportunity to explore potential drivers of *Salmonella* subpopulation and AMR relationship in cattle and their environment without antibiotic selection pressure.

Methods: A total of 720 cattle were randomized into treatment pens (n=48), and each pen received either a high- or low-starch diet combined with regular or erratic feeding schedule over approximately 168 days. Fecal and composite pen samples were collected on days 56 and at slaughter (day 168-200). In addition, hide swabs and subiliac lymph nodes were only collected at slaughter. We performed whole-genome sequencing (WGS) on selected isolates using Illumina reagents and the NextSeq1000 platform. In-silico analyses were used to identify serotype, 7-gene multi-locus sequence type, and antibiotic resistance genes. In addition, subspecies-level core genome comparisons were performed using SNP-based maximum-likelihood phylogeny, and the tree was inferred on IQ-TREE using the best model determined by Model-test NG. The resulting tree file was visualized and annotated with iTOL.

Results: Among 527 *Salmonella* isolates sequenced, the most commonly identified serotypes were Anatum ST64 (36.8%), Montevideo ST138 (30.7%), Kentucky ST198 and ST314 (15.9%), and Lubbock ST138 and ST413 (14.2%). Among soil isolates, one Montevideo isolate carried resistance genes aac(6')-Iaa, floR, tet(A), aph(6)-Id, aph(3")-Ib, sul2, whereas an Anatum isolate harbored these genes and blaTEM-1A. Nearly all isolates (99.6%) carried a cryptic aminoglycoside resistance gene (aac(6')-Iaa). Maximum-likelihood phylogeny revealed three distinct clades, with close clustering of serotypes Montevideo, Lubbock, and Kentucky within treatment pen and sample collection day at slaughter age. Two isolates Lubbock were closely clustered in the same clade as serotype Montevideo from different sample types.

Conclusions: Collection day and pen were the most predictive factors for clustering within the *Salmonella* subpopulation; no clear treatment-related patterns emerged. The resistant Montevideo isolate was closely linked to non-resistant Montevideo strains, suggesting that AMR may not be tied to serotype or clonal lineage without antibiotic pressure. Our analysis also showed that the resistant Anatum isolate was more closely related to a reference Anatum strain, previously reported as pan-susceptible, than to other Anatum isolates in this study. Interestingly, two Lubbock isolates showed clonal association with Montevideo isolates rather than Lubbock isolates found in this study. To improve genomic resolution, we plan further phylogenetic analysis within each serotype, hierarchical clustering using core-genome multi-locus sequence typing (cgMLST), and comparison with other publicly available *Salmonella* WGS data.

Financial Support: Texas Tech University and Texas A&M University Start-up funds

Notes:

53 - Testing the pathogenesis of the Louse-borne Relapsing Fever pathogen in the immunocompetent mouse model

A. M. Gaber[1, 2], J. C. Blazier[3], C. Lui[4], D. J. Wiener[4], A. S. Rogovsky[1]

[1]Department of Pathobiology and Diagnostic Investigation, Michigan State University, [2]Department of Veterinary Pathobiology, Texas A&M University, [3]Texas A&M Institute for Genomics Sciences and Society, Texas A&M University, [4]Department of Veterinary Pathobiology, Texas A&M University. alhussiengaber@tamu.edu

Session: Disease pathogenesis 1, 2025-01-19, 10:30 - 10:45

Objective: Louse-borne relapsing fever (LBRF) is a vector-borne disease of public health significance in some African countries, and an emerging disease in Europe. LBRF is caused by the louse-transmitted spirochetal bacterium, Borrelia recurrentis, which affects only humans and has no known animal reservoir. The clinical signs of LBRF include fever relapses, which reflect the periodic antibody-driven re-emergence of spirochetes in the blood. Until recently, research on LBRF had been restricted by the lack of suitable animal model. Our lab was the first to develop an immunocompetent mouse model using the Collaborative Cross resource and *B. recurrentis* strain A17. This strain developed initial spirochetemia in mice over the first 3 days post infection but no culture-detectable relapses. The antigenic variation system (AVS), the hallmark of relapsing fever spirochetes, was shown to be crucial for Tick-borne Relapsing Fever spirochetes to evade anti-borrelial antibodies. In *Borrelia hermsii*, the AVS system was well characterized and is composed of two surface membrane lipoproteins of different sizes, Vsp and Vlp. The antigenic makeup of these proteins constantly changes through recombination events between the vsp/vlp gene and highly homologous silent cassettes. Previous study that sequenced genomes of several B. recurrentis strains via the Illumina MiSeq technology, suggested that the repertoire of vsp/vlp cassettes could vary greatly between the strains. We hypothesized that *B. recurrentis* strains that possessed a higher number of vsp/vlp cassettes would develop more relapses than the strains with a limited repertoire of these cassettes.

Methods: In the present study, by using our novel mouse model, we investigated the pathogenesis of five B. recurrentis strains, A1, A11, PAbJ, PBeK, and PAbN over a 20-day-long period. Twenty mice were used to test each strain. To detect spirochetemia in blood samples collected daily, we utilized dark-field microscopy (DFM) and culture methods. Using the PacBio sequencing technology, we also defined the exact numbers and locations of vsp/vlp cassettes in the genomes of the above strains as well as our previously characterized B. recurrentis A17. Two-way ANOVA was applied to compare levels of spirochetemia between strains for different time points.

Results: Overall, the data demonstrated that one strain was non-infectious, two strains had DFM and culture-detectable spirochetemia at day 1 and 2 post inoculation and no relapses, and the other two strains developed multiple DFM-detectable relapses. To our surprise, the sequencing data revealed comparable numbers of vsp/vlp cassettes in the six strains (n=49 (A1), n=54 (A17, PBeK, or PAbN), n=57 (PAbJ), n=58 (A11)) with 49 pseudogenes being conserved, which led us to reject the hypothesis.

Conclusions: The data demonstrated that the AVS was only functional in the two *B. recurrentis* strains that had developed multiple relapses. Further studies are warranted to explain the impairment of the AVS in the *B. recurrentis* strains that were infectious but produced no relapses.

Financial Support: The research was supported by NIH NIAID awards, R03AI163601, R21AI182876, T32OD011083, and a start-up provided to A.S.R. by Department of Pathobiology and Diagnostic Investigation, College of Veterinary Medicine, Michigan State University.

Notes:

54 - Inflammatory pathways in *Clostridioides difficile* infection: A murine spatial transcriptomics study

N Ghahari[1], D Pillai[1, 2], S Narayanan[1], A Abdelkhalek[1]

[1]Department of Comparative Pathobiology, Purdue University, [2]Animal Disease Diagnostic Laboratory, Purdue University. nghahari@purdue.edu

Session: Disease pathogenesis 1, 2025-01-19, 10:45 - 11:00

Objective: To investigate the differential gene expression in the cecum and colon of symptomatic and asymptomatic mice infected with Clostridioides difficile using spatial transcriptomics analysis.

Methods: An antibiotic-primed mouse model of *Clostridioides difficile* infection (CDI) was utilized. Six-week-old C57BL/6 mice (n = 6, Jackson Laboratory, Bar Harbor, ME, USA) were acclimatized for one week before receiving an oral antibiotic cocktail consisting of kanamycin (0.4 mg/mL), gentamicin (0.035 mg/mL), vancomycin (0.045 mg/mL), metronidazole (0.215 mg/mL), and colistin (850 U/mL) for three days. This antibiotic treatment depletes the normal gut microbiota, creating susceptibility to CDI. After a two-day washout period, the mice were injected intraperitoneally with clindamycin (10 mg/kg) to further enhance CDI susceptibility. One day later, the mice were orally infected with 5 × 105 CFU/mL *C. difficile* ATCC 43255 spores prepared in phosphate-buffered saline (PBS). Mice were monitored for clinical signs of CDI, including diarrhea, scuffed coat, hunching, lethargy, and unresponsiveness. Symptomatic mice were euthanized immediately upon showing severe symptoms, while asymptomatic mice were euthanized at the end of the experiment, one week after infection, using CO2 asphyxiation. Tissue samples were collected from the cecum and colon, processed into formalin-fixed paraffin-embedded (FFPE) sections, and analyzed using NanoString™ GeoMx Digital Spatial Profiler.Differential gene expression was analyzed using the GeoMx Digital Analysis Suite (DAS). Genes with a Log2 fold change (LFC) of ≥1 were considered significantly upregulated, while genes with an LFC ≤ -1 were considered significantly downregulated. Statistical significance for differential expression was determined using a p-value < 0.05.Pathway analysis was performed using the Reactome (https://reactome.org/) and KEGG (https://www.genome.jp/kegg/pathway.html) databases. Pathways were prioritized based on coverage, normalized enrichment score (NES), and adjusted p-value (< 0.05). NES was considered significant if the adjusted p-value was less than 0.05, accounting for gene set size and variation in enrichment scores across gene sets.

Results: The IL-17 pathway, including Lcn2, Cxcl2, and S100a8 genes, was significantly upregulated in symptomatic mice. Activation of the IL-17 signaling pathway triggered downstream signaling through NF-κB and MAPK pathways. Gene expression was markedly altered between the superficial and deep layers of the intestine, revealing layer-specific differences in gene expression patterns between symptomatic and asymptomatic mice.

Conclusions: Spatial gene expression patterns in the enteric mucosa are strongly associated with clinical signs and lesions observed in symptomatic CDI mice. These findings provide insight into the role of inflammatory pathways in the pathology of CDI and highlight the significance of tissue layer-specific responses in the disease progression.

Financial Support: Internal funding from the Department of Comparative Pathobiology, Purdue University.

Notes:

55 - The effect of *Clostridium perfringens* intracellular bile acid levels and growth

Tahrir Alenezi[1], Bilal Alrubaye[1], Samar Algehani[1], Janashrit Shrestha[1], Rohana Liyanage Liyanage[1], Xiaolun Sun[1]

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

Session: Disease pathogenesis 1, 2025-01-19, 11:00 - 11:15

Objective: Necrotic enteritis (NE) caused by *Clostridium perfringens* is one of the reemerging prevalent enteritis in the poultry industry. Dietary bile acid deoxycholic acid (DCA) reduced NE and improved chicken growth performance. It remains largely unclear what is the molecular mechanism of bile acids influencing *C. perfringens* infection. In this study, we hypothesized that intracellular bile acid levels impacted C. perfringens pathogenesis. We aimed to investigate the relationship between growth and intracellular bile acids levels in *C. perfringens.*

Methods: *C. perfringens* chicken isolate CP1 was incubated with 0.1, 0.5, and 1 mM chenodeoxycholic acid (CDCA), cholic acid (CA), DCA or lithocholic acid (LCA) in BHI. After 24 h, C. perfringens CP1 was enumerated by serial dilution and plating on C. perfringens agar plates (Tryptic Soy Agar (TSA) with sodium thioglycolate, D-Cycloserine, and sheep blood. To measure the intracellular bile acid levels, *C. perfringens* HN13 was cultured with 1 mM CDCA, CA, DCA or LCA. After 24 h, *C. perfringens* was centrifuged, and the cell pellet was washed for five times. The bile acid retained inside *C. perfringens* was extracted and quantified using HPLC/MS-MS. Differences between treatments were analyzed using One-way ANOVA followed by Fisher's LSD test using Prism 7.0 software. Differences were statistically significant if p-values were < 0.05.

Results: *C. perfringens* growth in BHI (control) reached 7.4 log10 CFU/ml after 24 h culture. Compared to the control, the bacterium growth was at 7.0, 7.2 and 6.3 ($p<0.05$) log10 CFU/ml in 0.1, 0.5, and 1 mM CDCA, respectively and 7.3, 7.1, and 6.2 ($p<0.05$) log10 CFU/ml in 1 mM CA, respectively. Notably, *C. perfringens* growth was all significantly reduced to 5.1, 0, and 0 log10 CFU/ml in 0.1, 0.5, and 1 mM DCA, respectively, while *C. perfringens* growth was all significantly reduced to 6.3, 6.1, and 6.1 CFU/ml in 0.1, 0.5, and 1 mM LCA, respectively. HPLC/MS-MS assay showed that intracellular CDCA reached 295 ng/mg in *C. perfringens* cultured in 1 mM CDCA. C. perfringens retained significantly lower at 91 ng/mg CA, compared to CDCA treatment, when the bacterium was cultured in 1 mM CA. *C. perfringens* retained >500 ($p<0.05$, compared to CDCA treatment) and 178 ng/mg LCA and CDCA when the bacterium was cultured in 1 mM LCA medium. Intracellular DCA was significantly higher at 366 ng/mg, compared to CDCA treatment, when *C. perfringens* was cultured in 1 mM DCA.

Conclusions: We found that *C. perfringens* was more susceptible to DCA and the intracellular bile acid levels were different among CDCA, CA, DCA or LCA. It would be important to determine the specific intracellular bile acid levels on *C. perfringens* pathogenesis and NE.

Financial Support: This research was supported by NIFA 1018699, 2020-67016-31346, and 2024-67015-42404, Arkansas Biosciences Institute and AAES Research Incentive Grant to X.S. This research was also supported by the Poultry Federation Scholarship to T.A.

Notes:

56 - Ergot alkaloid dynamics and physiological responses in steers rotating grazing toxic endophyte-infected tall fescue

N. M. Filipov[1], I. M. Llada[1], J. M. Lourenco[1], M. K. Ross[2], G. Suen[3], D. P. Jones[4], M. M. Dycus[1], N. S. Hill[1]

[1]University of Georgia, [2]Mississippi State University, [3]University of Wisconsin-Madison, [4]Emory University. filipov@uga.edu

Session: Disease pathogenesis 1, 2025-01-19, 11:15 - 11:30

Objective: Fescue toxicosis (FT) is an economically devastating pasture-related disease to the US beef herd. A symbiotically residing endophyte (*Epichloë coenophiala*) produces ergot alkaloids, such as ergovaline (EV), which are considered the causative agents of FT in steers grazing wild-type infected toxic (E+) fescue. The objective of the current study was to investigate the relationship between ergot alkaloid levels and signs of FT under rotational grazing conditions as rotational grazing practices, sometimes used to manage the negative impact of FT, have not been investigated.

Methods: Eighteen steers were initially randomly placed on E+, nontoxic (NT), or endophyte-free (E-) fescue fall pastures. After 14 days, there was a pasture switch of, respectively, E+ to E-or NT, E- or NT to E+. An additional group of steers were switched E- to NT or NT to E-. Body weight (BW) was measured pre, 14, and 28 days after pasture placement. Respiration rate, rectal temperature, urine, ruminal fluid, and plasma samples were collected before and on days 2, 7, 14, 16, 21, and 28. Total ergot alkaloids in the urine were measured by ELISA, whereas plant, rumen fluid, plasma, and urine EV and lysergic acid (LA) were determined by an optimized triple-quad mass spectrometry using authentic standards. Data were analyzed with repeated measures ANOVAs, followed by an SNK post hoc.

Results: During the first 14 days, weight gain was unaffected, but steers that were placed on E+ pastures for the second 14 days gained less than the rest. Rectal temperature and respiration rate were increased by E+ grazing beginning a week post E+ placement; both parameters decreased when the E+ steers were placed on NT or E- paddocks, while the reverse was true for the animals grazing E+ for the second half of the study. Both EV and LA increased in the rumen of E+ steers 2 days post pasture placement and remained elevated while steers were on E+ pastures; rumen LA was markedly higher than EV. Total ergot alkaloids and LA in the urine followed similar dynamics to that of rumen EV and LA; however, EV in the urine was not present. Plasma levels of LA and, especially, EV, were low, but when detected followed similar pattern to their rumen and urine dynamics.

Conclusions: These data suggest that (i) upon placement on E+ pastures, the increase in rumen EV and LA precedes changes in physiological signs of FT, (ii) high ruminal LA and low plasma EV levels suggest pre-systemic EV metabolism and possible low absorption, (iii) at least some signs of FT are reversible by placing the animals on non-toxic fescue pastures, (iv) significant portion of the total ergots excreted with the urine is in the form of LA. The practical implications of these findings are that rotational grazing between toxic and non-toxic cultivars using a two-week rotating duration might be a viable management option, but it would be desirable to monitor urinary ergot alkaloids or LA specifically.

Financial Support: This research was funded by a grant from the USDA, National Institute of Food and Agriculture (NIFA). Grant #67015-31301

Notes:

57 - Endotoxemia modulates endocannabinoid and ethanolamide profiles in dairy cows' plasma

Madison N. Myers[1], Miguel Chirivi[1], Jair Parales Giron[2], Jose dos Santos Neto[2], Adam L. Lock[2], G. Andres Contreras[1]

[1]Dept. of Large Animal Clinical Science, Michigan State University, [2]Dept. of Animal Science, Michigan State University. smit2477@msu.edu

Session: Disease pathogenesis 1, 2025-01-19, 11:30 - 11:45

Objective: In dairy cows, bacterial endotoxemia induces systemic inflammation and fatty acid (FA) mobilization from adipose tissues. Endocannabinoids (eCBs) are potent, FA-derived bioactive molecules that modulate metabolic and inflammatory processes. At present, the effects of endotoxemic events on eCB profiles in cattle remain to be established. Therefore, the objective of our study was to determine how circulating eCB levels are mediated by single and repeat exposures to endotoxin.

Methods: We assigned 32 multiparous, mid-lactation dairy cows to treatment groups (saline, SAL; lipopolysaccharide, LPS) using a 2x2 Latin square design and serial administration (SAL-SAL, SAL-LPS, LPS-SAL, LPS-LPS). The study began with a 7-d observation period in which cows received no treatments. From d 8-11, the start of the first of two periods (P1, P2), feed was restricted. On d 10, treatments were infused into the jugular vein of each cow. A 7-d washout period followed. Next, P1 events were repeated for P2. Using LC-MS/MS, we quantified concentrations of six eCBs in plasma samples collected prior to feed restriction (BAS), immediately before infusion 1 (PRE1), 4 h post-infusion 1 (POST1), immediately before infusion 2 (PRE2), and 4 h after infusion 2 (POST2).

Results: Relative to PRE1, POST1 arachidonoylethanolamide (AEA) and oleoylethanolamide (OEA) concentrations were elevated in LPS-treated cows ($P<0.01$) whereas linoleoylethanolamide (LEA) levels were greater in SAL ($P=0.01$). LPS did not alter plasma palmitoylethanolamide (PEA), stearoylethanolamide (SEA), or 2-arachidonoylglycerol (2-AG) in POST1. However, levels of 2-AG were lower in POST1 versus PRE1. Relative to PRE2, levels of AEA, PEA, and 2-AG were reduced in POST2. The effect of P1 treatment on POSTP2 AEA, LEA, SEA, and 2-AG concentrations was significant ($P<0.05$) while P2 treatment was significant to AEA and 2-AG ($P<0.05$). The interaction between P1 and P2 treatments on POSTP2 AEA and LEA levels was also significant ($P<0.05$). Animals challenged with single LPS exposures (SAL-LPS, LPS-SAL) exhibited lower levels of AEA and LEA than those challenged with repeat exposures (LPS-LPS; $P<0.05$). Compared to SAL-SAL, 2-AG levels were elevated in cows exposed to LPS at least once, with the greatest levels observed in SAL-LPS ($P<0.05$).

Conclusions: These results suggest that LPS exposure induces distinct changes in eCB levels, with repeat challenges elevating AEA, LEA, and 2-AG levels. The findings point to a nuanced response where certain lipid mediators are selectively elevated or suppressed depending on the timing and frequency of LPS exposure. This highlights the potential for LPS to differentially modulate inflammatory and metabolic pathways through its impact on bioactive lipid profiles, providing insights into how endotoxemia may drive adaptive or maladaptive physiological responses in dairy cows.

Financial Support: Research presented in this abstract was funded by MAAA #AA-23-0014; US-Israel BARD #IS-5167-19; USDA-NIFA #2024-67011-42947, #2021-67037-34657; NCRR NIH: S10RR027926, S10OD032292.

Notes:

58 - Cyclooxygenase activity determines TLR4-mediated lipolysis in bovine adipocytes during endotoxin challenge

Miguel Chirivi[1], G. Andres Contreras[1]

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

Session: Disease pathogenesis 1, 2025-01-19, 11:45 - 12:00

Objective: Bovine periparturient diseases are often accompanied by endotoxemia. Endotoxin (LPS) triggers lipolysis and reduces insulin sensitivity in adipose tissue (AT). These effects evidence a link between metabolic and infectious diseases. However, the mechanisms by which LPS activates adipocyte lipolysis are poorly understood. The goal of this study was to determine the role of cyclooxygenase (COX) metabolites on bovine adipocyte lipolysis triggered by TLR4-activation.

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 pro-adipogenic media for 7 d. Next, lipolysis was stimulated with the β-adrenergic agonist isoproterenol (ISO=1 µM, Basal=0 µM) or Lipopolysaccharide (LPS; O55:B5; 1 µg/mL) for 7h. To quantify TLR4 contribution to LPS-induced lipolysis, cells were transfected with siRNA targeting TLR4 (siTLR4) or a non-coding (ncRNA) control. To quantify the effect of COX activity on lipolysis, adipocytes were pre-incubated with COX inhibitor, flunixin meglumine (FM). Lipolysis was determined by quantification of glycerol release and results are presented as units relative to Basal (±SEM). Arachidonic acid (ArA) derived oxylipids were quantified by (LC/MS). Statistical analyses used a linear mixed model in JMP.

Results: Compared to Basal, ISO and LPS increased glycerol release by 86.91±16% and 72.66±16%, respectively ($P<0.01$). siTLR4 reduced LPS -induced lipolysis by 39.16±4.5% compared to ncRNA ($P<0.05$). siTLR4 reduced the transcription of COX-2 gene (PTGS2) by 83.89% compared to ncRNA. Incubation with FM inhibited LPS-induced lipolysis by 73.63%, reduced PTGS2 by 52.45% and reduced the amount of LPS-induced ArA in the culture media by 63.51%. Adipocytes treated with FM showed lower concentrations of ArA-derived oxylipids during LPS incubation, including PGE2, PGD2, 6-Keto PGF1a, and 5-HETE.

Conclusions: These data highlight the important link between TLR4 and COX activity in bovine adipose tissue during lipolysis. The inhibition of COX activity may represent a new approach to reduce inflammation-induced lipolysis in dairy cows. Future studies will evaluate the role of prostaglandins in adipose tissue function.

Financial Support: This research was supported by U.S. Department of Agriculture's National Institute of Food and Agriculture (Washington, DC) competitive grants 2021-67015-34563, Agriculture and Food Research Initiative Competitive Grants

Notes:

59 - Testing alternate biosecurity policy mechanisms to overcome moral hazard problem in indemnifying cattle producers

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

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

Session: Biosecurity and infection control, 2025-01-19, 10:30 - 10:45

Objective: Research on human decision-making has showed that policy makers can nudge behaviors for individual and collective social wellbeing. Alternate indemnity policies basically change the underlying incentive structures, which in turn serve as policy signals that nudge biosecurity adoption behaviors. However, the challenges induced by moral hazard and collective action dilemmas limit the ability of inducements and nudges. Well-known moral hazards in livestock industry include reporting suspicions of a Foreign Animal Disease (FAD), compliance with holding movement orders if a FAD is detected, and free riding on other producers to adopt biosecurity. This study harnesses serious gaming approach to compare baseline (current USDA) policy of unconditional indemnity with conditional indemnity as alternate policy mechanisms for cost effective minimization of FAD risk. We hypothesize that an unconditional indemnity policy reduces the adoption of biosecurity practices, while conditional indemnity policy, in which provision of indemnity is conditional upon adoption of biosecurity, increases the adoption of biosecurity practices.

Methods: We developed a serious gaming experiment, in which subjects received payments for their performance. The game is mounted online and more than 500 Amazon Turks are invited to play the game containing one control and 18 treatment rounds of play with each round spanning a maximum of 22 weeks of decision making horizon, leading to a maximum of 500x19x22 = 209,000 revealed choices. Compared with the control group of 75% unconditional indemnity, treatments test 30%, 75% and 90% conditional indemnity scenarios, each with three thresholds of biosecurity adoption scale (3, 5 and 7 on a scale from 1 to 10); and low and high network centrality structures of cattle production supply chains. The gaming data are analyzed with Latent Class Analysis.

Results: We discover three types of latent classes among the players, distributed 9.93%, 80.02% and 10% in the sample. Latent class 1 (9.93%) players adopt the least amount of biosecurity and strongly prefer unconditional indemnity policy. Latent class 2 (80.02%) players adopt significantly higher biosecurity compared with class 1 and prefer unconditional indemnity at 90% with biosecurity adoption threshold of 5. Latent class 3 (10%) players adopt the highest amount of biosecurity and prefer unconditional indemnity at 30% with biosecurity adoption threshold of 7.

Conclusions: Using a serious gaming experiment, we demonstrate that the current unconditional indemnity policy for cattle producers poses a moral hazard problem, leading to overall lower adoption of biosecurity to proactively mitigate FAD incursion risk. We discover that switching the current USDA policy from 75% unconditional to 90% conditional indemnity will very likely increase biosecurity adoption. In this game setting, we find that 10% of the players (class 1) will prefer to “free ride” on government absorbing the risk from FADs, and another 10% (class 3) are altruistic and will adopt biosecurity even with minimal incentives. Most importantly, we find that majority of the players (80% in class 2) will respond to “nudging” induced through switching policy from unconditional to conditional indemnity, adopt higher levels of biosecurity and reduce moral hazard problem embedded in the current USDA policy.

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:

60 - Enhanced biosecurity planning resources for foot-and-mouth disease preparedness for cattle veterinarians

Julia A. Herman[1]

[1]National Cattlemen's Beef Association. jherman@beef.org

Session: Biosecurity and infection control, 2025-01-19, 10:45 - 11:00

Objective: Enhanced biosecurity planning for foreign animal diseases like foot-and-mouth disease can be challenging for veterinarians and cattle producers. Producer education has potential to improve types of educational resources through different types of media. New resources will be developed for the Secure Beef Supply plan for business continuity to improve uptake by cattle producers, industry stakeholders, and veterinarians.

Methods: The National Cattlemen's Beef Association received USDA National Animal Disease Preparedness grant to improve educational resources for the Secure Beef Supply (SBS) plan. An advisory group of cattle producers and influencers from all cattle sectors were invited to conduct a gap analysis of the current SBS resources and guide resource creation. Trade and agricultural media and professional veterinary conferences were used to disperse information and increase network of biosecurity educators.

Results: Multiple resources were developed and made publicly available to stakeholders within the cattle industry. These include handouts, videos, continuing education modules available online, webinars, and podcasts that cover topics specific to sectors (cow/calf, stocker/backgrounder, feedlot, livestock hauler/transporter).

Conclusions: Streamlining educational information on the Secure Beef Supply website with industry education programs like the Beef Quality Assurance program provides consistent and up-to-date information for cattle producers and veterinarians. Preventive planning and disease preparedness prior to a foot-and-mouth disease outbreak will necessitate collaboration from the producer to private veterinarians, up to state animal health officials. These resources will help all tiers of cattle caretakers in biosecurity planning and disease preparedness, seeking to mitigate as much business continuity issues during a potential FMD outbreak.

Notes:

61 - Use of an electrostatic precipitator to decrease airborne transmission of viruses in experimentally infected pigs

Lan Wang[1], José Morán[2], My Yang[1], Bernard Olson[2], Chris Hogan[2], Montserrat Torremorell[1]

[1]Department of Veterinary Population Medicine, University of Minnesota, [2]Department of Mechanical Engineering, University of Minnesota. wang9036@umn.edu

Session: Biosecurity and infection control, 2025-01-19, 11:00 - 11:15

Objective: Airborne viruses pose a threat to both human and animal health. Airborne viruses spread rapidly in animal premises, which makes them difficult to contain and protect against. Electrostatic precipitators (ESP) are air cleaning devices that ionize airborne particles using high voltage electrodes and electrophoretically deposit them on the grounded plates. ESPs have a much lower pressure drop, and potentially lower energy consumption compared to air filtration systems. In our study, we evaluated the effect of a single-stage wire-plate ESP on mitigating airborne transmission of two important viruses of swine, influenza A virus (IAV) and porcine reproductive and respiratory syndrome virus (PRRSV), using experimentally infected pigs.

Methods: In each test, four IAV or PRRSV inoculated pigs were placed in two connected isolator chambers (2 pigs per isolator) upstream of the ESP, and two uninoculated pigs (sentinels) were placed downstream in one isolator chamber. An airflow of 51 m3/h moved unidirectionally from the inoculated pigs, and passed through the ESP and then to the sentinel pigs. For each virus, we conducted a positive control test with the ESP powered off, and three tests with the ESP operating at 12 kV or 14 kV. Nasal swabs of pigs, air samples and wipes of surfaces from all the isolators were collected daily. Air samples were collected with two Andersen cascade impactors (ACI) which sampled air simultaneously from upstream and downstream of the ESP, respectively. Samples were tested by RT-qPCR, and PCR positive air samples were incubated with cell culture to assess virus viability.

Results: When the ESP was not powered (positive control test), sentinel pigs tested positive within 1 day of exposure to the IAV aerosols generated by inoculated pigs and 2 days to the PRRSV aerosols. Airborne IAV RNA was detected both upstream and downstream of the ESP in particles ranging from 0.22 μm to > 8 μm. However, almost no or only low concentration of PRRSV RNA was detected in air samples. In contrast, when the ESP operated at 12 kV or 14 kV, sentinel pigs tested positive for IAV after 5 - 6 days of exposure to ESP-treated aerosols, and tested positive for PRRSV after 7 - 8 days of exposure to ESP-treated aerosols. Only limited levels of IAV RNA were detected in air samples collected in the downstream isolator before sentinel pigs tested positive, mainly in particles 1 - 3.5 μm. The RNA-based virus removal efficiency (RE) of the ESP was estimated as 96.91% to 99.97%, with RE observed in particles > 8 μm significantly higher than that in particles < 3.5 μm. No viable viruses were isolated from the air samples.

Conclusions: The ESP delayed the onset of infection of IAV and PRRSV in the sentinel pigs under the conditions of this study, most likely due to the removal of virus-laden particles at different size ranges, resulting in a lower infectious dose in the air.

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:

62 - A scoping review of Foot-and-Mouth Disease biosecurity practices and farm-level risk factors

MaRyka R. Smith[1], Christy J. Hanthorn[1], Michael W. Sanderson[1]

[1]Center for Outcomes Research and Epidemiology, Kansas State University. marykasmith@vet.k-state.edu

Session: Biosecurity and infection control, 2025-01-19, 11:15 - 11:30

Objective: Numerous countries have developed plans to protect their food supply and livestock populations in response to a Foot-and-Mouth Disease (FMD) outbreak. These plans typically involve implementation of heightened biosecurity practices by producers and livestock caretakers. The purpose of this scoping review was to identify all published literature that described or evaluated biosecurity practices to mitigate FMD risk as well as risk factors for FMD infection that producers can manage at the farm-level.

Methods: Systematic search of multiple online databases and deduplication on title returned 3,153 records. To be considered for inclusion in this review, reports had to be original research, published in a peer-reviewed journal, published in English, and include information about biosecurity interventions to mitigate FMD risk or FMD risk factors at the farm-level. The reports also had to address at least one of the major domestic livestock species in the United States (cattle, sheep, goat, swine, American bison). Reports describing results generated entirely in-silico were not included. After screening, 35 reports were included in this review.

Results: The reports included in this review described data from 23 unique countries published from 1998 to 2024. The biosecurity practices were categorized into groups depending on where the measure was being applied (e.g., Personal Protective Equipment Use, People Management, Disinfection, Vehicle Management, Animal Management, and Fomite or Other Control). Two reports took place within a laboratory setting, all others (n=21) were from field data. The laboratory reports evaluated handwashing, showering, and changing outerwear as biosecurity practices. No reports from field data included an assessment of the efficacy of any biosecurity practices. Many reports (n=12) described isolation or quarantine of livestock or farms but failed to define specific quarantine practices including the distance from other animals or duration of separation. The reports that included risk factors were also categorized (e.g., Herd Composition, Management Decisions, Communal Actions, Animal Movement, Contacts with Other Farms). Multiple reports assessed herd size (n=11) and herd species composition (n=8) as risk factors, however there was not a clear consensus on what herd size or species composition increased FMD risk or was protective against FMD infection.

Conclusions: The current extent of the published literature on FMD biosecurity provides little data on the quantitative efficacy of specific biosecurity practices and highlights the difficulty of measuring the impact of biosecurity in the field. The limited data and incomplete reporting regarding details of the implemented practices makes meta-analysis infeasible at this time. Improved reporting of biosecurity practices and risk factor details would improve understanding; however, methods to evaluate biosecurity practice efficacy and implementation remains difficult and novel research protocols may be needed to meet the demands of advancing FMD response plans.

Financial Support: National Animal Disease Preparedness and Response Program 2020 Grant: USDA-APHIS-10025-VSSP0000-20-0001

Notes:

63 - Assessing ultrasound probe disinfection in veterinary clinics: A hidden risk for bacterial transmission?

Alexandria Lane[1], Smriti Shringi[1], Asmita Shrestha[1], Vishakha Kulkarni[1], Phillipa Gibbons[1], John Dascanio[1], Devendra Shah[1]

[1]Texas Tech University School of Veterinary Medicine. devendra.shah@ttu.edu

Session: Biosecurity and infection control, 2025-01-19, 11:30 - 11:45

Objective: Ultrasound imaging is widely used in veterinary medicine as a non-invasive and cost-effective diagnostic tool. Two types of ultrasound probes are routinely used in veterinary clinical practices: rectal (linear) and non-rectal (linear, convex, micro-convex, cardiac) probes. Bacterial contamination of ultrasound probes poses a serious risk of transmitting bacterial infectious agents between animals and from animals to humans (zoonoses). Proper disinfection of probes after each use is crucial to prevent such transmission, yet little is known about current practices in veterinary clinics. The objective of this study was to evaluate probe disinfection methods and the extent of bacterial contamination in veterinary settings.

Methods: Twenty-one veterinary clinics (2 equine, 6 mixed animal, and 12 small animal) and two academic units at Texas Tech University School of Veterinary Medicine (1 equine and 1 small animal) participated in the study through a standardized questionnaire regarding probe disinfection methods employed in veterinary clinical settings. A total of 52 ultrasound probes (41 non-rectal and 11 rectal), including 30 from small animals and 11 from mixed animal and equine practices, were swabbed during storage at the time of visit to each clinic. All swab samples were processed to assess bacterial contamination, followed by quantitative culturing for total aerobic bacteria and total *Staphylococcus* spp.

Results: Although all clinics reported cleaning probes after each use, the methods varied significantly, and none of the clinical practices, except two academic units, had a written standard operating protocol (SOP) for disinfection. Nearly all probes (50/52, 96.1%) were contaminated with aerobic bacteria, with levels ranging from 1.18 to 5.65 log10 CFU. Additionally, 65.4% (34/52) of probes were contaminated with *Staphylococcus* spp. with levels ranging from 1.17 to 3.55 log10 CFU. Rectal probes exhibited higher contamination levels, with a mean of 4.1 $\log_{10}$ CFU for aerobic bacteria and 2.42 $\log_{10}$ CFU for *Staphylococcus* spp., compared to non-rectal probes, which had mean contamination levels of 2.46 $\log_{10}$ CFU for aerobic bacteria and 1.1 $\log_{10}$ CFU for *Staphylococcus* spp. Probes from clinics without SOPs showed similar contamination levels to those from clinics with SOPs, suggesting either insufficient disinfection or recontamination during storage.

Conclusions: Ultrasound probes in veterinary practices are potential vectors for bacterial transmission, regardless of species or SOPs. Clinics with SOPs must ensure proper implementation and address potential recontamination during storage, while clinics without SOPs need to establish and rigorously follow standardized disinfection protocols to minimize infection risks.

Financial Support: Alexandria Lane received a Veterinary Research Scholarship from the Judy Calder Foundation. The research was funded by the Texas Tech University School of Veterinary Medicine.

Notes:

64 - What would it take to inspire ambulatory veterinary practitioners to make a just-in-time decision to use PPE?

Brandy Burgess[1], Nichelle Jasper[1]

[1]University of Georgia. brandy.burgess@uga.edu

Session: Biosecurity and infection control, 2025-01-19, 11:45 - 12:00

Objective: There are over 1,400 agents known to be infectious to humans with approximately 60% being classified as zoonotic agents. While practitioners may be more likely to use precautions to prevent transmission when a zoonotic agent is suspected, almost 75% of newly emerging or re-emerging agents are considered to be zoonotic. The risk this poses to practitioners is exemplified by the high consequences of the re-emergence of Hendra virus in Australia. This reality is exacerbated by practitioners often not taking standard prevention practices in their daily practice. With this in mind, what would it take to inspire practitioners to make a just-in-time decision to don personal protection equipment? The objectives of this study were to assess perceptions of zoonotic disease risk among ambulatory practitioners and to characterize factors that influence decision-making relative to infection prevention efforts.

Methods: A cross-sectional study was performed using an on-line, anonymous survey. Ambulatory veterinarians practicing in the United States were eligible to participate in a survey that was distributed via state veterinary medical associations and professional associations. The survey collected information on demographics, practice characteristics, and perceptions of zoonotic risks and prevention practices. Further, participants were asked to describe the measures they would take to prevent disease transmission in regard to clinical vignettes. Descriptive statistics were performed and thematic analysis was undertaken to evaluate open-ended questions.

Results: The majority of participants exclusively practiced on equids with fewer engaging in mixed practice. Clinical experience varied between 1 and 59 years, with a majority of respondents having at least 20 years of experience and were private practitioners engaging in at least some ambulatory practice. In general, practitioners ranked assisting with parturition and contact with feces or other bodily fluids to be high risk practices; and most did not report having experienced a zoonotic infection. Interestingly, environmental factors greatly influenced decision-making relative to donning PPE.

Conclusions: Overall, ambulatory practitioners have knowledge of factors associated with zoonotic disease exposure risk. However, many do not take minimum precautions to prevent transmission during daily practice and display a lack of concern regarding posing a risk to others.

Notes:

65 - RNA therapy beyond vaccination: Possibilities and challenges

Philip J. Santangelo[1]

[1]Wallace H. Coulter Department of Biomedical Engineering, Emory University and Georgia Institute of Technology. Philip.j.santangelo@emory.edu

Session: CRWAD Keynote Speaker, 2025-01-19, 1:30 - 2:30

In 2019, the emergence of SARS-CoV-2 reminded us of the potential potency and transmissibility of a respiratory pathogen. This prompted a worldwide response to find a vaccine. Prior to 2019, a number of efforts, focused on the development of nucleic acid-based vaccines, solely to find a way of providing a faster vaccine response to an emerging pathogen. Messenger RNA based vaccines were part of those early efforts including work by Moderna, Curevac, Sanofi Pasteur, Pfizer, and others. As of 2017, Moderna, Curevac, and BioNTech had mRNA-based vaccines in Phase 1 trials, priming their push towards COVID vaccines in 2020. Regardless of one's opinion regarding their success, the data clearly showed their ability to mitigate severe disease and limit hospitalization. Durability and cell-mediated responses, though, still need improvements. Overall, this effort brought mRNA-based technology into the forefront and demonstrated levels of efficacy, safety and manufacturability.

The question, though, is can this general approach be used for treatments, not just vaccines? What is different about treatments? For treatments, there is an increased burden on the safety of delivery formulations, potency, as doses are typically higher, and on the cargo, to be as translationally efficient as possible. For a treatment you typically do not have the amplification of the immune system in your favor, and therefore the whole approach has to be more efficient.

My lab has focused, the last 6 years, on local delivery of mRNA, to the respiratory tract, as a means of expressing various cargos to prevent and treat respiratory infections. I'm going to walk you all through the "scientific battle" to achieve the potency we need and the progression to a formulation that is likely going into the clinic, in humans, in 2026. I'll discuss using this approach to express antibodies, interferon lambda, and CRISPR proteins as a means of preventing and treating RSV, influenza and SARS-CoV-2. This work has relevance to animals, as we have successfully delivered mRNA into mice, hamsters, ferrets, swine, cows, horses and non-human primates, and are working on both respiratory and intramammary delivery for cows, in the context of H5N1 infections. Potency and safety are critical and much of our success to date has been because of the formulation choices we have made. Throughout the talk, I will discuss the methods we use to interrogate our mRNA-based therapies including qPCR, RNAscope (single RNA sensitive in situ hybridization), and spatial transcriptomic platforms as a means of interrogating delivery to specific cell types. In addition, I will also address some odd claims on the "internet" regarding mRNA technology. These claims are often not substantiated with statistically significant data, and often utilize methods and models incorrectly to arrive at their conclusions.

Notes:

66 - Development of countermeasures for high consequence zoonotic and human pathogens

R. Jeff Hogan[1], Eric Lafontaine[1]

[1]University of Georgia. jhogan@uga.edu

Session: CRWAD Special Symposium, 2025-01-19, 2:45 - 3:30

Objective: To underscore the essential role of academic institutions equipped with Biosafety Level 3 (BSL3) laboratories in developing countermeasures against high-consequence zoonotic and human pathogens. This includes their contributions to workforce training and advancing therapeutics and vaccine development for pathogens such as SARS-CoV-2, *Burkholderia mallei*, and *B. pseudomallei.*

Methods: We review the functions and capabilities of BSL3 laboratories within academic institutions, focusing on their dual role in training scientists to safely work with high-risk agents and supporting research. Selected examples of therapeutics and vaccines for SARS-CoV-2 and *Burkholderia* spp. will be presented.

Results: Research efforts within these facilities have yielded significant progress in the development of countermeasures for SARS-CoV-2 and *Burkholderia* species, demonstrating their critical role in addressing emergent public health threats.

Conclusions: While academic BSL3 laboratories are crucial for preparing the next generation of scientists and advancing therapeutic and vaccine research, they face considerable economic and regulatory challenges. Addressing these barriers is essential to fully realize the potential of BSL3 laboratories in enhancing public health preparedness and response capabilities for future pandemics.

Financial Support: We wish to thank DARPA, NIH, and DTRA for funding.

Notes:

67 - mRNA transduction of mucosa generates rapid and potent barrier to infection

Philip J. Santangelo[1], Jae Yeon Joo[1], Peng Xiao[2], Younghun Jung[1], Daryll Vanover[1], Mariluz Arainga[2], Deepanwita Bose[2], Kenneth Rogers[2], Chiara Zurla[1], Francois Villinger[2]

[1]Coulter Department of Biomedical Engineering, Emory University, [2]New Iberia Research Center, UL Lafayette. Francois.villinger@louisiana.edu

Session: CRWAD Special Symposium, 2025-01-19, 4:00 - 4:45

A number of pathogens are transmitted via mucosal routes, primarily through the respiratory, gastrointestinal or sexual routes. While immunity at these vast portals of pathogen entry are critical, inducing protective barriers at that level via immunization has proven challenging for many recent viral and bacterial infections, e.g., flu, COVID, Ebola, dengue, HIV, HSV and many more. As an alternative prevention method for instances where either time is critical, for patients unable to mount robust immune responses or for infections against which no preventive vaccine currently exists, we have used mRNA formulated either in water or with polymers to generate local protective antibodies lining the mucosal surface in nonhuman primate models of infection.

To induce achieve such barrier to respiratory infections, our team has compared various polymers to promote expression of protein in the lower respiratory tract following nebulization. As proof of concept, this approach resulted in rapid expression of nanoluciferase in as little as 4 hours post administration, and reached complete distribution across both lungs in 24 hours. Conversely, for the prevention of sexually transmitted disease, an ideal product would be the use of microbicides effective at the portal of entry, yet their development has been marred with recurrent failures against HIV infection. Alternatively, broadly neutralizing antibodies (bnAbs) delivered at mucosal interfaces have been shown to protect monkeys from SHIV infection, providing proof of concept that drug free protection may be achieved, though such protection is very short lived, as the bnAbs are rapidly excreted. mRNA transduction of vaginal epithelium presents multiple challenges including a multilevel squamous rapidly regenerating epithelia with its top layer dead and cornified. However, using a spray generating high velocity droplets was able to induce transduction of epithelia to produce HIV bnAbs anchored to the epithelium via a glycosylphosphatidylinositol (GPI) moiety. Analysis of vaginal fluids and biopsies demonstrated the extended presence of bnAbs even after 4 weeks of administration, suggesting durable protection afforded even by single treatments. In fact, mucosal explants from mRNA induced bnAbs were protected from infection challenged in vitro with clade A, B and C SHIVs. Moreover, unlike systemic delivery, mucosal delivery of proteins allows for the design and transduction of synthetic bnAbs with enhanced bioactivity and affinity, without the risk of ADA, offering a highly flexible platform to design effective barriers to infection and even contraception devoid of hormonal treatment. While such optimization is ongoing, the next step will be to test our approach in vivo measuring protection of female rhesus macaques from viral acquisition upon vaginal challenges.

Notes:

68 - Opportunities for RNA therapy in animal health

Amelia Woolums[1]

[1]Department of Pathobiology and Population Medicine, Mississippi State University. amelia.woolums@msstate.edu

Session: CRWAD Special Symposium, 2025-01-19, 4:45 - 5:30

Objective: The objective of this presentation is to prevent an overview of research testing the use of messenger ribonucleic acid (mRNA) treatment of animals to induce expression of proteins that may have therapeutic or prophylactic effect against animal diseases. While the use of mRNA to induce expression of antigens for the purposes of inducing an immune response, in the context of vaccination, is well recognized, less well known is the use of mRNA therapy to induce production of proteins that can have direct therapeutic or prophylactic effect. Examples of this application of mRNA for the potential control and treatment of animal diseases that are not necessarily of zoonotic concern will be reviewed.

Methods: Research trials have demonstrated that mRNA can be applied to cells of domestic animals, typically with cationic transfection reagents, to induce uptake of mRNA and expression of the encoded protein in vitro. Moreover, mRNA in water or with polymeric or lipid nanoparticle carriers, has been delivered to mucosal surfaces of live sheep, cattle, swine, and horses, with no adverse effects noted.

Results: In vitro treatment of cells of domestic animals, and in vivo treatment of livestock, with mRNA in water or with various carriers, has resulted in expression of antibodies, antimicrobial peptides, and cytokines within 24 hours of treatment. Modification of mRNA so that expressed antibody remains tethered to the cell expressing the antibody has led to persistence of the antibody for up to 90 days following a single treatment. Expressed antibodies have been shown to bind and neutralize virus, confirming that that mRNA treatment could be used to induce rapid passive immunity at the mucosal surface. Antiviral cytokines such as interferon lambda have also been expressed, providing the possibility of multi-modal antiviral therapy induced with mRNA treatment. Mucosal expression of antibodies against the parasite *Tritichomonas foetus* have also been induced by mRNA therapy.

Conclusions: While the development of mRNA therapy to treat or prevent diseases of animals is in the early stages, the results to date indicate the potential of the approach to lead to rapid onset of local responses to a variety of types of infection. Going forward, research will need to confirm the optimal delivery approach to ensure expression of adequate durability for various indications.

Financial Support: USDA NIFA AFRI

Notes:

69 - Immune response after vaccination - peculiarities of the porcine immune system

Armin Saalmüller[1]

[1]University of Veterinary Medicine Vienna. Armin.Saalmueller@vetmeduni.ac.at

Session: AAVI - Featured Speakers, 2025-01-20, 8:30 - 9:15

Pigs are an economically important species and vaccination to protect their health is of paramount importance. In addition to antibodies and neutralizing antibodies, the cellular immune system plays a central role in an immune response following vaccination and infection. In this case, the pig's immune system has some special features. Three of these peculiarities will be discussed. Natural killer cells, which play a role especially in immune reactions against viral antigens and in the polarization of the T-cell response, are composed of three subpopulations in pigs, which are characterized by the differentiation antigens CD8α and CD335. Can we increase their activity? Within the T cells, there is a very large population of T-cell receptor (TCR) γδ T cells in pigs. These represent a very heterogeneous population whose potential is only moderately utilized. Can the effectiveness of vaccines be improved by stimulating TCR-γδ T cells? The classical TCR-αβ T cells are characterized by four subpopulations defined by the differentiation antigens CD4 and CD8. Activated and differentiated CD4+ T cells express CD8α molecules and can be distinguished from resting CD4+ T cells. The expression of CD27 also enables the differentiation of central and effector memory cells.A detailed understanding of the porcine T-cell response after immunization will help to improve the efficacy of existing and the development of new vaccines.The immunology team at the University of Veterinary Medicine in Vienna has been working on the cellular immune system of the pig for many years. In addition to basic research on NK cells, TCR-γδ T cells and the classical TCR-αβ T cells and their differentiation into corresponding functional T-cell populations and memory cells, applied research questions also play a central role. Of importance is the reaction of the cellular immune system after vaccination with various vaccines and the reaction of the cellular immune system after infection with different viral and bacterial pathogens.

Notes:

70 - Potentiating the application of immunoinformatics tools to animal health

Anne S. De Groot[1]

[1]EpiVax, Inc. dr.annie.degroot@gmail.com

Session: AAVI - Featured Speakers, 2025-01-20, 9:15 - 10:00

The recent breakthroughs recognized by the 2024 Nobel Prizes in Physics and Chemistry underscore the transformative potential of advanced computational tools in scientific innovation. In animal health, immunoinformatics tools such as PigMatrix and EpiCC for swine vaccine design are similarly expanding the potential for immunization strategies targeting economically impactful swine diseases and spillover risks. Now that these tools are integrated in the iVAX vaccine design platform, which was originally developed for human vaccines, they are helping to advance novel swine vaccine design and swine vaccine efficacy analysis. PigMatrix predicts T cell epitopes for swine leukocyte antigen (SLA) classes I and II by scoring pathogen sequences for SLA binding potential, enabling targeted selection of T cell epitopes that balance protective immune response, cross-conservation within strains of the same pathogen, and self-tolerance. EpiCC complements PigMatrix by evaluating epitope relatedness between vaccines and circulating pathogen strains, facilitating vaccine updates to counter evolving strains within herds.This talk will provide two important examples showcasing the use of these tools for veterinary applications, such as developing cross-conserved T cell epitopes for swine influenza vaccines and guiding strain selection for vaccines against swine influenza, PRRSV, and porcine circovirus type 2. The first example is a case study that will describe how the EpiCC tool can be used in the selection of a vaccine strain for autogenous vaccination of a single farm. Second, a retrospective case study will highlight both PigMatrix and EpiCC to explore the rationale for the protective efficacy of a single PRRSV vaccine against several well defined PRRSV outbreak strains. Together, these computational tools represent a leap in infectious disease research as applied to animal health, empowering infectious disease researchers with tools to evaluate strain evolution and swine vaccine developers with targeted insights on swine vaccines efficacy. Given the importance of swine health to global economic activity, the integration of informatics tools into the scientific pursuit of improved vaccines and therapeutics for swine is both timely and necessary.

Notes:

71 - Balancing farm antimicrobial resistance genomic research and data privacy

Charles Adegbole[1], Togzhan Seilkhanova[2], Rebecca Smith[3]

[1]Department of Pathobiology, University of Illinois Urbana-Champaign, [2]School of Information Sciences, University of Illinois Urbana-Champaign, [3]Carl R. Woese Institute for Genomic Biology, Urbana-Champaign. caa7@illinois.edu

Session: Antimicrobial resistance 1, 2025-01-20, 8:30 - 8:45

Objective: Genomic antimicrobial resistance (AMR) research allows us to unravel the intricacies of antimicrobial resistance and devise strategies to counter it. However, the complexities of this type of research raise a concern of privacy breaches, resulting in stigma to the farmers and other stakeholders providing the research data. The objective of this study is to identify acceptable scenarios for safeguarding farm AMR genomic data and its providers, while still advancing critical research.

Methods: In this study, we interviewed researchers who interact with antimicrobial resistance genomic data from farms. The interviews were conducted in a semi-structured format by an author with experience in qualitative research. The interviews lasted 25 to 60 minutes and were performed via a secure video conference platform. The videos from the interviews were auto-transcribed, de-identified, and stored in a secure folder accessible to the study investigators, who then checked the transcription to ensure accuracy. The interview transcripts were analyzed using the thematic analysis approach. The steps followed include identifying concepts, building concepts, analyzing data for context, and integrating the categories. A two-pronged approach was used in conducting the analysis. Initially, two authors coded the first four interviews independently and thereafter agreed to a set of descriptive categories and codes. The authors then proceeded to analyze different interviews independently on MAXQDA. A third author consolidated the efforts of the two independent coders by reviewing the different codes and categories that emerged in the analyses process.Answers to closed-ended questions were also extracted on a categorical basis and analyzed in R to make summary plots of participant characteristics. Participants of this study engaged farm genomic AMR data in the United States as PhD students, University lecturers, and members of government agencies such as the California Department of Food and Agriculture, the United State Department of Agriculture, the US Food and Drug Administration, and the US Environmental Protection Agency.

Results: Preliminary findings underscore a prominent theme: a preference for implementing a multilevel security system. Such a system would allow access to varying levels of data and metadata at different levels of security, depending on the sensitivity of the data involved and the needs of the research project. Other emerging themes are distrust among farmers, researchers, and the government, and the usefulness of state level data aggregation for most research.

Conclusions: For a data sharing platform to be worthwhile, it must be both trustworthy enough for stakeholders to submit data and useful enough for researchers to utilize. This research contributes valuable insights into developing comprehensive and secure systems for managing and sharing farm animal AMR genomic data, fostering collaboration between stakeholders, and addressing concerns related to privacy and trust in this critical domain.

Financial Support: This project is funded by the United States Department of Agriculture (Award #RPT-131874). We thank the participants for their time and contributions to this effort.

Notes:

72 - Antimicrobial resistance and virulence reservoirs: evolution, distribution and persistence of *R.equi* in horse farms

Alinne L. R. Santana-Pereira[1], Courtney Higgins[1], Nathan Slovis[2], Pankaj Gaonkar[1], Laura Huber[1]

[1]Auburn University, [2]Hagyard Equine Medical Institute. alp0051@auburn.edu

Session: Antimicrobial resistance 1, 2025-01-20, 8:45 - 9:00

Objective: *Rhodococcus equi* is a soil saprophyte responsible for lethal respiratory infections in livestock, especially in young fouls, which are infected by inhalation of soil particles. Widespread prophylactic treatment of subclinical infections has led to an increase in antimicrobial resistance (AMR) in *R.equi*, especially to macrolides and rifampin. These are shed onto the soil, where the pathogen can survive, reinfect foals and spread AMR due to its remarkable niche-adaptability. Recent description of a distinct environmental population with a new macrolide resistance gene (erm51) in soil indicates that *R.equi* is more genomically diverse than previously thought. Genomic variability, albeit small, can correlate with niche specification, infection outcomes and persistence ability of different *R.equi* lineages. This study seeks to characterize 100 isolates of soil *R.equi* in order to identify phylogroups and their origin, virulence, AMR load, distribution and persistence across 44 commercial race-horse breeding farms.

Methods: *R.equi* was isolated from soil samples from 44 commercial race-horse breeding farms in 2021, for a total of 100 environmental isolates and 6 clinical isolates. Isolates were confirmed via 16S sequencing, and Whole Genome Sequenced using Illumina. Genomes were assembled for all isolates. Due to the high conservation of *R.equi* genome, relatedness was inferred using high- quality SNPs, called with GATK. SNP phylogroups were identified using Jaccard distances and hierarchical clustering. Virulence and macrolide resistance were determined using PCR amplification of the genes VapA and erm(46/51), respectively.

Results: SNP analysis reveals that the soil isolates belong to 5 distinct phylogroups. Group A formed a divergent branch of avirulent isolates carrying erm51, present in 72.1% of the farms. This lineage seems to be separate from virulent lineages and are specialist soil saprophytes. Group B is clonal resistant clinical isolate PAM 2287, and all isolates carried erm46 and VapA. Group B were present in the soil of 25.6% of the farms. Group C are highly clonal to resistant clinical isolate CGG, but interestingly CGG is distant to PAM2287, showing diversity within virulent lineages. Group C was less widespread, recovered from only 5 farms. Group D, despite being genomically related to pathogenic phylogroups, is avirulent, susceptible and rare. Group E are formed by avirulent, resistant (erm46) isolates found in 13.9% of the farms. 44% of the farms had isolates from different phylogroups co-existing in the soil, most commonly Group A (Bonafide soil lineage) and B (virulent lineage).

Conclusions: *R.equi* presents detectable diversity reflected in genomically and functionally distinct phylogroups. A spread Bonafide soil saprophyte phylogroup was identified which is genomically distant from clinical strains. Clinical isolates were clustered into different phylogroups which are differentially spread across farms, indicating differences in their ecology, and possibly pathogenicity. Phylogroups were found not to be competitively exclusive, which offers opportunities for plasmid sharing and resistance/virulence persistence in soil but also points towards plasmid trophism, especially in Group A. Further studies will focus on full annotation of the genomes and correlation between genomic variation and persistence, virulence, resistance, pathogenicity and plasmid biology across phylogroups.

Notes:

73 - Whole genome sequence analysis and molecular characterization of virulence and antimicrobial resistance profiles of *Salmonella* isolated from necropsied horse

Ajran Kabir[1], Grant William[1], Cheyenne Glover[2], Steve Locke[3], Erdal Erol[3], Yosra A. Helmy[1]

[1]Department of Veterinary Science, University of Kentucky, [2]College of Veterinary Medicine, Lincoln Memorial University, [3]Veterinary Diagnostic Laboratory, University of Kentucky. ajran.kabir@uky.edu

Session: Antimicrobial resistance 1, 2025-01-20, 9:00 - 9:15

Objective: *Salmonella* is a foodborne pathogen that represents a serious risk to global public health. It impacts various animal species, including horses. In horses, *Salmonella* infections can range from being asymptomatic to causing severe clinical disease. Infections caused by *Salmonella* are presently controlled with antibiotics. Due to the formation of biofilms and the emergence of antimicrobial resistance, the treatment became more complicated. Our study focused on investigating the prevalence of *Salmonella* enterica in necropsied horses, assessing the capability for biofilm formation, motility, determining the antibiotic resistance profile and perform whole genome sequencing for in-depth genotypic study.

Methods: A total of 2,182 samples were tested for the presence of *Salmonella* infection. Samples were enriched in tetrathionate broth and then cultured on XLT4 plates. Confirmation of the serotypes was performed according to the Kauffmann-White-Le Minor Scheme followed by biofilm formation screening using crystal violet assay. The resistance profile of the isolates was determined by broth microdilution assay using the Sensititre™ Vet (Equine EQUIN2F). The genotypic AMR and virulence profiles were detected using polymerase chain reaction (PCR). Whole genome sequencing was conducted using the Illumina MiSeq platform, followed by genome assembly and annotation, which led to the identification of additional genomic features.

Results: The overall prevalence of *Salmonella* was 1.19% with 11 different serotypes identified. *Salmonella* Typhimurium was the most prevalent serotype with 19.23% prevalence. All of the isolates were identified as biofilm producers and motile. An overall 11.4% of the isolates were identified as multidrug-resistant (MDR), with resistance to gentamicin, amikacin, ampicillin, ceftazidime, ceftiofur, chloramphenicol, and trimethoprim/sulfamethoxazole. Whole genome sequence of these isolates revealed that all of our isolates contained more than 100 virulence genes and more than 30 AMR genes. Multi-locus sequence typing (MLST) revealed that our isolates have 11 different sequence types and ST-19 was the most prevalent. Phylogenetic analysis found that these isolates are closely related to other *Salmonella* species isolated from other sources.

Conclusions: The detection of multi-drug resistant, motile, and biofilm-forming *Salmonella* in horses poses a significant risk to both human and animal health. The presence of over 30 antimicrobial resistance genes in these isolates heightens the potential for horizontal gene transfer to other commensal bacteria. This study underscores the role of horses as possible sources of pathogenic *Salmonella* transmission, particularly to humans. Therefore, increased monitoring and surveillance are crucial for tracing the origins of *Salmonella* infections and implementing effective preventive strategies.

Notes:

74 - Targeted resistome sequencing and colocalization analysis for assessing antimicrobial resistance genes and their bacterial hosts

Tara Nath Gaire[1], Jacob Singer[1, 2], Noelle Noyes[1]

[1]Department of Veterinary Population Medicine, University of Minnesota, [2]Department of Physics and Astronomy, Northwestern University. tgaire@umn.edu

Session: Antimicrobial resistance 1, 2025-01-20, 9:15 - 9:30

Objective: The detection of diverse antimicrobial resistance genes (ARGs) and the accurate identification of their bacterial hosts are crucial for understanding the health risks posed by specific ARGs within microbial communities. However, current standard metagenomic sequencing often lacks the sensitivity to detect low-abundance microbes and their associated ARGs. This study aimed to improve ARG detection within microbiomes while supporting the accurate identification of bacterial hosts for each detected ARG. To achieve this, we utilized previously generated data from pigs, sampled from their arrival at the abattoir to the end of carcass processing and food safety interventions (N = 42 pooled skin and 63 carcass swabs).

Methods: Targeted-enriched shotgun sequencing data from 105 samples were subjected to resistome analysis using the MEGARes.v2 database via the AmrPlusPlus pipeline. Bioinformatic colocalization analysis was performed to identify bacterial sources of ARGs, specifically metal/mercury ARGs. First, host-associated reads were removed from both forward and reverse reads. The filtered paired-end FASTQ files were converted to single FASTA files, assembled into contigs, and then aligned to a database to detect mercury ARGs. Second, contigs that aligned with mercury ARGs were further processed by excising the target ARG sequences (i.e., removing all nucleotides identified in the alignment) and then taxonomically classified using Kraken2.

Results: We detected a diverse profile of ARGs on pig carcasses, which were significantly influenced by food safety interventions and environmental exposures within the abattoir. The colocalization analysis indicated that approximately 73% of contigs containing mercury-associated ARGs were assigned to a taxonomic rank at the phylum level. Proteobacteria was the dominant bacterial phylum harboring these ARGs, with most mercury-related ARGs associated with genomes from the Pseudomonadaceae and Enterobacteriaceae families. However, nearly 17% of the mercury ARG-containing contigs were not long enough to include flanking sequences, making taxonomic assignment impossible.

Conclusions: Accurate identification of the bacterial hosts of ARGs using short-read metagenomic sequencing data is challenging (often incomplete), even with enriched metagenomic data and de novo assembly; thus, results require careful interpretation when applied to AMR surveillance or in designing food safety measures.

Financial Support: Acknowledgments: This work was supported by the National Pork Board (NPB) (Grant No. 19-038), USA.

Notes:

75 - Assessment of antimicrobial resistance genes in the bovine gastrointestinal tract

Levi Svaren[1], Wenli Li[1]

[1]US Dairy Forage Research Center, USDA-Agricultural Research Service. wenli.li@usda.gov

Session: Antimicrobial resistance 1, 2025-01-20, 9:30 - 9:45

Objective: Antibiotics are widely used in cattle industry to prevent and treat diseases as well as boost growth and production. However, application of antibiotics in food animals promotes the development of microbial antimicrobial resistance (AMR), and subsequently wide spread of AMR bacteria throughout food chain, endangering the wellbeing and health of both animals and humans. In humans, it's well documented that the gut microbiome harbors a diverse range of AMR bacteria, called the resistome. Yet, similar knowledge in the gastrointestinal tract (GIT) of cattle is largely lacking. This study aimed at identifying the distribution and prevalence of AMR genes in cattle GIT.

Methods: We gathered bulk RNA-seq data from 10 publicly available datasets and 14 internal datasets in cattle. These datasets include liver, blood, esophagus, caecum, four stomach chambers, and small intestine samples and include studies monitoring feed, infection, fertility and fiber intake. The cattle reference genome is used to remove the host sequences and retain the non-host reads, which are of microbial origin. AMR genes from the MEGARes database were used to quantify the abundance of AMR in the tissue samples, differential abundance analysis of these AMR genes among different cohorts and feed treatments.

Results: Our analysis identified that AMR associated drug classes MLS, aminoglycosides, cationic antimicrobial peptides, tetracyclines, and beta lactams were the most prevalent across all datasets surveyed. Differential gene expression analysis revealed that AMR gene expression from these classes varied greatly in between cohorts with various feeding regimens.

Conclusions: Our findings suggested that feeding treatments may impact the distribution and abundance of AMR in the GIT. Our study shed light on the critical drug classes associated with AMRs and highlight important areas for future microbial research of the cattle GIT. Financial Support:

Financial Support: The funding for this experiment is provided by projects 5090-31000-026 and 5090-31000-028 from USDA Agricultural Research Service, US Department of Agriculture.

Notes:

76 - The impact of diet and commingling on the fecal resistome of production pigs exposed to metaphylactic antibiotics: a randomized controlled trial in a commercial flow

Tara Nath Gaire[1], Jared Young[1], Gerardo R Diaz[1], Mariana Meneguzzi[1], Valeria Lugo Mesa[1], Ammar Yasir[1], Yale Deng[1], Katie Culhane[1], Avery Cheeseman[1], Garret Drum[1], Claire Mitchell[1], Serena May[1], Mark Schwartz[1, 2], Randall Singer[3], Maria Pieters[1], Noelle Noyes[1]

[1]Department of Veterinary Population Medicine, University of Minnesota, [2]Schwartz Farms Inc, [3]Department of Veterinary and Biomedical Sciences, University of Minnesota. tgaire@umn.edu

Session: Antimicrobial resistance 1, 2025-01-20, 9:45 - 10:00

Objective: This study aims to evaluate whether dietary fiber and/or commingling can be used to mitigate the development and persistence of antimicrobial resistance genes (ARGs) within the fecal microbiome after metaphylactic antibiotic use in production pigs.

Methods: A total of 84 sows from two rooms within a single sow farm were enrolled in this 2x2 factorial design randomized controlled trial. From each litter, up to ten piglets were randomly selected and enrolled into the study as they were born within an hour to no more than 24 hours after birth (n=833 piglets across 84 litters). At enrollment, each piglet was tagged with a unique ID, weighed, and given a single dose of Excede (ceftiofur, 100 mg/mL, 0.5 mL intramuscularly; Zoetis Animal Health). Following enrollment, all piglets were processed together within the room at 2-5 days post-birth (dpb), which included an iron injection, tail docking for all piglets, and castration for barrows. Immediately after processing, the first "commingling" factor treatment was initiated, wherein enrolled litters were either kept within a single farrowing pen with their sow and littermates ("CONV") or they were allowed to move freely across multiple pens and suckle from multiple sows ("MSCC" for multisuckle, common creep). On days 17-18 dpb, all enrolled piglets received Baytril (enrofloxacin, 100 mg/mL, 0.35 mL intramuscularly; Elanco US Inc) immediately prior to the introduction of pre-weaning creep feed, which was the second study factor in the 2x2 design. Creep feed was either standard feed or supplemented with potato starch, depending on the pre-allocated treatment group. At 23-24 dpb, all piglets were weaned and moved to the nursery facility, where they were weighed upon arrival. Piglets were sampled by inserting a sterile swab into the rectum at 0-12 hours post-birth (hpb) and then daily until 2-4 dpb. Additionally, fecal swabs were collected before and after the commingling and diet interventions, and at key production points. These timepoints included: the day of "commingling" intervention (5-6 dpb), and at 8, 11, and 14 dpb; the day of the Baytril injection and creep feed initiation (17-18 dpb), as well as one and three days afterwards; and one day before moving to the nursery facility (22-23 dpb). Further samples were taken at the nursery facility one day post-weaning (dpw), 4 dpw, 7 dpw (one day before moving to the finisher). Pigs were then moved to a grow-finish facility and final samples were collected one day before marketing (149-151 dpb). Each swab was placed in an individual sterile Whirl-Pak and stored at -80°C. Total gDNA was extracted from each swab, and extracted gDNA was pooled by sow/litter ID and sampling time point. A total of 1197 DNA samples were submitted for metagenomic library preparation and shotgun sequencing.

Results: We are currently awaiting the metagenomic sequencing data.

Conclusions: We expect the results to provide insights into whether commingling and dietary creep feed can be effective interventions to reduce the persistence of ARGs within fecal microbial communities following metaphylactic antibiotic exposure, while also supporting overall pig performance in commercial production systems.

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:

78 - Mapping the neutralizing epitopes of Shiga toxins

Sai Simha Reddy Vakamalla[1], Siqi Li[1], Aashwina Madhwal[1], Ipshita Upadhyay[1], Chongyang Zhang[1], Bedaso Edao[1], Weiping Zhang[1]

[1]University of Illinois Urbana Champaign. sv46@illinois.edu

Session: Parasitology & Vaccinology, 2025-01-20, 8:45 - 9:00

Objective: Shiga toxin (Stx) is one of the most potent bacterial toxins, found in *Shigella dysenteriae* 1 and certain *Escherichia coli* serogroups, where it is known as Stx1. In addition to or in place of Stx1, some *E. coli* strains produce a second type, Stx2, which shares the same mode of action but is antigenically distinct. This study advances vaccine development against Shiga toxin-producing *Shigella dysenteriae* and *Escherichia coli* (STEC) including O157:H7, by identifying and characterizing key B-cell epitopes from Stx1 and Stx2. These toxins are critical virulence factors responsible for severe foodborne illnesses and life-threatening complications such as hemolytic uremic syndrome (HUS). The Stx toxins are comprised of an A subunit that targets and disrupts eukaryotic ribosomes, inhibiting protein synthesis in host cells, and a B subunit that forms a pentamer to bind to the globotriaosylceramide (Gb3) receptor, primarily located on endothelial cells. Given its crucial role in cell attachment, this study focused on the B subunit (with ongoing study targets the A subunit).

Methods: Using in silico tools, we identified seven immunodominant B-cell epitopes on the B subunits of both Stx1 and Stx2 toxins. These seven epitopes were embedded into a carrier protein, resulting in the production of recombinant fusion proteins, which were then expressed, purified, and subjected to analysis via SDS-PAGE and Western blotting. Each epitope was evaluated for immunogenicity by immunizing five mice per group and further for antibody response measurement by i-ELISA.

Results: Results showed that all the tested epitopes triggered an immune response on performing i-ELISA, underscoring their potential as vaccine candidates. The data was analyzed by one-way Anova in GraphPad Prism. Further analysis will focus on the neutralizing capabilities of these epitopes, utilizing Vero cell cytotoxicity neutralization assays and GB3 receptor-binding ELISAs.

Conclusions: This research represents a significant step toward creating broadly effective vaccines to prevent infections caused by *Shigella dysenteriae* and STEC. By focusing on the key virulence factors shared by these pathogens, we aim to address a critical public health need and reduce the burden of severe foodborne illnesses worldwide.

Financial Support: This work was financially supported by the University of Illinois at Urbana-Champaign and NIH

Notes:

79 - Unique glycolytic enzymes as targets for novel anti-*Cryptosporidium* drugs in bovine calves

Md M. Mia[1], Abdur R. Azam[1], Akansha Singh[1], Jennifer M. Reinhart[1], Jozef Stec[2], William H. Witola[1]

[1]University of Illinois Urbana-Champaign, [2]Marshall B. Ketchum University. whwit35@illinois.edu

Session: Parasitology & Vaccinology, 2025-01-20, 9:00 - 9:15

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 globally. There are neither effective drugs nor vaccines available against *C. parvum* infection. In our preliminary work, we found that inhibitors of *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 thus prevent disease development in infected mice models. In this project, we aim to derivatize and optimize CpLDH and CpPyK inhibitor combinations that are efficacious and safe in treating *C. parvum* infection in bovine calves.

Methods: First, we investigated the safety and anti-cryptosporidial synergistic efficacy of CpPyK and CpLDH inhibitors in an immunocompromised mouse model. Subsequently, we tested the efficacy of combinations of CpPyK and CpLDH inhibitors in treating cryptosporidiosis in neonatal Holstein bovine calves. Further, we determined the pharmacokinetics of CpPyK and CpLDH inhibitors in bovine calves following oral administration of single tolerable doses.

Results: In infected mice, the CpPyK+CpLDH small molecule inhibitor combinations of NSC303244+NSC158011 and NSC252172+NSC158011, depicted enhanced efficacy against *C. parvum* reproduction, and ameliorated intestinal lesions at doses 4-fold lower than the effective doses of individual inhibitors. Notably, combination of NSC303244+NSC158011 completely cleared the infection without relapse in immunocompromised mice. In neonatal bovine calves, the combination of NSC638080+NSC158011 showed the most significant efficacy against cryptosporidiosis, with amelioration of clinical signs, intestinal pathology and parasite oocysts loads in feces. Pharmacokinetic analyses showed that NSC638080 is rapidly absorbed, attaining maximum systemic levels 2 hours post-administration, and is eliminated over a 10-hour period, with a half-life of 1.58 hours. On the other hand, NSC158011 attains maximum systemic levels 10 hours post-dosing, but is slowly eliminated with a systemic half-life of 31.5 hours. Studies are ongoing to test derived analogs of NSC638080 for improved anti-cryptosporidial efficacy.

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 compound combinations are, therefore, potential lead-compounds for the development of a new generation of efficacious drugs for treating cryptosporidiosis.

Financial Support: This work was funded in-part by USDA-NIFA-AFRI grant number 2022-67015-36347.

Notes:

80 - Aerosolized synthetic mRNA elicits antibodies against *Tritrichomonas foetus* in bovine preputial skin

Kenzie McAtee[1], Merrilee Thoresen[1], Heath King[1], Santiago Cornejo[1], Cassandra Barber[1], Daryll Vanover[2], Jae Yeon Joo[2], Hannah Peck[2], J.M. Feugang[3], Philip Santangelo[2], Amelia Woolums[1]

[1]College of Veterinary Medicine, Mississippi State University, [2]Georgia Institue of Technology and Emory University, [3]Department of Animal and Dairy Science, Mississippi State University. km3415@msstate.edu

Session: Parasitology & Vaccinology, 2025-01-20, 9:15 - 9:30

Objective: *Tritrichomonas foetus* (Tf) is a urogenital parasite that causes trichomoniasis, a venereal disease characterized by infertility in cattle. Significant economic impacts are due to reduced pregnancy rates, extended calving seasons, and decreased calf crops. Our goal was to identify Tf-specific antibodies in preputial skin and secretions of bulls treated with synthetic mRNA encoding antibodies directed against the TF1.17 cell surface antigen. Our specific aim was to investigate whether antibody expression varied depending on the dose and type of mRNA constructs administered, either secreted or membrane-anchored versions of anti-TF1.17. We hypothesized that calves treated with either dose would exhibit measurable antibody levels, with a dose-dependent increase.

Methods: Twelve calves were assigned to 4 treatment groups (n = 3 per group) that received either a low (1.5 mg) or high (3 mg) dose of synthetic mRNA designed to produce antibodies targeting TF1.17, in secreted or membrane-anchored form, with reporter molecule NanoLuc luciferase fused to the antibody light chain. Synthetic mRNA was suspended in molecular-grade water and was administered using a mucosal atomization device inserted into the preputial fornix. Two additional calves served as controls, receiving only water. The prepuce of the calves was swabbed to collect secretions before treatment (day 0) and on days 2, 4, 7, 10, 14, and 21 post-treatment. Swabs were transferred to phosphate buffered saline (PBS) and stored at -80°C. Later samples were vortexed, transferred to a homogenizer column, centrifuged and the homogenate was analyzed for antibody by immunofluorescence assay (IFA). Tf were cultured, fixed and applied to slides, blocked, incubated with preputial secretions, then with conjugated secondary antibody to detect bovine IgG. Fluorescence intensity was measured to quantify antibody binding to Tf. Calves were euthanized on day 21. Preputial epithelium was collected postmortem and frozen at -80°C. Later NanoGlo (Promega) substrate was applied to the thawed tissue which was imaged by in vivo imaging system (IVIS). Tissue was collected from areas of high radiance, lysates were prepared and tested in capillary-based western blots for NanoLuc-labeled bovine IgG using the Jess automated system (Biotechne).

Results: Preputial tissue collected postmortem from calves treated with a high dose (3 mg) of secreted or anchored mRNA showed radiance levels between 1.8-2.4 x 10^5 via IVIS, while low-dose calves exhibited no detectable radiance after background correction. Despite this, NanoLuc-labeled bovine IgG was identified in western blots of skin lysates from all mRNA-treated calves. Chemiluminescent signals corresponded to bands of the expected size for bovine IgG with NanoLuc-labeled light chains. Testing preputial secretions for antibody by IFA was inconclusive, likely due to sampling method and high background in control calf secretions.

Conclusions: The detection of NanoLuc in preputial skin samples from treated calves, through both IVIS and western blot, confirmed that aerosol application of synthetic mRNA successfully induced the production of bovine IgG against TF1.17 surface antigen. Inducing antibody production at the site of infection with aerosolized mRNA has the potential to become a novel and effective strategy to prevent or clear *Tritrichomonas foetus* infection in bulls.

Financial Support: This project was supported by Agriculture and Food Research Initiative Competitive Grant Number 2021-67016-34571 from the USDA National Institute of Food and Agriculture.

Notes:

81 - Pathogenicity of *Tritrichomonas foetus* following exposure to anti-TF1.17 produced via synthetic mRNA transfection

Merrilee Thoresen[1], Kenzie McAtee[1], Elmer Heath King[1], Daryll Vanover[2], Jae Yeon Joo[2], Hannah Peck[2], Philip John Santangelo[2], Amelia Woolums[1]

[1]Mississippi State University, [2]Georgia Institute of Technology and Emory University. mt1657@msstate.edu

Session: Parasitology & Vaccinology, 2025-01-20, 9:30 - 9:45

Objective: Aerosol application of synthetic mRNA to the urogenital mucosa of bulls to induce expression of antibodies against *Tritrichomonas foetus* (Tf) could offer a new approach to treating or preventing bovine trichomoniasis. Utilizing synthetic mRNA transfection of bovine cells to induce their expression of a monoclonal bovine IgG1 against the Tf cell surface adhesin TF1.17, we evaluated the extent to which expressed antibodies, 1) bound to live parasites, 2) protected bovine cells from cytopathic effects of Tf and 3) reduced rates of Tf attachment to cells.

Methods: Primary bovine preputial keratinocytes (PK) and a bovine kidney cell line (BK) were transfected with synthetic mRNA encoding anchored or secreted bovine IgG1 against TF1.17 with the reporter protein NanoLuc luciferase fused to the antibody light chains. Tf were incubated in 24 and 48 h post-transfection supernatants and antibody binding was measured via NanoGlo (Promega) luminescent assay. Viability of transfected BK exposed to Tf was determined using neutral red uptake by BK at 12, 18 and 24 h following the addition of 1 million/cm2 Tf at 48 h following transfection. Attachment of fluorescently labelled (Invitrogen CellTrace) Tf was assessed after introduction of 0.5 million Tf/cm2 to BK at 48 h following transfection. Twenty-four hours later, non-adherent Tf were removed, cultures were fixed in 4% PFA and nuclei were stained with DAPI. An automated fluorescence microscope was used to enumerate BK cells and attached Tf. To identify differences among groups, means of parametric data were compared with ANOVA and LSD; for non-parametric data, Kruskal-Wallis tests and pairwise comparisons were performed (SPSS), with significance set at $p < 0.05$.

Results: There was a significant increase in relative light units (RLU) of parasites incubated in 24 and 48 h post-transfection supernatants containing anchored or secreted anti-TF1.17, as compared to transfection controls ($p<0.001$), indicating binding of expressed antibody to Tf. Twenty-four hours following co-culture with Tf, BK cells transfected with anchored or secreted anti-TF1.17 had significantly higher viability (51 or 43%, respectively) as compared to transfection controls (31%) ($p=0.005$). Adherence of Tf to BK cells that were expressing anchored or secreted anti-TF1.17 was not significantly different from transfection controls ($p=0.222$), although variability among replicates was high.

Conclusions: Antibody to TF1.17 produced by mRNA-transfected bovine cells bound to Tf. Cells expressing either anchored or secreted anti-TF1.17 had higher viability following 24-h exposure to Tf as compared to non-transfected controls. There was no detectable effect of expressed antibody on attachment of Tf to bovine cells, though accuracy of this assessment was likely influenced by high inter-replicate variability. Antibodies produced by bovine cells transfected with synthetic mRNA bind Tf and reduce cytotoxicity of the parasite to host cells in vitro.

Financial Support: This project was supported by Agriculture and Food Research Initiative Competitive Grant Number 2021-67016-34571 from the USDA National Institute of Food and Agriculture.

Notes:

82 - A trans-amplifying SARS-CoV-2 mRNA vaccine generates broad antibody responses against multiple virus variants

Abhinay Gontu[1], Sougat Misra[2, 3], Shubhada K. Chothe[2, 3], Santhamani Ramasamy[2, 3], Padmaja Jakka[4], Maurice Byukusenge[4], Lindsey C. LaBella[2, 3], Meera S. Nair[5], Marco Archetti[1, 6], Ruth H. Nissly[1, 4], Suresh V. Kuchipudi[2, 3]

[1]Huck Institutes of the Life Sciences, Pennsylvania State University, [2]Department of Infectious Diseases and Microbiology, University of Pittsburgh, [3]Center for Vaccine Research, University of Pittsburgh, 4Animal Diagnostic Laboratory, Pennsylvania State University, [5]MARS Veterinary Health, Antech Diagnostics, [6]Department of Biology, Pennsylvania State University. abhinay@psu.edu

Session: Parasitology & Vaccinology, 2025-01-20, 9:45 - 10:00

Objective: With the ongoing evolution of SARS-CoV-2 variants, there is an urgent need for vaccines that generate broadly cross-neutralizing antibodies. This study developed and evaluated a trans-amplifying(TA) mRNA vaccine encoding a consensus spike protein. The objective was to induce broad antibody responses against multiple variants using a reduced antigen dose compared to traditional mRNA vaccines.

Methods: A consensus spike protein sequence was generated by aligning spike protein sequences from multiple SARS-CoV-2 variants. Several additional sequence modifications were introduced to enhance the stability of the spike protein's pre-fusion state. The TA mRNA platform, comprising two sets of mRNAs—one for replicase and the other for the antigen was developed and produced. Special design elements ensured that the replicase specifically amplified the mRNA encoding the spike antigen. The impact of mRNA amplification on the cellular transcriptome was also evaluated in an epithelial cell model to investigate the off-target effect of the TA mRNA vaccine. Transgenic humanized(h) ACE-2 mice were immunized with the TA mRNA encoding the consensus spike protein, using either 40 or 400 times less antigen than a full-dose traditional mRNA vaccine. Post-immunization, neutralizing antibody levels against multiple SARS-CoV-2 variants were measured. Additionally, hACE-2 transgenic mice vaccinated with the TA mRNA vaccine were challenged with the SARS-CoV-2 Omicron BA.1 variant to assess the vaccine's protective efficacy.

Results: Amplification of the antigen mRNA by the replicase and efficient translation was confirmed in cells using western blot analysis of cells transfected with the vaccine mRNA constructs. Immunization studies in hACE-2 transgenic mice demonstrated that the consensus spike TA mRNA vaccine elicited neutralizing antibody levels comparable to those induced by a full-dose mRNA vaccine, despite using a lower antigen dose. Upon challenge with the SARS-CoV-2 Omicron BA.1 variant, the TA mRNA vaccine reduced lung viral titers by over 10-fold, indicating robust protective efficacy. The TA mRNA vaccine also induced cross-neutralizing antibodies in immunized mice against multiple SARS-CoV-2 variants, indicating broad immunogenicity. Transcriptome analysis of cells transfected with the vaccine mRNA constructs revealed no significant change in the cellular mRNA expression, confirming the vaccine platform’s safety profile.

Conclusions: The consensus spike TA mRNA vaccine induced robust neutralizing antibodies against a broad range of SARS-CoV-2 variants while utilizing a markedly lower antigen dose. The scalability and dose-sparing capability of the TA mRNA platform are especially advantageous for veterinary applications.

Financial Support: We gratefully acknowledge the financial support from the Huck Chair in Emerging Infectious Diseases Endowment (SVK), the Huck Innovative and Transformational Seed Fund (MA), Interdisciplinary Innovation Fellowship at the One Health Microbiome Center (AG), Pennsylvania State University.

Notes:

83 - Analysis of risk factors for Valley Fever in Arizona: Application of negative binomial model

D. Areda[1]

[1]Department of Applied Sciences, Ottawa University. demelash.biffa@gmail.com

Session: Epidemiology, 2025-01-20, 6:00 - 8:00

Objective: To assess risk factors of Valley fever in Arizona using negative binomial model.

Methods: Data on Valley Fever cases and potential influencing factors in the Phoenix Metropolitan Area (PMA) from 2000 to 2023 were collected from various local, state, and federal institutions. Data was checked for over-dispersion using the Dispersion Index (Variance-to-Mean Ratio). A negative binomial regression model was used to fit the data. Cases and incidence rate of valley fever were considered as response variables, while all other recorded variables were considered as predictor variables. Goodness-of-Fit of the model was assessed using the dispersion parameter (the difference in deviance between full and null models).

Results: The study found that Particulate Matter 10 (PM10) (μg/m³) was the main risk factor for an increase in valley fever cases and incidence (P=0.00528). Conversely, higher mean humidity (%) and wind speed (mph) were associated with lower risk of valley fever cases. The dispersion index (DI) of the negative binomial model revealed significant over-dispersion in the data (2021.08 for Valley Fever cases and 45.61 for incidence), suggesting that the use of a Negative Binomial model was appropriate.

Conclusions: the analysis identified PM10 levels as a significant risk factor for increased Valley Fever cases in AZ, while higher mean humidity and wind speed were associated with a lower risk. Negative Binomial model, validated by a significant reduction in deviance and a low AIC, effectively explained the variability in Valley Fever cases and incidence.

Notes:

84 - Effect of antioxidant supplementation on health and growth of pre-weaned dairy calves in a multi-herd study

Abigail Reid[1], Hannah Carlson[1], Natalie Prichard[1], Faith Cullens-Nobis[1], Angel Abuelo[1]

[1]Dept. of Large Animal Clinical Sciences, Michigan State University. amr436@cornell.edu

Session: Epidemiology 3, 2025-01-20, 8:45 - 9:00

Objective: Within the first few weeks of life, dairy calves experience significant oxidative stress, which can impact lymphocyte functioning with implications for vaccine efficacy and generalized health status. The aim of this study was to determine the impact of parenteral antioxidant supplementation at birth on both health and growth outcomes with a multi-herd study. We hypothesized that calves receiving parenteral antioxidant supplementation at birth will demonstrate greater average daily gain (ADG) and experience reduced health events compared to those not receiving supplementation.

Methods: Female dairy calves (n = 756) were enrolled from four different farms. Enrollment criteria included receiving minimal to no calving assistance as well as having at least fair transfer of passive immunity as measured by ≥5.1 g/dL serum total protein at 1 week of age. An electric random number generator was used to assign one of four treatment groups for each calf: Multimin90 (Multimin North America Inc.), Bo-Se (Merck Animal Health), Vitamins E-AD (VetOne), Saline (ICU Medical). Treatments followed the recommended dose for each commercial antioxidant supplement. Farm staff were blind to treatment allocation. Weekly measurements (every 7 days) included body weight, hip height, and health data (using University of Wisconsin Calf Health Scorer) such as clinical respiratory exam, thoracic ultrasound, general appearance, and fecal observation. RStudio was used to build a linear mixed model with repeated measures. Treatment, time (in weeks), and their interaction were included as fixed effects, while calf ID and farm were included as random effects. Additionally, disease or absence thereof was determined for each calf such as bovine respiratory disease (BRD), lung consolidation, diarrhea, all-cause morbidity, and mortality. Survival analysis was performed on each disease using a Cox proportional hazards model.

Results: As expected, growth measurements (body weight, hip height) increased across all treatment groups from week 1 to week 8, during the weaning period. However, there were no statistically significant differences between treatment groups for growth or health parameters (total respiratory score, temperature). Similarly, there were no significant differences observed between treatment groups regarding disease hazard ratio during the trial period.

Conclusions: Based on the statistical analysis, we fail to reject the null hypothesis and determined there was no effect of parenteral antioxidant supplementation at birth, between treatments and against the saline control, on growth parameters and health events throughout the weaning period. Under the management conditions of the four farms included in this study, a single dose of antioxidant supplementation did not result in improved growth or health outcomes for pre-weaned dairy calves. Nonetheless, the benefits of antioxidants are well known at the cellular level and may still be beneficial for calves in a greater challenge situation. Further investigation is warranted to determine if frequency of antioxidant supplementation can demonstrate a clinically significant change in growth or health.

Financial Support: This study was supported by competitive grant no. 2018-67015-2830 from the USDA National Institute of Food and Agriculture and a grant from the Michigan Alliance for Animal Agriculture.

Notes:

85 - 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], Carol Leitch[2], Samantha Lycett[2], Andrea Doeschl-Wilson[2]

[1]USDA-ARS, [2]Roslin Institute. john.dunn@usda.gov

Session: Epidemiology 3, 2025-01-20, 9:00 - 9:15

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. We will focus on Objective 1 in this report.

Methods: In Objective 1, four high-resolution empirical datasets for detailed analyses of the effects of vaccination and host genetics on MDV transmission and virulence evolution were generated from four large-scale MDV transmission experiments. These experiments assessed both the direct and indirect effects of vaccination or host genetic resistance to Marek's disease (MD) on the virus transmission, and monitored differences between vaccinated and non-vaccinated birds or birds that are genetically resistant or susceptible to MDV, with regards to changes in the virus sequences and associated changes in virulence over 10 successive generations (passages) of virus transmission between naturally infected shedder birds and naïve contact birds. Each passage and treatment included 10 birds with 6 biological replicates (>1,000 birds per experiment).

Results: Sequencing indicates high genetic stability of MDV with few polymorphisms discovered across the MDV genome. The statistical analyses by Linear Mixed Model has shown that vaccination against MD does not block infection and virus transmission but significantly reduces virus shedding, MD incidence and mortality of vaccinated birds. In addition, transmission of vaccinated bird significantly reduces virus shedding, MD incidence and mortality of non-vaccinated Contact birds. Posterior distributions for the epidemiological transition rates give evidence that infection outcome depends on exposure dose and vaccination status of the recipient birds and the shedder birds, but appear relatively stable across passages.

Conclusions: 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 vaccination does not prevent MDV transmission within all 10 subsequent passages. However, vaccination with the full recommended 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. The ability to naturally transmit Marek's disease through 10 passages of vaccinated birds highlights that unless the environment is deep cleaned, Marek's disease transmission will persist.

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:

86 - A quantitative model of the risk of FMD transmission following cattle movements during an outbreak

Torre Dunlea[1], Shankar Yadav[1], Michael Sanderson[1]

[1]Center for Outcomes Research and Epidemiology, Kansas State University. tdunlea@vet.k-state.edu

Session: Epidemiology 3, 2025-01-20, 9:15 - 9:30

Objective: Foot and Mouth Disease (FMD) presents a major economic threat to the U.S. cattle industry. A key pathway for FMD transmission between farms is through direct contact, primarily driven by the movement of livestock between operations. While the Secure Beef Supply (SBS) project provides disease control guidelines—including biosecurity protocols and daily active observational surveillance (AOS)—to curb further spread, there is no quantitative assessment of FMD transmission risk under current suggested SBS protocols. Overly restrictive movement control measures could severely disrupt business continuity and hinder the development of balanced and effective outbreak management strategies. This study addresses this gap by estimating the transmission risk associated with cattle movements during an FMD outbreak.

Methods: Simulations from InterSpread Plus (ISP, a spatially explicit, stochastic FMD spread model) were used to estimate local, indirect, and direct daily transmission risk for cattle farms within 200 km of an infected premises (IP). Daily incidence risk was used to inform the farm risk of infection in the model. Biosecurity effect on transmission risk was modeled and analyzed at three different effectiveness levels (low, medium, and high). For infected farms, within-herd disease dynamics and active observational surveillance (AOS) processes were used to estimate the probability of detecting infection by days since infection. Movement farms were categorized as uninfected, infected but not detected or infected and detected.

Results: Local transmission was predominant within 3 km of an infected premises (IP), with infection risk decreasing sharply as distance increased. Additionally, increasing the number of direct cattle shipments received increased the infection risk for receiving farms. The low, medium, and high levels of biosecurity modeled reduced infection probability. However, farms within 3 km of an IP still faced substantial risk even at the highest level of biosecurity. This highlights the potential risk posed by movements from farms near an IP, despite the benefits to business continuity. Conversely, for farms situated more than 3 km from an IP, baseline risk was much lower and further reduced by biosecurity measures. Allowing movements from farms more distant from an IP could help maintain business continuity at a lower risk. The implementation of AOS significantly impacted the detection of Foot-and-Mouth Disease (FMD) infected herds prior to movement. Baseline AOS parameters (75% of the herd observed and 60% probability of clinical disease recognition) resulted in a high probability of detection by day 8.

Conclusions: In response to an FMD outbreak, prompt and focused disease control programs will be necessary for disease eradication, however, robust controls can impede business continuity. Quantitative assessment of cattle movement risk based on the risk of herd infection and probability of detection prior to movement can help to estimate the risk distribution of specific cattle movements in an FMD outbreak scenario. The results from this study will improve our ability to make safe, informed cattle movement decisions, minimizing the impact of an FMD outbreak as well as mitigating business continuity impacts on the cattle industry.

Notes:

87 - Evaluating FMD spread among U.S. dairy cattle premises: Findings from the InterSpread Plus model

Johnbosco U. Osuagwu[1], Julia M. Smith[1], Columb Rigney[2]

[1]Department of Animal and Veterinary Sciences, University of Vermont, [2]United States Department of Agriculture's Animal and Plant Health Inspection Service. josuagwu@uvm.edu

Session: Epidemiology 3, 2025-01-20, 9:30 - 9:45

Objective: Foot-and-mouth disease (FMD) is a highly contagious viral infection impacting cloven-hoofed animals, leading to massive production and economic losses. Understanding its transmission dynamics is crucial for effective control and prevention. This study evaluated FMD transmission in dairy cattle, comparing two outbreak scenario sets: scenario set one focused solely on dairy farms and scenario set two included other dairy cattle premises- cattle dealers, markets, and slaughter plants. The hypothesis posited was that the set of outbreak scenarios involving other dairy cattle premises would significantly increase the average proportion of infected dairy farms when compared to the set of outbreak scenarios that involved only dairy farms.

Methods: The study utilized the InterSpread Plus model (version 6.02.55), a robust spatially explicit and stochastic simulation tool for epidemiological modeling. Key parameters included FMD infectivity, direct and indirect contact movements, and normal and heightened local spread, all tailored to reflect the complexities of disease dynamics. Airborne transmission through cattle was assumed in the model. Data from the Farm Location and Animal Population Simulator (FLAPS), which disaggregates county-level census data collected by the USDA National Agricultural Statistics Service, was used to create a farm file encompassing 65,779 dairy cattle premises. The model simulated high-risk live animal transfers based on frequency, distance, and transmission probabilities. Indirect movements were categorized by low and medium risk levels to reflect non-physical interactions. Local spread mechanisms and airborne transmission probabilities were integrated to consider biosecurity practices and environmental factors. Eighty iterations of the model were run for each scenario set: one with only dairy farms (64,057 premises) and the other including 4,728 additional premises (comprising 3,031 cattle dealers, 1,033 cattle markets, and 664 slaughterhouses). A two-sample proportions test was conducted using RStudio (version 2022.07.2 Build 576) to test the difference between the proportion of infected dairy farms in the two outbreak scenario sets.

Results: In the first set of outbreak scenarios involving only dairy farms, an average of 2 farms (approximately 0.003%) were infected, ranging from 1 to 8 infected farms. In contrast, the second set of outbreak scenarios that included other dairy cattle premises showed an average of 83 infected farms (about 0.12%), with a range of 1 to 628 infected farms. The analysis indicated a significantly higher proportion of infected dairy farms in the set of outbreak scenarios containing other dairy cattle premises ($P < 0.001$).

Conclusions: The inclusion of other dairy cattle premises significantly increased the proportion of infected dairy farms, highlighting the crucial roles of dealers, markets, and processors in the disease's spread. These findings provide essential insights for stakeholders in animal health and agriculture, aiding the development of effective contingency plans and biosecurity strategies tailored to specific risks associated with various livestock operations. This research underscores the importance of evaluating other potential points of disease spread when informing plans for containing and controlling FMD outbreaks.

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:

88 - State-level geographic disparities and annual changes in milk somatic cell counts across the United States, 2011-2023

Jessica Vidlund[1, 2,] Agricola Odoi[1], Chika C. Okafor[1]

1Department of Biomedical and Diagnostic Sciences, University of Tennessee, [2]East Tennessee AgResearch and Education Center-Little River Animal and Environmental Unit, University of Tennessee. jvidlund@utk.edu

Session: Epidemiology 3, 2025-01-20, 9:45 - 10:00

Objective: The dairy industry faces challenges related to mastitis, which significantly impacts milk quality. Monitoring somatic cell counts (SCC) is critical, as SCC levels serve as indicators of udder health, legal thresholds, and the presence of mastitis. Despite SCC's importance, limited research has explored its geographical disparities and temporal changes across the United States. This study aims to investigate state-level geographic disparities annual changes in SCC from 2011 to 2023.

Methods: This study analyzed records from Dairy Herd Improvement Association (DHIA) from January 2011 to December 2023. The DHIA obtained monthly information, including SCC, from selected dairy farms across the United States that subscribe to their service. States must have at least 3 herds with over 1000 animals per herd per year to be included in the analysis. Median weighted SCC percent differences were calculated to assess temporal changes. Wilcoxon-signed rank test, adjusted for multiple comparisons using the Benjamini and Hochberg procedure, was used for the temporal analysis.

Results: The study analyzed data from 42 to 45 states annually between 2011 and 2023. The sample times in states varied from 23 to 451,083 with median value at 6,247. The overall median weighted SCC decreased from 234,000 cells/mL in 2011 to 176,000 cells/mL in 2023, representing a 24.8% reduction. Notable reductions were observed in select states with Virginia and Georgia experiencing the most significant decreases of 36.6% and 34.5%, respectively. However, some states only saw a marginal decline in SCC levels, such as Tennessee (15.6%) and South Carolina (14.0%). Conversely, Texas, California, and Colorado saw significant increases in median weighted SCC, with Colorado experiencing a 147.9% rise.

Conclusions: Overall, the United States has seen a steady decline in composite SCC over the study period. However, the decline in some states was not comparably optimal. By identifying states with little decline in SCC or persistent high SCC, we have the ability to explore potential factors influencing these lower milk qualities. Addressing these issues will lead to healthier herds and higher-quality milk production nationwide.

Financial Support: Data provided by the Dairy Herd Improvement Association

Notes:

89 - Hepatic transcriptome analysis reveals candidate genes in liver abscesses of beef × dairy crossbred steers

Luana D. Felizari[1], Sydney M. Bowman[1], Chiquito J. Crasto[2], Jhones O. Sarturi[1], Dale R. Woerner[1], Bradley J. Johnson[1]

[1]Department of Animal and Food Sciences, Texas Tech University, [2]Center for Biotechnology and Genomics, Texas Tech University. luana.felizari@ttu.edu

Session: Disease pathogenesis 2, 2025-01-20, 8:30 - 8:45

Objective: Liver abscesses are a significant concern in the cattle feeding industry due to their higher prevalence and association with visceral condemnation and carcass trimming. However, the molecular mechanism of development and progression of liver abscesses is still unknown. Therefore, the objective of this study was to evaluate the hepatic transcriptomic profile, immunohistochemistry, and IGF-I circulation from beef × dairy (Angus × Holstein) steers with and without liver abscesses.

Methods: A total of twelve beef × dairy steers (average final body weight of 719 ± 5.8 kg) were selected for blood and liver collection at the packing plant, based on liver scores. The animals were divided into abscessed (n = 6) and healthy livers (n = 6). The sera from the blood samples were used to quantify the circulating insulin-like growth factor I (IGF-I) levels with an ELISA kit. The liver samples were divided into two portions. The first portion was used in immunohistochemistry (IHC) to identify the insulin-like growth factor I receptor (IGF-IR) in the liver tissue. The second portion was used for total RNA extraction, library preparation, and sequencing using the Illumina NovaSeq 6000 platform. Differentially expressed genes (DEGs) were identified using the DESeq2 R package, with an adjusted p-value ≤ 0.05 and fold change > 1.5. The DEGs were evaluated for gene ontology (GO), pathway enrichment, and protein-protein interaction (PPI) network analyses.

Results: Overall, IGF-I levels did not differ in the sera regardless of liver condition; however, the IGF-IR abundance in the liver tissue was up-regulated for liver abscesses. A total of 568 DEGs were identified with 372 up-regulated and 196 down-regulated genes in liver tissue with abscesses. Among these, the most highly up-regulated genes were FGF23, NXPH4, and CYP7A1, while EPHA6, CD70, and INHBA showed the most significant downregulation. Following a PPI network analysis, THBS1 and COL1A2 were the most significant hub genes. Moreover, DEGs showed significant enrichment in biological processes including angiogenesis, cell migration, cell adhesion, and extracellular matrix organization. Also, DEGs were associated with the up-regulation of several pathways signaling, including hepatic fibrosis, interleukin, and IGF-I signaling.

Conclusions: Our study revealed candidate genes and pathways associated with liver abscesses in cattle, including key genes in inflammatory responses and tissue remodeling. These findings provide valuable evidence that could enhance our understanding of the progression of liver abscesses in cattle.

Financial Support: This project was supported by Gordon W. Davis Reagent's Chair in Meat Science and Muscle Biology Endowment at Texas Tech.

Notes:

90 - WGS of intrauterine *E. coli* from cows with metritis reveals a non-unique genotype and the lack of virulence factors associated with disease

Adriana Garzon[1], Carl Basbas[1], Cory Schlesener[1], Noelia Silva del Rio[2], Betsy Karle[3], Fabio Lima[1], Bart C. Weimer[1], Richard Pereira[1]

[1]Department of Population Health and Reproduction, University of California, Davis, [2]Veterinary Medicine Teaching and Research Center, University of California, Davis, [3]Cooperative Extension, University of California, Davis. amgarzon@ucdavis.edu

Session: Disease pathogenesis 2, 2025-01-20, 8:45 - 9:00

Objective: Metritis is a common infectious disease in dairy cattle and the second most common reason for treating a cow with antimicrobials. The pathophysiology of the disease is complex and is not completely understood. *Escherichia coli* has been traditionally attributed to playing a major role in a cascade of events that affect the prevalence, severity, and persistence of uterine disease in cattle. Specific endometrial pathogenic *E. coli* have been reported to be adapted to the endometrium and sometimes lead to uterine disease. Unfortunately, the specific genomic details of the endometrial-adapted isolates have not been investigated using enough genomes to represent the genomic diversity of this organism to identify specific virulence genes that are consistently associated with disease development and severity. The objectives of this project were to 1) define the association between the prevalence of specific AMR and VF genes in *E. coli* with the clinical status related to uterine infection; 2) identify the genetic relationship between *E. coli* isolates from cows with diarrhea, mastitis, and with and without metritis; and 3) determine the association between the phenotypic and genotypic AMR identified on the *E. coli* isolated from postpartum cattle in California.

Methods: Bacterial isolates (n = 162) were obtained from a cross-sectional field study that collected intrauterine swabs from post-partum cows between 3 and 21 DIM from 25 commercial dairy farms in CA. Cows were diagnosed and categorized into one of three clinical groups before enrollment: metritis (MET), as a cow with watery, red or brown colored, and fetid vaginal discharge (VD); cows with purulent VD (PUS) as a non-fetid purulent or mucopurulent VD; and control cows, (CTL) as cows with either no VD or a clear VD. For genomic comparison with other *E. coli* strains, public genomes (n=130) from cows with diarrhea, mastitis, and metritis were included in GWAS, and PERMANOVA analyses were used to evaluate differences between the drug classes or the VF category among clinical groups.

Results: A distinct *E. coli* genotype associated with metritis could not be identified. Instead, a high genetic diversity among the isolates from uterine sources (metritis, pus, or control group) was present. A virulent factor previously associated with metritis (fimH) using PCR methods was not associated with metritis. Genomic comparison with isolates from the public domain revealed that extended-spectrum beta-lactamase (ESBL) genes were more abundant in genomes from diarrhea and metritis clinical groups than isolates from mastitis, pus, or control. Genomes from the metritis clinical group had higher odds of carrying the CTX-M-124 ESBL gene. In comparison, genomes from the diarrhea group had higher odds of carrying TEM-105, TEM-1B_1, and TEM-1 ESBL genes when compared to those isolated from mastitis, pus, or control. There was moderate overall accuracy for WGS to predict phenotypic resistance, which considerably varied depending on the specific antimicrobial tested.

Conclusions: Findings from this study using population WGS that represent the genomic diversity of the organism contradict the traditional pathotype classification and the unique intrauterine *E. coli* genotype associated with metritis in dairy cows.

Financial Support: Funding for this research was provided by the U.S. Department of Agriculture Project number CALV-DHHFS-059. Any opinions, findings, conclusions, or recommendations expressed in this publication are those of the authors and do not necessarily reflect the view of the USDA

Notes:

91 - Heat stress alters innate immune function of the endometrial mucosa in dairy cows and is associated with increased uterine disease

Arslan Tariq[1], John J. Bromfield[1]

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

Session: Disease pathogenesis 2, 2025-01-20, 9:00 - 9:15

Objective: As environmental temperatures and food insecurity increase globally, it is imperative to determine the impact of heat stress on the physiology of food producing animals. Dairy cows are susceptible to uterine disease, with 40% of animals developing disease that increases culling and reduces fertility and milk production. We hypothesized that environmental heat stress would increase the susceptibility of cows to develop disease and alter the innate immune function of the endometrial mucosa of the uterus.

Methods: To evaluate the seasonal incidence of uterine disease, clinical records of 3,507 cows were evaluated in north central Florida. To assess the innate immune function of the endometrial mucosa, endometrial epithelial cells were isolated from cows using a cytobrush at d 21 after calving (n = 7) and cultured at 38.5°C or 40.0°C to mimic heat shock conditions. Cells were simultaneously exposed to 1 μg/mL of the bacterial endotoxin, LPS, for up to 24 h and gene expression of inflammatory mediators was measured. The effect of repeated heat shock on LPS responses was examined when endometrial epithelial cells (n = 10) were cultured at 40.0°C during two 24-h periods prior to exposure to LPS.

Results: Calving during the hot season increased the development of uterine disease in cows. During the period of April through September when the average monthly temperature-humidity index exceeded the physiological threshold of 68, the incidence of uterine disease increased ($P = 0.03$) by 14.2% compared to cows that calved in the cooler months of October through March.Isolated endometrial epithelial cells increased ($P < 0.001$) the expression of the heat shock protein HSP1A1 by 2.2-fold after being cultured at 40.0°C for 12 h compared to cells cultured at 38.5°C, indicating a functional heat shock response. Interestingly, the maximal LPS induced expression of the chemokine CXCL8 and cytokine IL6 were reduced ($P < 0.01$) by 2.7-fold and 3.7-fold in cells cultured at 40.0°C compared to cells cultured at 38.5°C. Culture temperature had no effect on LPS induced expression of the cytokine IL1B. Repeated exposure to heat shock for two 24-h periods had the same effect on endometrial epithelial innate immune responses as exposure to a single period of heat shock.

Conclusions: These data indicate that the development of clinical uterine disease increased when cows calve in hotter months of the year compared to cooler months. In addition, we show that innate immune function of the endometrial mucosa is reduced when cells are cultured at 40.0°C, which mimics the temperatures of cows under heat stress conditions. These findings suggest that heat stress of dairy cows alters mucosal innate immunity which is associated with the increased development of uterine disease. Understanding and mitigating the effects of heat stress on dairy cow health will improve animal welfare, health and productivity while collectively enhancing food security and environmental impacts of dairy production.

Financial Support: This material is based upon work that is supported by the National Institute of Food and Agriculture, U.S. Department of Agriculture, under award number 2024-67015-42414.

Notes:

92 - Using fluorescent *Salmonella* serovars to evaluate trafficking and tissue preference in orally challenged calves

Stephenie M. Bradley[1], Alexis M. Carey[2], Claudia Ossa-Trujillo[2], Ashlyn H. Strickland[2], Ingrid M. Leon[2], L. Garry Adams[1], Sara D. Lawhon[1], Keri N. Norman[2], Nikki Shariat[3], Cheryl Herman[2], Roberta A. Pugh[1], Javier Vinasco-Torres[1], H. Morgan Scott[1]

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

Session: Disease pathogenesis 2, 2025-01-20, 9:15 - 9:30

Objective: Non-Typhi serovars of *Salmonella* enterica subspecies enterica cause foodborne illness in humans which are often associated with undercooked ground meats. Despite slaughter-plant advances, the incidence of salmonellosis remains high. *Salmonella* colonization of lymph nodes, which may be incorporated into ground beef, remains a critical challenge in the ongoing effort to control *Salmonella*.We aimed to determine how fluorescent *Salmonella* colonize the challenged bovine host, providing an enhanced method to study potential serovar differences in invasion potential, tissue affinity and distribution, and bacterial quantification in peripheral lymphatic tissues. We also aimed to understand how *Salmonella* delivered orally can travel to and colonize peripheral lymphatic tissue. We used a pansusceptible *S.* Newport CDC strain isogenic to the novel MDR *S.* Newport strain implicated in a current multi-year and multi-state US outbreak. Additionally, a wild type *S.* Lubbock strain isolated from healthy bovine lymph node tissue was selected for comparison.

Methods: *Salmonella* strains were modified with the Tn7 method to chromosomally encode a fluorescent mCherry gene (S. Newport) or a fluorescent sfGFP (super folder green fluorescent protein) gene (S. Lubbock). Holstein steers (n=12) were orally challenged under ABSL-2 conditions with *Salmonella* strains at 109 CFU in three replicates of four and housed individually by replicate for 7 days after challenge. Within replicates, one calf was randomly assigned to each treatment group: sfGFP S. Lubbock only, mCherry S. Newport only, both serovars at a 1:1 ratio, and a negative control. Blood and feces were collected daily during challenge, and several different peripheral lymphatic tissues, mesenteric lymph nodes, bone marrow, spleen, and liver were collected at necropsy on day seven.Blood cultures were incubated for 96-hours and plated onto *Salmonella* selective media (XLT4). A portion of tissue samples were fixed and prepared for fluorescent microscopy. The remaining tissue samples and feces were enriched for *Salmonella* and plated onto XLT4 media. *Salmonella* positive cultures were imaged on a ChemiDoc MP Imaging System to determine the presence of fluorescent colonies. The fixed tissue samples were mounted and imaged with either a Zeiss fluorescent upright microscope or a Zeiss LSM 780 confocal microscope.

Results: Fluorescent *Salmonella* strains were recovered in both the feces and tissues of all challenged calves. The sfGFP *Salmonella* was recovered in the feces of a calf challenged with only S. Lubbock. As expected, both sfGFP and mCherry *Salmonella* were recovered in the feces of calves challenged with both serovars. Fluorescent *Salmonella* were recovered in cultures of tissue samples from all challenged calves. The mCherry S. Newport was recovered more frequently in tissue than the sfGFP S. Lubbock. Microscopically, *Salmonella* was observed in multiple different tissue samples.

Conclusions: Intrachromosomally fluorescent *Salmonella* proved an effective method for studying the trafficking of *Salmonella* throughout the bovine host and for evaluating differences in invasion potential and bacterial quantities between serovars. Fluorescent challenge strains were recovered in the feces and tissues of orally challenged calves, demonstrating that *Salmonella* consumed by cattle travel to the peripheral lymphatic tissues, including lymph nodes, and can become a risk to food safety.

Financial Support: Texas A&M AgriLife Research; USDA NIFA Animal Health and Disease Research Capacity; College of Veterinary Medicine & Biomedical Sciences, Texas A&M University, Merit & Excellence Fellowship

Notes:

93 - Interactions of *Fusobacterium necrophorum, Bacteroides pyogenes* and *Porphyromonas levii* in bovine metritis

Dianjun Cao[1], Bindu Subhadra[1], Mehwish Ammad[1], Thomas Inzana[1], SooJin Jeon[1]

1Department of Veterinary Biomedical Sciences, Long Island University. dianjun.cao@liu.edu

Session: Disease pathogenesis 2, 2025-01-20, 9:30 - 9:45

Objective: Metritis is a multifactorial disease caused by polymicrobial bacterial infection. The uterine microbiota of cows with metritis undergoes divergence and dysbiosis postpartum characterized by an increase in *Fusobacterium necrophorum*, *Bacteroides pyogenes*, and *Porphyromonas levii*. Moreover, in animals that recover from metritis, the abundance of all three bacterial species decreases, implying that the interaction among *F. necrophorum*, *B. pyogenes*, and *P. levii* is essential for the development of metritis. To identify potential synergistic relationships between *F. necrophorum*, *B. pyogenes*, and *P. levii*, we investigated the growth, biofilm formation, and interactions of these three bacteria cultured in vitro.

Methods: *F. necrophorum*, *B. pyogenes*, and *P. levii* were isolated from the uterus of Holstein dairy cows with metritis and characterized using Gram staining, scanning electron microscopy (SEM), and metabolite profiling. The suspensions of these isolates prepared from log-phase cultures were then analyzed with growth curve, coaggregation, adherence to bovine endometrial epithelial (BEND) cells, and biofilm assays. Biofilm assays were performed anaerobically in 96-well or 24-well culture-treated plates at 37°C. The resulting biomass of biofilms was evaluated by crystal violet staining. For analysis of the spatial organization of in vitro biofilms composed of *F. necrophorum*, *B. pyogenes*, and *P. levii*, all three species were mixed at a 1:1:1 ratio and grown anaerobically on coverslips for 5 days at 37 °C. The biofilms were labeled using fluorescence in situ hybridization (FISH) with 16S rRNA-targeted oligonucleotide probes specific to *F. necrophorum*, *B. pyogenes*, and *P. levii* conjugated with Fam, Cy5, and Cy3, respectively, and analyzed using confocal laser scanning microscopy (CLSM).

Results: *F. necrophorum* thrived at pH 7, while *B. pyogenes* preferred pH 6. In contrast, *P. levii* adapted well across a broader pH range (pH 6 to 9). *F. necrophorum*, *B. pyogenes*, and *P. levii* influenced the environmental pH of the culture media differently. *F. necrophorum* and *B. pyogenes* created acidic environments, while *P. levii* could alkalinize its surroundings under certain conditions (initial pH 6 and 7). Each bacterium developed unique strategies for energy acquisition in the uterine environment. Notably, *F. necrophorum* displayed strong autoaggregation and coaggregation with the other two species, while *B. pyogenes* and *P. levii* exhibited minimal coaggregation. This coaggregation may contribute to mixed-species infections. *B. pyogenes* and *P. levii* formed single-species biofilms within three days of inoculation, whereas F. necrophorum did not form biofilms efficiently under similar conditions. Consistent results were observed across three different broths: brain heart infusion, Wilkins Chalgren, and chopped meat broth. CLSM imaging of FISH-labeled biofilms cultured in chopped meat broth for five days confirmed these findings, revealing a multispecies biofilm dominated by P. levii, with B. pyogenes and F. necrophorum distributed throughout. The biofilm depth ranged from 30 to 60 μm, illustrating cooperative interactions among the three species.

Conclusions: *F. necrophorum* plays an important role in the interaction and formation of biofilm with *B. pyogenes* and *P. levii*. The presence of all three species results in the most significant coaggregation and biofilm formation, implying a cooperative or synergistic interaction among three species.

Financial Support: This project was supported by Agriculture and Food Research Initiative Competitive Grant: 2021-67015-34420 from the USDA National Institute of Food and Agriculture

Notes:

94 - 3D bovine endometrium model for studying host-pathogen interactions associated with metritis

M.K Panda[1], B Subhadra[1], T Inzana[1], SJ Jeon[1]

[1]Department of Veterinary Biomedical Sciences, Long Island University, Brookville, NY. mrunmaya.panda@liu.edu

Session: Disease pathogenesis 2, 2025-01-20, 9:45 - 10:00

Objective: Metritis is a prevalent inflammatory uterine disease, impacting about 20% of dairy cows, primarily resulting from bacterial infections following calving. Our previous research highlighted a significant increase in *Bacteroides*, *Porphyromonas*, and *Fusobacterium* in the uterine microbiota of cows affected by metritis. However, the specific mechanisms through which these bacteria contribute to the disease remain largely unknown. In this study, we aimed to develop a 3D cell culture model to explore the interactions among these three bacterial species and gain insights into the development of metritis in postpartum dairy cows in vitro.

Methods: To develop a 3D cell culture model, bovine endometrial epithelial (BEND) cells were cultured in Dulbecco's modified eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 10% horse serum (HS). A total of 7.5 x 104 cells were mixed with 17.5% collagen (5 mg/mL) and evenly distributed into the wells of a 24-well plate by adding 125 µL per well, followed by a 15-minute incubation. Once solidified, 300 µL of DMEM per well was added and incubated at 37°C for 24 hours. To prepare the bacterial inoculum, uterine bacteria including *Bacteroides pyogenes* (Bp), *Porphyromonas levii* (Pl), and *Fusobacterium necrophorum* (Fn) were cultured anaerobically on BHI agar plates and then grown overnight in chopped meat broth. The next day, the 3D BEND cells were inoculated with the overnight bacterial cultures, either individually or in combinations, at a multiplicity of infection (MOI) of 10 and incubated for 5 days under anaerobic conditions. After incubation, the 3D BEND cells were rinsed with Dulbecco's phosphate-buffered saline (dPBS), fixed using 2% glutaraldehyde, and stained with Hoechst for imaging analysis with a confocal microscope.

Results: We observed that BEND cells became polarized over time, indicating their progression and maturation within the 3D environment and they remained viable throughout the experiment. BEND cells infected with F. necrophorum formed several clusters, indicating that F. necrphorum could adhere and invade host cells. In contrast, B. pyogenes and P. levii were scattered and showed no clustering around the BEND cells. When all three uterine bacteria were present, larger clusters formed compared to infection with F. necrophorum alone. This suggests that F. necrophorum plays a key role in interacting with both other uterine bacteria and BEND cells. RNA-Seq will be performed to characterize the transcriptomes of both the bacteria and BEND cells in the infection model. DESeq2 (v.1.34.0) will be used for differential expression analysis with significant differences defined as a log2 fold change ≥ 1 and $P < 0.05$.

Conclusions: The establishment of a 3D culture of bovine endometrial cells is a crucial first step toward modeling the complex in vitro microenvironment. This proposed model will ultimately provide a way to study how these anaerobic uterine bacteria interact with the host and how these interactions increase the risk of infections in cows with metritis. We will use this model to investigate host gene expression in response to polymicrobial infections involving these three uterine pathogens, thereby elucidating the underlying mechanisms of metritis.

Financial Support: This project was supported by Agriculture and Food Research Initiative Competitive Grant no. 2021-67015-34420 from the USDA National Institute of Food and Agriculture.

Notes:

95 - *Listeria monocytogenes* infection in the bovine ligated ileal loops

Yuxing Chen[1], Trina Westerman[1], Mariana Paiva Rodrigues[1], Cheta Siletti[1], Zepeng Tu[1], Shreya Kumar[1], Johanna Elfenbein[1], Tu-Anh Huynh[1]

[1]University of Wisconsin Madison. ychen2328@wisc.edu

Session: Bacteriology 1, 2025-01-20, 8:30 - 8:45

Objective: *Listeria monocytogenes* is an invasive pathogen in humans and more than forty animal species, including ruminants. Although acquired orally, L. monocytogenes can breach the intestinal barrier to systemically infect multiple organs, causing highly lethal clinical listeriosis in cattle of all ages. The gastrointestinal (GI) phase of *L. monocytogenes* infection precedes systemic spread, and is a determinant of listeriosis outcome. Very little is known about *L. monocytogenes* pathogenesis in the bovine GI tract, and such knowledge is greatly hampered by the lack of a suitable bovine infection model. To address this knowledge gap, we developed a bovine intestinal infection model for L. monocytogenes, using calf ligated ileal loops. The objectives of this project are to determine *L. monocytogenes* replication and invasion kinetics, and perform preliminary assessments of mucosal immunity against *L. monocytogenes* infection.

Methods: Neonatal calves at 3 - 6 weeks of age were placed under general anaesthesia, and abdominal surgeries were performed to ligate the ileum into ~ 38 loops of ~ 5 cm long, with spacers between adjacent loops. The loops were injected either with phosphate-buffered saline (PBS) containing *L. monocytogenes* or PBS alone as mock controls. The ileum was returned to the abdomen and the calf maintained under general anesthesia. Loops were harvested at 5 and 8 hours post infection (hpi) for L. monocytogenes quantification, histopathology, and RNAseq.

Results: At an inoculum dose of $5x10^7$ cfu per loop, *L. monocytogenes* strain 10403S consistently infected the lumen and intestinal tissue in all of the six animals in our study. Luminal burdens were rather variable, with medians of ~103 cfu/g at 5 hpi and 106 cfu/g at 8 hpi. Intestinal tissue burdens were rather consistent, with medians of 102 cfu/g at 5 hpi and 5 x 103 cfu/g at 8 hpi. Increasing inoculum dose to 108 cfu per loop did not increase *L. monocytogenes* burdens. Interestingly, a *L. monocytogenes* mutant that is defective for general stress response (Sigma B) did not exhibit an infection defect. Preliminary histopathology revealed no overt mucosal inflammation in response to L. monocytogenes infection. RNAseq is in progress to quantify bovine gene expression at the infection sites.

Conclusions: These data suggest that: i) *L. monocytogenes* can replicate in the bovine GI tract within 8 hours of infection, ii) Although tissue invasion is consistent, the vast majority of *L. monocytogenes* remains in the intestinal lumen. There appear to be “upper limits” for *L. monocytogenes* burdens in the calf intestines, which are approximately 108 cfu/g of luminal contents and 105 cfu/g intestinal tissue. Despite such high burdens, *L. monocytogenes* does not elicit visible tissue inflammation at this early stage of infection (8 hpi). The general stress response factor, Sigma B, which is important for many GI tract-relevant stress conditions in vitro, is dispensable for *L. monocytogenes* colonization of the cattle intestines. Our infection model will be used to interrogate the genetic determinants of *L. monocytogenes* colonization in the bovine GI tract.

Financial Support: Funding was provided by USDA NIFA project WIS05035

Notes:

96 - Exploring biofilm formation in *Mycoplasma ovipneumoniae*: attachment, nuclease activity, and disaggregation

B. Tegner Jacobson[1], Eli T. Selong[1], Jessica DeWit[1], Noah Adams[1], Sobha Sonar[1], McKenna Quirk[1], Diane Bimczok[1], Erika R. Schwarz[2]

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

Session: Bacteriology 1, 2025-01-20, 8:45 - 9:00

Objective: *Mycoplasma ovipneumoniae* (*M. ovi*) is a facultative respiratory pathogen associated with atypical pneumonia in domestic sheep. Recent work has shown that *M. ovi* can form biofilms in vitro, and extracellular DNA (eDNA) produced by many biofilms is a key component of adherence and immune resistance. The objectives of this study were to determine (1) whether biofilm formation is associated with altered nuclease activity in *M. ovi* biofilms compared to planktonic bacteria and (2) whether exogenous nuclease is effective in disaggregating mature *M. ovi* biofilms.

Methods: Biofilm formation by *M. ovi* was tested using a reference strain (Y98, ATCC 29419) that was cultured on glass-bottom 96-well plates (12 replicate cultures). Biofilms were compared to planktonic cells grown on 96-well polystyrene plates. Whole cell nuclease activity was assessed by observing the digestion of phage DNA on an agarose gel (2 replicates/treatment). We also exposed M. ovi to exogenous nuclease to see whether this would prevent the adherence of a new biofilm on the glass surface, or whether exogenous nuclease could mediate disaggregation of established biofilms (12 replicates/treatment).

Results: The M. ovi biofilms showed a peak in maturity at 3 days of incubation, with a confluence of 38.97 ± 4.03% on the glass-bottom plate. These biofilms showed increased levels of whole-cell nuclease activity with degradation of the added phage DNA seen on the agarose gel, with a visible DNA smear. In contrast, the planktonic cells showed a lower nuclease activity than the biofilms, with no visible DNA degradation on the agarose gel. Treatment of the planktonic cells with exogenous nuclease significantly (Student's t-test, $p < 0.001$) decreased the ability of *M. ovi* to adhere to the plates and form new biofilms, with a 15.0 ± 7.8% reduction in biofilm confluence at 3 days of incubation. Conversely, we observed a significant 3.4 ± 2.9% increase (Student's t-test, $p < 0.05$) in biofilm confluence when the mature biofilms were treated with the nuclease.

Conclusions: Our data suggest that eDNA is an important component of *M. ovi* biofilm adherence, with reduced biofilm confluence observed when the cells were treated with exogenous nuclease before adherence. Whole cell nuclease activity was increased in the biofilms, which might be due to the inability of *M. ovi* to synthesize nucleic acid. Scavenging of environmental nucleic acids might allow the organism to recycle the environmental DNA in a controlled manner to contribute to continued growth and reshaping of the biofilm. Further supporting this hypothesis is the increase in confluence observed when treating the mature biofilms with the exogenous nuclease. This was surprising, but previous work with other species has observed instances where the addition of nucleases can act to modulate biofilm formation.

Financial Support: Funding for this research was provided by the USDA award 2022-67016-36503, by USDA-NIFA award 2023-67011-40356 (to BTJ), by a MT INBRE Undergraduate Scholar Award (to ES), and the Montana Agricultural Experiment Station.

Notes:

97 - Molecular characterization of uncommon historical isolates of *Salmonella*

Nigatu A. Atlaw[1], Jessica Hicks[1], Patrick Camp[1], Tonya Mackie[1], Edward Palmer[1], Dana Ludwick[1], Matt Eiben[1], Karthik Shanmuganatham[1], Brenda Morningstar-Shaw[1]

[1]USDA-APHIS-NVSL. Nigatu.Atlaw@usda.gov

Session: Bacteriology 1, 2025-01-20, 9:00 - 9:15

Objective: Multidrug-resistant *Salmonella* can be a challenge for treatment in infected people and animals. The National Veterinary Services Laboratories (NVSL) receives *Salmonella* isolates from multiple sources including domestic animals, wildlife, environmental samples, and food/feed items. The objective of this study was to characterize uncommon historical isolates of *Salmonella* kept at the Bacterial Typing Section (BTYP) of the NVSL. These isolates were accumulated over a period of about 50 years, 1967 to 2017.

Methods: A total of 457 *Salmonella* were analyzed in this study. The isolates were serotyped using classical serotyping complimented with the xMAP *Salmonella* Serotyping Assay for detection of 'O' and 'H' antigens. Of these, 85 isolates were selected for whole-genome sequencing using the Illumina MiSeq platform. The WGS data was analyzed for common antimicrobial resistance (AMR) determinants (AMR genes, plasmids, and AMR-associated point mutations) and virulence factors using the AMRFinder and ABRicate with the virulence factor database. Mobile genetic elements and *Salmonella* Pathogenicity Islands were obtained from in silico databases on the Center for Genomic Epidemiology. WGS-based serovars were predicted using Seqsero2, and a kSNP phylogenetic tree was used to compare relative relationships.

Results: No AMR genes were found in 81.2% of the isolates. Sixteen isolates (18.8%) were predicted to be resistant to at least one antimicrobial. Of these, three *Salmonella* isolates were predicted to be multi-drug resistant (resistant to three or more classes of antimicrobials). Interestingly, FosA7 gene was detected in eight isolates, three of which were recovered in the 1990's. Predicted resistance to phenicols, tetracyclines, beta-lactams, quinolones, trimethoprim, and aminoglycosides was detected in three to five isolates. Fifty-four different types of mobile genetic elements were detected. Fifty of the isolates had at least one plasmid commonly associated with AMR genes including IncFIB, IncFII, IncI, Col_types, IncHI_types, IncA/C, and IncX. Sixteen different types of *Salmonella* Pathogenic Islands were detected; and SPI-1, SPI-2, SPI-3, SPI-5, and SPI-9 were detected in all sequenced isolates. At least 88 virulence genes were detected per genome of the isolates.

Conclusions: These rarely detected and historical *Salmonella* isolates possess important AMR determinants, and the detection of key virulence factors indicates their pathogenic potential.

Financial Support: USDA/APHIS - The National Veterinary Services Laboratories (NVSL)

Notes:

98 - Identification of virulence genes in *Salmonella* isolates from cattle.

Ethan P. Dudley[1], Sal Lamsal[1], Paul S. Morley[1], Matthew A. Scott[1], Lee J. Pinnell[1], Brandy A. Burgess[2], Robert Valeris-Chacin[1]

[1]VERO program, Texas A&M University, [2]University of Georgia. edudley01@exchange.tamu.edu

Session: Bacteriology 1, 2025-01-20, 9:15 - 9:30

Objective: *Salmonella* enterica subsp. enterica is one of the leading causes of foodborne illness in the United States (U.S.). Beef products account for a relatively small proportion of cases, but increased interest among U.S federal agencies in reducing salmonellosis has highlighted the lack of information regarding the genetic determinants of virulence among *Salmonella* circulating in cattle. Therefore, our objective was to characterize the virulence genes present in clinical *Salmonella* isolates from cattle.

Methods: Three-hundred *Salmonella* isolates from the Colorado State University's James L. Voss Veterinary Teaching Hospital collected during a cattle health surveillance program between 2002 and 2012 were received at the VERO research laboratory. Genomic DNA was extracted using the DNeasy Ultraclean Microbial Kit (Qiagen, Hilden, Germany) and whole-genome sequencing was performed using the native barcoding kit v14, R10.4.1 flow cells, and the MinION Mk1C sequencer (Oxford Nanopore Technologies, Oxford, United Kingdom). Long-read sequence quality was assessed using LongQC. Genome assemblies were derived using Flye and polished by Medaka. Genomic DNA from each isolate was also sent to the North Texas Genome Center at the University of Texas at Arlington for short read sequencing. Short read sequence quality was assessed using FastQC and MultiQC and low-quality reads were trimmed using Trimmomatic. Pypolca was used to further correct the Medaka-polished assemblies using the short reads. Prokka was used to generate annotated genome assemblies, which were used as input for a pan-genome analysis using Roary. The gene presence-absence matrix from Roary was used to create a phylogenetic tree visualized in Interactive Tree Of Life. ABRicate was employed to search the assemblies for virulence genes using the virulence factor database (VFDB). Scoary was utilized to detect associations between virulence genes and the metadata using a Fisher's exact test. The false discovery rate was controlled with the Benjamini-Hochberg method according to a p-value cutoff of 0.2 and adjusted for the population structure.

Results: The most prevalent *Salmonella* serotypes were Montevideo (n=43, 14.33%), Muenster (n=40, 13.33%), and Newport (n=36, 12%). In this study, *Salmonella* isolates harbored 148.3 virulence genes on average, with most genes (n=137) present in at least 80% of the isolates. We detected 26 virulence genes that occur in less than 20% of the isolates and code for metabolic factors, effector delivery systems, toxins, and proteins involved in adhesion and stress survival. A set of virulence genes were significantly associated with increased fitness to induce diarrhea and fever in older animals. These genes were more prevalent in isolates collected between 2002 and 2007, suggesting a possible temporal trend. Interestingly, a different set of genes encoding exotoxins was found to be significantly associated with milder cases in younger cattle.

Conclusions: Our results show high diversity of *Salmonella* virulence genes and associations between different sets of virulence genes depending on the age of the host. Differential carriage of virulence genes may reflect further adaptations to the host or other stressors related to the environment and husbandry. More research is needed to understand how these less common virulence genes contribute to *Salmonella* fitness in cattle.

Financial Support: Research coordinated by the National Cattlemen's Beef Association, a contractor to the Beef Checkoff

Notes:

99 - Unraveling the antibiotic-dependent evolutionary changes in globally emerging multidrug-resistant *Salmonella*

Samuel Ajulo[1], Smriti Shringi[1], Devendra H. Shah[1]

[1]Texas Tech University School of Veterinary Medicine. sajulo@ttu.edu

Session: Bacteriology 1, 2025-01-20, 9:30 - 9:45

Objective: The evolution of antimicrobial resistance (AMR) and multidrug resistance (MDR) in bacterial pathogens depends on the balance between the fitness advantages and costs imposed by AMR traits. Most laboratory-based studies on AMR have focused on the effects of single antibiotics, primarily using *E. coli* as models. However, the differential impact of various classes of antibiotics on the evolution of AMR, especially in clinically relevant bacterial strains, remains unclear. This study aims to investigate how different antibiotic classes influence AMR evolution and uncover the molecular mechanisms driving this process. The study uses the clinically relevant, multi-drug resistant (MDR) *Salmonella* Kentucky ST198 strain PU131 (MDR-SK198).

Methods: Duplicate clonal evolutionary lineages (CELs) of MDR-SK198 were passaged for over 4000 generations (265 days) with or without exposure to five antibiotic classes: amoxicillin, chloramphenicol, ciprofloxacin, sulfamethoxazole-trimethoprim, and tetracycline, in intestinal-mimicking media. Evolved lineages under ciprofloxacin, sulfamethoxazole-trimethoprim, and non-antibiotic conditions were selected (representative CELs for different trajectories) to determine genotype-phenotype associations and the impact on bacterial fitness in different metabolic and stressor conditions. Genotypic changes of evolved CELs (deletions and SNPs) including AMR, were identified through comparative genomic analysis followed by functional classification using NCBI BLAST and STRING databases using strain PU131 as a reference (NZ_CP026327). The phenotypic changes of evolved CELs were assessed via AMR MIC, growth assays, biofilm production, motility assays, and phenotype microarray (PM) analysis (380 phenotypes under metabolic, oxidative, and pH stress conditions).

Results: The AMR phenotype and genotype of evolved CELs showed an antibiotic-dependent evolution of CELs with three distinct evolutionary trajectories after a 265-day passage. Under ciprofloxacin (CIP) and streptomycin (STR) pressure, there was accelerated decay of non-CIP and non-STR AMR traits in respective CELs. However, sulfamethoxazole-trimethoprim (STrim), amoxicillin (AMOX), and tetracycline (Tet) pressure maintained their respective non-STrim, non-Amox, and non-Tet AMR traits. Interestingly, the no-antibiotic (NOABX) passage displayed varied AMR persistence. The non-AMR genetic changes mirrored the AMR trajectories. The comparative genomic analysis identified 157 gene deletions and 40 single nucleotide polymorphisms (SNPs) across three selected lineages with the most changes in the CIP lineage followed by NOABX and STRIM. Functional enrichment analysis revealed that most (>70%) of the genetic changes in CIP lineages likely impacted bacterial fitness, including virulence (e.g., SPI-3), metabolism, and stress responses. Preliminary PM microarray analysis (RA >/< 50 compared to WT) revealed several phenotypic changes (CIPL1 (n=13), CIPL2 (n=33), STRIML1 (n=15), STRIM L2 (n=21), NOABXL1-TetS (14), NOABXL2-TetS (44), and NOABXL2-TetR (9) compared to wild type. The growth assay, biofilm, and motility assays also showed differences in a lineage-specific manner.

Conclusions: Our study demonstrates antibiotic pressure-dependent evolution of AMR traits, along with non-AMR genetic and phenotypic changes in bacterial fitness. We are evaluating additional phenotypic changes in CELs. These findings offer insights into the antibiotic-dependent evolution of AMR and improve our understanding of bacterial fitness and AMR evolution in the emerging MDR-SK198 pathogen. This knowledge may aid in developing strategies to combat MDR bacterial pathogens and guide future AMR studies.

Financial Support: Texas Tech University School of Veterinary Medicine

Notes:

100 - The development of an axenic medium to support *Anaplasma marginale* metabolic activity and replication

Shelby M. Jarvis[1, 2], Susan M. Noh[1, 2, 3], Anders Omsland[2]

[1]Animal Disease Research Unit, USDA-Agriculture Research Service, [2]Department of Veterinary Microbiology and Pathology, Washington State University, [3]Paul G. Allen School for Global Health, Washington State University. Shelby.jarvis@usda.gov

Session: Bacteriology 1, 2025-01-20, 9:45 - 10:00

Objective: Bovine anaplasmosis, caused by *Anaplasma marginale*, is widespread in the United States and estimated to cost approximately $660 per head of cattle. A licensed, efficacious, low-cost vaccine is not currently available. Native outer membrane proteins isolated from infected bovine erythrocytes provide protection from clinical disease. However, because *A. marginale* is an obligate intracellular bacterium, our ability to culture the organism and thus produce large quantities of native outer membrane proteins for use in vaccines is expensive and requires cultured tick cells to support *A. marginale* growth. To address this limitation, our goal is to develop axenic media, a culture that supports *A. marginale* replication in the absence of host cells, to enable cost-effective production of immunogens for vaccine development.

Methods: Following isolation from tick cells, we measured the metabolic activity *of A. marginale* by the uptake and incorporation of [35S] cysteine-methionine into bacterial proteins in response to various medium components at 24 hours. Intracellular Phosphate Buffer (IPB), supplemented with amino acids and fetal bovine serum (FBS) was used as the basal medium. Scintillation counting was used for quantitative analysis and phosphor imaging to verify incorporation of the radiolabel into de novo synthesized proteins. One-way ANOVA was used to determine statistically significant differences between treatments.

Results: Pyruvate enhanced protein production of *A. marginale* by 2.5-fold increase and with the addition of iron a 3.7-fold increase ($P < 0.0001$) compared to the basal medium. When normalized to pyruvate, iron has a 1.37-fold increase ($P < 0.01$). Other carbon sources including succinate, glucose, glucose-6-phosphate, and UDP glucose did not enhance ($P > 0.05$) A. marginale metabolic activity. Phosphor imaging confirms production of a broad array of *A. marginale* proteins.

Conclusions: Established *A. marginale* axenic activity serves as the foundation for continuous improvement of the medium, in which combinatorial testing of vitamins, minerals and other carbon sources are likely required to maximize the metabolic activity of *A. marginale* to achieve replication. If achieved, this medium will allow production of native proteins for vaccine development. Additionally, this work will provide valuable insights into the metabolic requirements of *A. marginale* and may help identify additional targets for treatment or prevention of bovine anaplasmosis.

Financial Support: This research is fully funded by the USDA-ARS CRIS #2090-32000-043.

Notes:

101 - Emergence and spread of viruses in new hosts - the examples of canine parvovirus and H3N8 canine influenza virus

Colin R. Parrish[1]

[1]Department of Microbiology and Immunology, Cornell University. crp3@cornell.edu

Session: AAVI - Featured Speakers, 2025-01-20, 10:30 - 11:15

Viruses are generally restricted in their host ranges, and are only rarely able to transfer to a new host to cause outbreaks or epidemics. However, those that do emerge in humans or other animals can cause extensive disease and death. Here I describe and compare two viruses that have emerged to cause epidemics in animals where we can directly analyze and compare the newly emerged virus with its ancestor in the original hosts.Canine Parvovirus. In 1978 a new disease caused by a parvovirus (canine parvovirus - CPV) was observed in dogs, and that virus was recognized as being closely related to the long-known feline panleukopenia virus (FPV). The CPV and FPV have continued to circulate in dogs and cats, respectively, and we have been able to follow and understand their spread and evolution during the subsequent 46 years. The canine host range of CPV involved a number of mutations that mostly fell within the capsid protein gene, and resulted in changes in key sites on the capsid surface. We showed that the receptor on host cells for the FPV and CPV capsids were the transferrin receptor type-1, and differences in the receptor - in particular a new glycosylation site within the apical domain - controlled the differences in susceptibility of cats and dogs to the two viruses. The structures of complexes between the capsids and the transferrin receptor show that the binding site contains most of the host range controlling mutations, and also overlaps the main antigenic epitopes recognized by host antibodies.Canine Influenza Virus (H3N8). Another example of an emerging virus is the H3N8 canine influenza virus, which arose around 1999 when an H3N8 equine influenza virus transferred to dogs and caused an epidemic. The virus spread widely among dogs in the USA until it died out around August 2016. The equine influenza virus continues to circulate among horses, mainly in the USA, and it also spreads to other regions of the world to cause more outbreaks. We examine the epidemiology and evolution of the equine and canine viruses as they circulate separately in their different host populations. There are different patterns of variation in each host, with limited spread of the virus in dogs compared to the global spread of the virus in horses.

Notes:

102 - Perspectives on vaccination as an approach for control of highly pathogenic avian influenza viruses

Shayan Sharif[1]

1Department of Pathobiology, University of Guelph, Canada. shayan@uoguelph.ca

Session: AAVI - Featured Speakers, 2025-01-20, 11:15 - 12:00

Highly pathogenic avian influenza (HPAI) virus of H5N1 subtype (clade 2.3.4.4b) has been circulating in North America since 2021, first among wild birds and, subsequently among domestic poultry. More recently, the virus has shown unprecedented transmission to various species of domestic and wild mammals, including dairy cows. Additionally, spillover to humans has been documented, resulting in mild to severe clinical disease. Given the wide range of species that can become infected with H5N1 clade 2.3.4.4b, it is now critical more than ever to devise and implement ways for control of this virus among domestic animals, especially among poultry. Vaccination is an effective method for reducing the impact of HPAI on poultry and the possibility of transmission between poultry and also between poultry and other animals. Despite the promising innovations in vaccination strategies, the use of vaccines against HPAI has been limited, due to some of their inherent shortcomings. More specifically, vaccines need to be cost-effective and be easy to manufacture and deploy. Moreover, they must not only prevent or mitigate clinical disease but also significantly reduce viral transmission. Over the past two decades, significant progress has been made toward developing vaccines that fulfill these criteria. In this talk, I will provide an overview of avian influenza vaccines, where we are now and where might be heading, in terms of vaccine development and application.

Notes:

104 - A survey of emergency preparedness and disaster response in animal shelters located in the United States of America

Amber T Cleggett[1], Amie Hufton[1], Jeanette O'Quin[1], Andréia G. Arruda[1]

[1]Ohio State University. cleggett.1@osu.edu

Session: One health / Public health 1, 2025-01-20, 10:45 - 11:00

Objective: To describe emergency preparedness and disaster response plans and activities in animal shelters across the United States and to identify minimum requirements shelters believe should be in an effective plan for animal shelters interested in creating a or updating their emergency preparedness and disaster response plans utilizing data obtained through the literature review and this project's survey.

Methods: A Qualtrics survey was developed and distributed through fifteen target list serves and one social media site to animal shelter personnel from December 4, 2023 to February 15, 2024. Survey questions covered demographic information and the shelters' understanding of emergency preparedness and disaster response, the presence of an emergency preparedness/disaster response plan and its contents, and how that plan is practiced outside of emergencies. The survey was considered exempt by the Ohio State University Institutional Review Board (Protocol Number: 2023E1182).

Results: Fifty-seven shelters consented to the survey, but only twenty-eight responded to any of the survey questions. Two responses were discarded since they failed to answer any vital survey questions. Twenty-six respondents from 18 states represented animal shelters of varying sizes and types. Our results showed that at least 21 shelters across the United States (N=25; 84% of respondents) have a plan that protects humans in their shelter, 18 (N=24; 75% of respondents) reported having a plan that protects the animals in their shelter, and 14 shelters reported having a plan that covers both humans and animals. Thirteen (N=18; 72%) shelters with a plan covering humans or animals held trainings regarding their plan, 7 (N=18; 39%) have used discussion-based scenarios, 6 (N=18; 33%) have run drills, 1 (N=18; 6%) had an actual emergency or evacuation at least once a year, and 1(N=18; 6%) shelter reported no trainings, exercises, or incidents that have tested their plans. Based on data from the survey and themes identified from the literature, the proposed minimum requirements for an effective emergency preparedness and disaster response plan are hazard identification and analysis, emergency contacts/partnerships, communication plan, resource management, evacuation/temporary sheltering, and training.

Conclusions: Animal shelters provide a myriad of vital services to their communities, and it is imperative that they continue to do so during crises, while simultaneously limiting any public health concerns they may pose through adequate response plans. To our knowledge, this is the most recent attempt in the past decade to gather information regarding emergency preparedness and disaster response activities in animal shelters, and the first of its kind at this scale, seeking information from shelters across the United States. Though participation was low, the survey provided insight on how shelters define "emergency preparedness" and "disaster response", provided baseline statistics regarding how many shelters have an emergency preparedness and disaster response plan (and who that plan covers), while allowing themes for the minimum requirements of an effective emergency preparedness and disaster response plan to be identified (and possibly simplifying the creation of these plans). The results of this research identified the need for additional resources to assist animal shelters in preparing disaster response plans.

Financial Support: This research was not sponsored.

Notes:

105 - Are antimicrobial resistance genes transferred to consumers through beef consumption?

Molly McClurg[1], Enrique Doster[1], Cory A. Wolfe[1], Lee J. Pinnell[1], Noelle R. Noyes[2], Tara Gaire[2], Keri Norman[1], Emily Rice[3], Adam Byers[4], Eric Davis[5], Harrison M. Cottingham[4], Mahesh Nair[5], Keith E. Belk[3], Wayne W. Campbell[4], Paul S. Morley[1]

[1]VERO Program, Texas A&M University, [2]University of Minnesota, [3]Colorado State University, [4]Purdue University, [5]Marian University. mcclurgmc@tamu.edu

Session: One health / Public health 1, 2025-01-20, 11:00 - 11:15

Objective: Despite consumer concerns about animal production practices, research is not available about whether antimicrobial drugs (AMDs) used in cattle promote antimicrobial resistance (AMR) in bacteria that are transferred to humans via the food chain. A nutritional trial was performed in human subjects to investigate differences in the fecal resistome and microbiome when people eat beef from cattle raised conventionally, including prudent use of AMDs, vs "raised without antibiotics" (RWA) systems. The potential transmission of AMR from beef sources into human consumers was evaluated using multiple analyses, including SourceTracker models, to assess the source of AMR transmission.

Methods: Twenty-six human participants were enrolled in a masked, randomized, controlled cross-over trial. The diets provided were identical in the two treatment arms except for the beef source (conventional or RWA production). Fecal samples were collected throughout the 16-week trial period, and meat rinsates were collected weekly throughout the 32-week study. Target-enriched shotgun sequencing was used to characterize AMR genes, and 16S rRNA gene sequencing was used to characterize microbial community structures. SourceTracker was used to assess the potential transmission of microbial amplicon sequence variants and AMR gene variants from beef sources into human consumers through the trial.

Results: Resistome and microbial community structures in human feces were richer and more diverse than those of beef products, as expected. Shifts in microbial community structures over time were evident but not statistically significant. The resistome analysis found Tetracycline resistance to contribute to the majority (~74%) of the resistance profile of beef and fecal samples, with fecal samples having more profile diversity than beef samples. The SourceTracker analysis showed that a majority of suggested sequence variants were found in participants' feces before consuming beef products. A significant proportion of ARG sequence variants identified in participant's feces after trial initiation were not found in baseline fecal samples or in beef products, suggesting that low abundance sequence variants may have been common, resulting in intermittent detection in after trial initiation, and should be considered interpretation of putative transmission from beef. Only a small portion of sequence variants (≤5%) were identified in beef products before identification in participants' feces.

Conclusions: This study is among the first to assess the risk of AMR transfer to consumers through beef products. These findings suggest that beef products are not a significant source of AMR genes transferred to consumers.

Financial Support: Research supported by the Beef Checkoff

Notes:

106 - An Investigation into the suitability of household pets as sentinels for human exposure to Per-and Polyfluoroalkyl Substances (PFAS)

Heather D. Brake[1], Melinda J. Wilkins[2], John B. Kaneene[1]

[1]Michigan State University College of Veterinary Medicine, [2]University of Minnesota, College of Veterinary Medicine. bairheat@msu.edu

Session: One health / Public health 1, 2025-01-20, 11:15 - 11:30

Objective: Pets, particularly household dogs and cats may serve as valuable sentinels for human exposure to per-and-polyfluoroalkyl substances (PFAS) in residential environments. Given the proximity of young children and household pets to PFAS-treated surfaces like carpets and floors and the tendency of children and pets to engage in mouthing behaviors, both are at similar risk of exposure. An ongoing study of dogs in West Michigan is being conducted to evaluate the biomarkers of exposure and biological effect, the most likely sources of exposure in the household, and the similarities to human PFAS test results from the same catchment area. The specific goal of our ongoing study is to assess the suitability of dogs as sentinels for PFAS contamination in homes. Our central hypothesis is that household dogs are reliable indicators of human PFAS contamination because they share the same environmental exposures and show similar alterations in biomarkers of exposure and biological effects.

Methods: The cities of Belmont, Rockford, and Parchment, MI were selected as catchment areas because of historical contamination of PFAS in private wells and public water systems in these areas. Whole blood was collected from dogs and cats while samples of pet food, pet bowl water, and dust were collected from each household. The samples were sent to both human and veterinary accredited diagnostic laboratories for analysis. PFAS concentrations in all substrates were analyzed by an EPA approved solid-phase extraction (SPE) liquid chromatography/tandem mass spectrometry method for 40 different species of PFAS. Standard blood chemistry analysis and thyroid panels were conducted on pet blood.

Results: Our results to date indicate that PFAS is present in all of the pets sampled, most of the household dust, and only a small proportion of the water collected from pet bowls. When serum PFAS concentrations of dogs were compared to human data provided by the Michigan Department of Health and Human Services and to PFAS concentrations in household dust samples, the measure of relationship was stronger between pets and people than between either pets and dust or people and dust. In terms of blood chemistry, we found that the presence of certain species of PFAS were significantly associated with changes in blood chemistry and electrolytes.

Conclusions: Preliminary results suggest that pets do share similar blood PFAS concentrations as humans in the same catchment and changes in blood chemistry in exposed pets may be able to indicate exposure. Details of the study, including likely household exposures will be discussed.

Financial Support: This study is supported by the Michigan State University, College of Veterinary Medicine, Endowed Research Fund and through a grant provided by the Michigan Animal Health Foundation.

Notes:

107 - Genetic relatedness of beta-lactamase-producing *Escherichia coli* from different One Health sources.

Yamima Tasnim[1], Md. Kaisar Rahman[1], Babafela Awosile[1]

[1]Texas Tech University School of Veterinary Medicine. ytasnim@ttu.edu

Session: One health / Public health 1, 2025-01-20, 11:30 - 11:45

Objective: Multidrug-resistant *Escherichia coli* isolated from food animals, pet animals, and environments are considered a potential threat to human, animal, and environmental health. In recent years, there have been many significant reports of the spreading of resistance and bacterial potential to transmit mobile genetic elements that give resistance to many drugs. We aimed to determine the genetic relatedness among beta-lactamase-producing *Escherichia coli* isolated from soil, lake, and feces of geese, pigs, coyotes, wild hogs, and horses at one health interface in West Texas.

Methods: 43 *E. coli* isolates were included in this study, previously isolated using ESBL chromogenic agar. These isolates were previously recovered from the feces of geese (n=13), pigs (n=9), horses (n=10), coyotes (n=4), and wild hogs (n=1), as well as from environmental samples (soil (n=5) and water (n=1) from recreational parks) from different locations in West Texas. Genetic relatedness between the isolates was determined using whole-genome sequencing (WGS), core genome multilocus sequence typing (cgMLST), and phylogenetic single nucleotide polymorphism (SNPs) analysis using the publicly available bioinformatic platforms.

Results: All the 43 *E. coli* isolates were carriers of beta-lactamase genes including blaCTX-M-1 (n=11), blaCTX-M-55 (n=10), blaCTX-M-15 (n=7), blaCTX-M-65 (n=5), blaCTX-M-27 (n=3), blaCTX-M-32 (n=3), blaCTX-M-14 (n=1), blaCMY-2 (n=2), and blaSHV-2 (n=1). Among the 43 isolates, the predominant phylogroups were B1 (n=19), and A (n= 14). Among the various serogroups identified, O101:H9 (n=5) was mostly found in isolates from pigs. We found 33 different sequence types (STs) with ST10 (n=5) mostly found in isolates from pigs. Based on cgMLST, 34 clonal lineages were identified. By using 100 maximum number of SNPs between the isolates to define a clone, 13 clones were identified in 17 isolates (8 clones with 1-20 SNPs difference and 5 clones with 21-100 SNPs difference). Among the 17 isolates belonging to the 13 clones, 6 clones were from pig isolates (n=6), 3 clones from horse isolates (n=6), 3 clones from isolates from geese and soil samples from the recreational parks (n=3), and 1 clone from two *E. coli* isolates derived from a coyote and a lake sample from a recreational park. The clonally related *E. coli* isolates were clustered based on the phylogenetic tree.

Conclusions: The findings from this study provide limited evidence of clonal dissemination of beta-lactamase-producing *E. coli* between different animal sources.

Notes:

108 - Close genetic relatedness of carbapenem-resistant Enterobacterales from companion animals and humans.

Allison E. James[1], Lingzi Xiaoli[1], Anna Stahl[2], Stephen D. Cole[3], Maho Okumura[3], Jaclyn M. Dietrich[3], Molly M. Lapeer[1], Jordan K. Putney[1], Maroya S. Walters[2], Richard A. Stanton[2]

[1]Division of Foodborne, Waterborne, and Environmental Diseases, Centers for Disease Control and Prevention, [2]Division of Healthcare Quality Promotion, Centers for Disease Control and Prevention, [3]School of Veterinary Medicine, University of Pennsylvania. hwj7@cdc.gov

Session: One health / Public health 1, 2025-01-20, 11:45 - 12:00

Objective: Carbapenemase-producing carbapenem resistant Enterobacterales (CP-CRE) are urgent public health threats because they cause difficult-to-treat infections associated with high mortality in humans. CP-CRE are increasingly being reported in dogs and cats (i.e., companion animals) in the U.S. A few reports from Europe provide evidence for isolated CP-CRE transmission events between humans and animals, but the zoonotic risk is unknown. To understand whether strains of CP-CRE from companion animals could pose a risk to human health, we analyzed publicly available whole genome sequence data for CP-CRE isolated from companion animals and compared their relatedness to those isolated from humans.

Methods: The National Center for Biotechnology Information (NCBI) Pathogen Detection database was queried to identify clusters of genetically closely related CP-CRE from U.S. companion animals and humans. Phylogenetic relatedness of clustered isolates was evaluated using core genome multi-locus sequence typing (cgMLST). Metadata from NCBI and de-identified patient data from CDC's Multi-drug Resistant Organism Database and the University of Pennsylvania's Veterinary Diagnostic Laboratory were analyzed to explore epidemiological connections between genetically similar isolates.

Results: As of Feb 23rd, 2024, 170 CP-CRE sequences from three different bacterial species (*Escherichia coli* (n=111), Klebsiella pneumoniae (n=22), and Enterobacter cloacae (n=37)) were identified from companion animals (160 from dogs and 10 from cats). These included five *E. coli* sequence types (ST162, ST167, ST361, ST410, and ST617), two from K. pneumoniae (ST11 and ST307), and one from Enterobacter cloacae (ST171). Of the 170 companion animal isolates, 169 possessed blaNDM-5 and one possessed blaNDM-7. Four hundred and six closely related human CP-CRE sequences from the U.S. were identified on the same clusters as the animal isolates. Analysis by cgMLST showed that most of the animal-source isolates did not cluster distinctly but were instead dispersed among clusters with human sequences. Nearly 72% (n=122/170) of the companion animal isolates were from animals residing in the northeastern portion of the U.S., and many of these were clustered closely with human isolates from the same region.

Conclusions: The detection of multiple species and strains of genetically related CP-CRE from both humans and companion animals suggests that zoonotic transmission of multiple CP-CRE pathotypes is possible and may already be occurring in the U.S., either through direct contact or common reservoirs. The shared strains identified in this study have been previously recognized as internationally disseminated, high-risk clones in humans, and many have previously been isolated from different animal species and the environment. The presence of the emerging carbapenemase gene family blaNDM in these ecologically diverse strains might increase the potential for amplification of carbapenem resistance across the One Health continuum and therefore warrant additional monitoring and prevention efforts. Lastly, while the geographical clustering of human and animal isolates suggests a foci of undetected community transmission, these results could have been impacted by sampling bias. Further studies are needed to understand the geographic distribution and transmission dynamics of CP-CRE in companion animals.

Notes:

109 - Testing for heavy metals in drinking water collected from Dog Aging Project participants

Courtney Sexton[1], Janice O'Brien[1], Justin Lytle[2], Sam Rodgers[1], Amber Keyser[3], Mandy Kauffman[3], Matt Dunbar[3], Leigh-Anne Krometis[2], Marc Edwards[4], Audrey Ruple[1]

[1]Population Health Sciences, Virginia-Maryland College of Veterinary Medicine, [2]Dept. of Biological Systems Engineering, Virginia Tech, [3]Center for Studies in Demography and Ecology, University of Washington, [4]Civil & Environmental Engineering Department, Virginia Tech. sextonc@vt.edu

Session: Companion animal health 2, 2025-01-20, 10:30 - 10:45

Objective: Access to potable water is essential for animals' survival, yet drinking water source, quality, and availability all vary greatly across species and populations. Most companion animals depend on the people with whom they live for access to drinking water, 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 standardized monitoring, 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 pilot, we analyzed drinking water for dogs participating in the Dog Aging Project (DAP), a large cohort study, who lived in homes with private wells. Our aim was to determine whether heavy metals could be detected by at-home sampling of dogs' drinking water.

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 in the pilot. Dog owners filled out a pre-sampling survey with any known information about their dog's drinking water. They were mailed kits including 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 Thermo Electron X-Series ICP-MS to analyze the samples and determine the presence and concentrations of 28 different elements, including eight heavy metals with EPA-designated maximum contamination levels (MCL) and five metals that have EPA health guidance levels for people. Regression models built in R studio v.4.4.0 using the glm function examined associations between: water source variables as reported by owners in survey and the quantification of each metal (Gaussian); numerical metal value and the total burden of developed disease for each dog (Poisson); water treatment system installed and total burden of developed disease (Poisson).

Results: 178/200 (89%) sample kits sent were returned by dog owners. We found detectable levels of all metals tested. There was wide variability in the types and amounts of metals present in dogs' drinking water. There were 126 instances when an analyte (arsenic, lead, copper, sodium, strontium, nickel, or vanadium) was above the EPA MCL or human health guidance level.

Conclusions: Drinking water toxicity from heavy metals can lead to acute and chronic health conditions including organ failure in dogs and people. Dogs, who have little awareness of the quality or control over their drinking water sources, may be especially susceptible to such risks. At-home testing of dogs' drinking water was successful, and our results prompt further investigation with a larger, stratified sample to analyze water composition and long-term health outcomes for dogs living in diverse geographies. These data may impact veterinary care decisions, and underscore the validity and importance of utilizing dogs as sentinels of human health outcomes in the context of drinking water contamination.

Notes:

110 - How can we achieve more accurate reporting of average dog lifespan?

Courtney Sexton[1], Audrey Ruple[1]

[1]Population Health Sciences, Virginia-Maryland College of Veterinary Medicine. sextonc@vt.edu

Session: Companion animal health 2, 2025-01-20, 10:45 - 11:00

Objective: Despite major advances in our understanding of dogs as a biological system (including genetics/epigenetics, physiology, cognition, and behavior), the veterinary field lacks consensus around a critical piece of information: namely, the average lifespan/life expectancy of a domestic dog. In recent years, some have argued that the dog lifespan is shortening, especially in some specific breeds, and despite the sparseness of evidence for the conjecture, the suggestion has undoubtedly influenced human decision-making regarding care of companion animals. Conversely, improvements in diagnostics and care and overall quality of life for companion animals would point toward increased longevity. Our aim is to review the currently available data to determine recent changes in average dog lifespan.

Methods: In an effort to review historical and current reports of dog lifespan to determine whether the domestic dog's lifespan has changed over time, we found that incongruous data were prohibitive to conducting a formal meta-analysis of dog lifespan reports, and instead we present a brief account of these challenges, along with our qualitative findings.

Results: Contrasting reports regarding dog lifespan are primarily due to significant gaps and inconsistencies in the way that mortality data for household dogs are recorded and reported. Existing life expectancy datasets primarily include geographic- or breed-specific cohorts. Many other studies focus on specific causes of death, risk factors, and/or rates of death in specific breeds. Additionally, it is difficult to compare available data between studies, as they rely on varying sources such as cemetery records, electronic medical records (EMRs) from private practices, breed registries, and insurance records. Different methodological approaches also introduce source-specific biases, and existing datasets may include missing or incorrect data. Keeping these limitations in mind, in our examination of a dozen large-cohort studies on dog aging and mortality covering a span of ~40 years (1981 to 2023), the median lifespan of domesticated dogs has not recently decreased, as has been reported in the popular press, but rather has increased steadily over that time frame.

Conclusions: With limitations considered, available data suggest that the lifespan of the domestic dog has not declined over the last 40 years, nor do we know it to be in a current state of decline. However, dogs' lifespans may be affected more than they have been previously by new and/or different risk factors, especially those related to the shared human-dog exposome. The inconsistencies in historic reporting of mortality in dogs make a fully accurate determination of the average dog lifespan difficult to ascertain and as a result also impact our ability to assess the urgency of responding to such risk factors. We therefore recommend the adoption of a comprehensive, standardized method for reporting and recording dog mortality so that more accurate reporting of dogs' lifespans can be made moving forward.

Notes:

111 - Assessing the application of infrared thermography for measuring unrestrained dog body surface temperature

Sahar Rostami[1], Abigail McBride[1], Kimberly Woodruff[1], David Smith[1]

[1]Department of Pathobiology and Population Medicine, Mississippi State University. sr2116@msstate.edu

Session: Companion animal health 2, 2025-01-20, 11:00 - 11:15

Objective: Infrared thermography (IRT) might provide a more efficient method for detecting disease in animal populations compared to rectal thermography (RT). The objective of this study was to evaluate the performance of IRT for measuring body temperatures of shelter dogs.

Methods: Study 1. We recorded the maximum eye temperature (ET) of 97 unrestrained dogs on 4 separate days, using IRT. Three measures of ET were recorded every 10-minutes, followed by a single RT measurement. Study 2. To assess the effect of time on IRT performance, we used a Styrofoam box, to record the temperature of a specific spot on the inner wall, at 5-minute intervals for 1 hour. Ambient temperature and humidity were recorded in both studies. Data were analyzed using repeated measure linear regression. (Alpha = 0.05).

Results: Study 1: The final model included RT, repeat, and age (adult or pup) as fixed and day as random effects. RT was associated with ET (β=0.62). ET of adults was 0.35 °C less than puppies. Mean ET decreased over each repeated measure (39.29, 38.54, 38.35°C, respectively). Study 2: With each consecutive IRT reading, the temperature dropped by -0.1250 °C.

Conclusions: ET was associated with RT, and puppies had higher ET than adults, adjusting for RT. The systematic decrease in ET readings over time was confirmed in the environmentally controlled study that demonstrated the effect was due to the camera. It is important to recognize that thermal cameras can be a potential source of error in estimating body temperature of dogs.

Financial Support: The research was supported by the USDA ARS Non-Assistance Cooperative Agreement.

Notes:

112 - Environmental exposures and health outcomes differ according to geographic region among Dog Aging Project participants

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

[1]Virginia-Maryland College of Veterinary Medicine. janiceobrien@vt.edu

Session: Companion animal health 2, 2025-01-20, 11:15 - 11:30

Objective: To determine any environmental exposure differences that may vary according to geographic region within the United States to determine whether dogs living in different places are exposed to different environmental exposures, including infectious diseases, traffic noise, household pollutants, outdoor pollutants, and water exposures (both for swimming and drinking).

Methods: Environmental exposures reported by dog owners in volunteer internet surveys were collected from 1/1/2020 to 12/31/2023 as part of the Dog Aging Project's initial enrollment survey. These owner-reported exposures were binned according to census region. Differences between the proportions of the exposures by region were compared using a chi-square test of independence in R studio.

Results: Indoor and outdoor environmental exposures differ across regions in the U.S., and these differences impact both dogs and their owners. In older homes typically found in the northeast, there's a higher likelihood of encountering metal pipes, wood-burning stoves, and materials like asbestos and lead. Newer homes in the South, however, tend to have plastic pipes and central air conditioning, with fewer reports of asbestos and lead. Weather patterns also influence regional differences, with the South experiencing more hot weather and the Midwest and Northeast facing colder months. Despite these variations, dog activity levels remain consistent across regions, suggesting that weather does not significantly impact their energy levels. Instead, dogs may engage in different activities based on weather conditions, or dogs and owners may adjust activity times to suit the temperature. Outdoor exposures for dogs vary significantly by region, including the types of surfaces they encounter and their swimming environments. The South sees more yard pest and weed treatments, exposing dogs to potentially harmful chemicals. Lastly, health diagnoses in dogs, including conditions affecting the mouth and skin and susceptibility to infectious diseases, toxins, and trauma, show regional differences, with certain conditions more prevalent in specific areas.

Conclusions: This geographically diverse cohort of pet dogs demonstrates differences in their exposures to indoor and outdoor toxicants and diseases. Despite the different weather temperatures reported between regions, dogs living in the regions showed no difference in their activity levels. These differences in regions will be helpful for future research examining the effect of these environmental exposures on health outcomes to better inform both dog and human health.

Notes:

113 - Companion animal health surveillance systems: an environmental scan

H. Davies[1], T. Epp[2], A.L. Greer[3], J.S. Weese[4], L. Grant[1]

[1]Department of Population Medicine, University of Guelph, [2]Department of Large Animal Clinical Sciences, University of Saskatchewan, [3]Department of Biology, Trent University, [4]Department of Pathobiology, University of Guelph. hdavie03@uoguelph.ca

Session: Companion animal health 2, 2025-01-20, 11:30 - 11:45

Objective: In Canada, there is no fully comprehensive companion animal health surveillance system for zoonotic diseases which limits our ability to implement risk monitoring and mitigation measures for both human and animal populations. There are several examples of large-scale informatics-based systems globally. To guide the development of a framework outlining how such a system could be developed in Canada, a thorough understanding of existing systems is necessary. Therefore, the objectives of this study were to identify and characterize existing companion animal health surveillance systems and to describe any integration of data relating to humans or the environment.

Methods: We searched academic and grey literature databases to identify companion animal health surveillance systems published in English. These searches were supplemented with targeted internet searching. The system characterization step of the SERVAL framework1 was adapted and used as a data extraction tool. Data relating to the scope of health outcomes and target population under surveillance, objectives, structure, data collection methods and stakeholders was extracted and used to describe the identified systems.

Results: In total, 11,981 unique sources were identified in academic and grey literature. Following title and abstract screening, the full text of 590 sources was screened for inclusion, of which 339 were excluded. For many sources the distinction between research and surveillance was unclear. An analysis of the remaining 251 sources identified 119 national or regional surveillance and control programs, the majority of which pertained to rabies (n=55), dracunculiasis (n=19), echinococcosis (n=18) or leishmaniasis (n=13). In general, the description of these notifiable disease programs did not provide in-depth detail relating to the surveillance portion of the program. Thirty examples of informatics-based companion animal surveillance systems or pilot systems were identified of which 43.3% (n=13) utilize electronic healthcare records and/or diagnostic laboratory results to conduct surveillance, with the remainder based on submission of data from veterinary professionals or other sources. Eleven systems focused solely on either specific or all infectious diseases, whilst others pertained to broader health outcomes, including cause of death (n=2), cancer (n=1) and toxin exposure (n=1). Only 36.6% (n=11) of systems clearly describe the integration of data relating to humans or the environment, of which 45.5% (n=5) of systems describe this integration at a system level, for example through the simultaneous collection of data relating to humans or wildlife, or through the integration of environmental data into forecasting models.

Conclusions: For many of the identified sources the distinction between research programs and surveillance informing action was unclear. In addition, many of the identified systems relate to notifiable diseases and national level, government led rabies control programs. There are few examples of fully integrated One Health informatics-based systems, however, characterization of the data sources and integration methods used by existing systems will inform development of a potential similar system in Ontario, Canada.

Financial Support: This project is funded by the Public Health Agency of Canada (PHAC) Infectious Disease and Climate Change fund (Grant Number 2324-HQ-000026).

Notes:

114 - Diagnoses in dogs with pet health insurance

Aliya McCullough[1], Janice O'Brien[1], Audrey Ruple[1]

[1]Virginia-Maryland Regional College of Veterinary Medicine. aliyam@vt.edu

Session: Companion animal health 2, 2025-01-20, 11:45 - 12:00

Objective: In the United States, only about 4% of pet owners have a pet insurance policy despite the fact that such policies mitigate the financial risk of veterinary expenses. Previous research has found that pet insurance policyholders spend more on medical expenses and wellness plan subscribers visit veterinary hospitals more frequently. Yet, it is not known if dogs with pet insurance have earlier disease diagnoses and improved health outcomes. In this work we compare the total number of diagnoses in dogs with pet insurance to those without.

Methods: Pet owners' responses from the Dog Aging Project (DAP) initial enrollment survey, Health and Life Experience Survey (HLES), and the Annual Follow Up Survey (AFUS) distributed approximately one year apart by the DAP were evaluated for this study. We categorized dogs into the following groups: no pet insurance reported on the HLES or AFUS (None); pet insurance reported on both the HLES and AFUS (Insured); pet insurance reported on HLES but not AFUS (Dropped); no pet insurance reported on HLES but reported on AFUS (Added). Disease diagnoses were quantified for the four categories of dogs after controlling for dog life stage, breed, weight, and sex.

Results: The results indicate that the Insured dog group received more diagnoses than those without pet insurance. The mean of health conditions in each group were: 4.3 (Insured), 3.91 (None), 3.64 (Dropped), 3.24 (Added). Additionally, when controlling for dog demographic variables, the difference between the pet insurance groups was found to be statistically significant ($P<0.0001$).

Conclusions: The higher number of diagnoses in the Insured dog group suggests that their pet owners are opting for diagnostic procedures when recommended by their veterinarian and not declining due to cost. Further investigation about the impact of pet insurance on disease is warranted, including the effect of pet insurance on prognosis and specific medical conditions.

Notes:

115 - Stress primes a proinflammatory respiratory mucosal immune response in beef stocker cattle

Grace Jakes[1], Dylan Ammons[1], Ediane Silva[2], Steven Dow[1, 3], Sarah Raabis[3]

[1]Dept. of Microbiology, Immunology, and Pathology, Colorado State University, [2]USDA-ARS, National Bio and Agro-Defense Facility, [3]Dept. of Clinical Sciences, Colorado State University. grace.jakes@colostate.edu

Session: Bovine Respiratory Disease 1, 2025-01-20, 10:30 - 10:45

Objective: Bovine respiratory disease (BRD) is a leading cause of morbidity and mortality in beef cattle globally. Stress due to transport and weaning is associated with BRD risk, yet the mechanism of immune susceptibility following stress is less clear. The mucosal immune response to BRD is integral in mitigating pathology and interacts with respiratory microbiota to maintain homeostasis. Here we evaluated host-microbe interactions from bronchoalveolar lavage fluid (BALF) with the goal of understanding interactome shifts in response to transport and auction stress.

Methods: Stocker calves were purchased at auction and transported to a commercial backgrounding facility. A subset of calves were sampled for BALF within 24 hours (STRESSED; n=7) or allowed to acclimate to the backgrounding facility for 2-3 months before BALF sampling (ACCLIMATED; n=7). Calves were randomly selected and screened for sampling based on an absence of lung consolidation on lung ultrasound and a rectal temperature <103.5 °F. BAL sampling was performed at the backgrounding facility, and BAL samples were processed for bulk transcriptomic (RNA-seq) and 16S rRNA microbiome sequencing separately. Sequences were processed for RNA-seq using the Partek Flow RNA analysis pipeline, and Qiime2 for the microbial sequences. Gene expression data was analyzed using Cibersortx to deconvolute bulk sequences to predict relative abundance of major cell populations.

Results: RNA-seq profiles of stressed calves demonstrated over 800 differentially expressed genes (DEGs), with 656 being upregulated, and 205 being downregulated. Key pathways upregulated included neutrophil and chemotaxis associated pathways, with an increase in neutrophil numbers predicted in STRESSED calves ($P<0.01$). Additionally, desmosome organization and epithelial differentiation pathways were upregulated in stress, while some antiviral genes including GZMA and IFNG were upregulated in ACCLIMATED calves. Microbiome analysis demonstrated no difference in alpha and beta diversity metrics ($P>0.05$) in spite of the immune activation signatures.

Conclusions: These data demonstrate that healthy stocker calves at arrival have an increase in neutrophil-associated gene expression relative to acclimated calves, even as microbial diversity metrics demonstrate no differences. STRESSED calves in this study were immunomodulated to a different inflammatory profile regardless of microbial diversity in the lower respiratory tract.

Financial Support: Funding for this project was provided by USDA Non-Assistance Cooperative Agreement #: 58-3022-3-02. Research reported was supported by the National Institutes of Health, NIGMS Award number T32GM136628

Notes:

116 - Multiomic investigation in beef cattle characterizes management-associated immune modulation in context of respiratory disease

Bradly Ramirez[1], Hudson McAllister[1], Sarah Capik[2], Robert Valeris-Chacin[1], Kelsey Harvey[3], Amelia Woolums[4], Brandi Karisch[5], Lee Pinnell[1], Paul S. Morley[1], Matthew Scott[1]

[1]VERO Program, Texas A&M University, [2]Tumbleweed Veterinary Services, [3]Prairie Research Unit, Mississippi State University, [4]Department of Pathobiology and Population Medicine, Mississippi State University, [5]Department of Animal and Dairy Sciences, Mississippi State University. ramirez_b@tamu.edu

Session: Bovine Respiratory Disease 1, 2025-01-20, 10:45 - 11:00

Objective: Bovine respiratory disease (BRD) is a multifactorial disease stemming from various viral and bacterial pathogens, often in accompaniment with host immune dysregulation. Understanding the etiology of BRD, as well as the response of the cattle immune system to an assortment of BRD-prevention measures such as vaccination and marketing, will lead to insights which can be exploited for improved BRD resistance and prognosis in beef cattle.

Methods: A multiomic approach combining whole-blood RNA-Seq and nasal swab metagenomics was employed to characterize the cattle immune and microbial response to vaccination, auction-induced stress, and BRD. Blood and mucus were collected from 73 bull calves enrolled in a split-plot randomized controlled trial. Thirty-three calves were randomly chosen to receive a attenuated viral vaccine (Pyramid 5), with 40 serving as unvaccinated controls. Additionally, 40 calves were randomly selected for direct transportation from cow-calf to backgrounding, while 33 spent time in an auction and order-buyer setting for three days prior to backgrounding. Blood was collected at six timepoints: immediately prior to vaccination or not (T1; ~107d), seven days post-vaccination (T2; ~114d), immediately prior to booster (T3; ~183d), at weaning prior to marketing enrollment (T4; ~230d), backgrounding arrival (T5; ~234d), and backgrounding end (T6; ~279d). Left and right nasopharyngeal (NP) swabs were collected at T1, T4, and T5 for shotgun metagenomic sequencing. Cattle were monitored daily for signs of BRD as well. RNA was extracted, sequenced (150 bp; ~35 million reads/sample), and bioinformatically processed using a HISAT2/StringTie2 pipeline. NP swab cuttings were homogenized, DNA was extracted via AllPrep DNA/RNA/miRNA Universal kit and sequenced (150 bp; ~90 million reads/sample). Differential expression and abundance were evaluated between treatment groups based on vaccination, marketing strategy, and BRD outcome, using glmmSeq/edgeR (FDR<0.05) and k-mer distribution approaches. Changes in gene expression over time were characterized with EBseq-HMM.

Results: At T4, 3, 4, and 18 DEGs were identified for marketing strategy, vaccination, and BRD during backgrounding, respectively. Genes at T4 involved in later BRD development enriched for oxygenation and lipid metabolism, cellular scavenging, and leukocyte migration. At T5, 834, 56, and 364 DEGs were identified for marketing strategy, vaccination, and BRD during backgrounding, respectively. Genes at T5 influenced by vaccination enriched for extracellular matrix organization and neutrophil degranulation, antimicrobial peptides, interleukin signaling, and class A receptor scavenging. Genes at T5 influenced by marketing enriched for neutrophil degranulation, interleukin signaling, type I and II interferon signaling, cellular stress response, cornified envelop formation, and keratinization. Genes at T5 involved in BRD development enriched for cytokine, interferon, and interleukin signaling, neutrophil degranulation, and phagocytosis. At T6, 135, 17, and 3 DEGs were identified for marketing strategy, vaccination, and BRD during backgrounding, respectively. Genes at T6 influenced by marketing strategy enriched for tight junction formation, cornified envelop formation, keratinization, and interleukin signaling.

Conclusions: Exposure to auction systems resulted in the largest differential gene expression of any evaluated metric, highlighting the importance of stress management in addressing BRD. The recurring theme of cytokine signaling hints at an exploitable BRD mitigation opportunity surrounding inflammatory mediation.

Financial Support: This work is supported by the USDA National Institute of Food and Agriculture (NIFA) Agriculture and Food Research Initiative Competitive Grant No. 2023-67015-39711.

Notes:

117 - Metagenomic detection of BRD viruses at and shortly after arrival in commercial beef feedlots in Western Canada

Emmanuel Donbraye[1], Lianne McLeod[1], Cheryl Waldner[1]

[1]University of Saskatchewan. emmanuel.donbraye@usask.ca

Session: Bovine Respiratory Disease 1, 2025-01-20, 11:00 - 11:15

Objective: Viral infections in feedlot cattle near the time of arrival can independently initiate bovine respiratory disease (BRD) or predispose calves to bacterial infection and pneumonia. This study aimed to describe the prevalence of viruses critical to BRD development and other viruses detected in respiratory samples from feedlot cattle at arrival and 14 days on feed (DOF). We compared the prevalence of BRD-associated viruses between calves and yearlings at arrival and 14 DOF and differences over time in each group. Additionally, the study assessed the potential for one-step analysis of viral and bacterial BRD pathogens by identifying respiratory bacteria and associated antimicrobial resistance genes (ARGs) from the sequencing data generated for the viral metagenomic analysis.

Methods: Nasal swabs were obtained from fall-placed calves and yearlings from commercial feedlots in Western Canada. The samples were collected from 760 cattle: 20 per pen from 19 pens (13 pens of calves and six pens of yearlings) at arrival (n = 380) and again at 14 DOF (n = 380). Respiratory viruses were characterized with Nanopore metagenomic sequencing. Generalized estimating equations identified differences in virus detection between arrival and 14 DOF samples for calves and yearlings, and between calves and yearlings at each time point.

Results: Twenty-one viruses from 12 viral families were detected, with multiple viruses in most samples. In calves, the most common viruses associated with BRD at arrival were bovine coronavirus (BCoV; 32%), influenza D virus (IDV; 17%), bovine respiratory syncytial virus (BRSV; 8.5%), and bovine parainfluenza virus 3 (BPIV3; 4.2%). The prevalence of BoHV1 (2.7%), BPIV3 (12%), BRSV (26%), and IDV (51%) increased at 14 DOF compared to arrival ($p<0.05$). Bovine viral diarrhea virus 1 (BVDV1) and bovine viral diarrhea virus 2 (BVDV2) were rarely detected.In yearlings, the most prevalent viruses at arrival were BRSV (39%), BPIV3 (20%), IDV (16%), BCoV (12%), and BVDV2 (7.5%). The prevalence of BRSV (60%), BPIV3 (39%), and BVDV2 (17%) increased at 14 DOF ($p<0.05$).BRSV (OR 7.0, 1.7-29) and BPIV3 (OR 5.7, 1.5-21) were more likely to be detected at arrival in yearlings than in calves ($p = 0.01$). BPIV3 (OR 4.9, 1.3-19, $p = 0.02$) and BVDV2 (OR 13, 2.0-83, $p = 0.01$) were more likely at 14 DOF in yearlings than in calves.Respiratory bacteria and 29 antimicrobial resistance genes (ARGs) were also identified. More ARGs were found in calves (57%) than in yearlings (43%) and at 14 DOF (82%) compared to arrival (18%). In calves, 83% of ARGs were detected at 14 DOF versus 17% at arrival. Similarly, 81% of ARGs were detected at 14 DOF versus 20% at arrival.

Conclusions: Improved identification of BRD viruses in newly arrived feedlot cattle can highlight deficiencies in current commercial vaccines and indicate the necessity for new vaccines. Samples collected at 14 DOF and post-vaccination can reveal actively circulating viruses but must be differentiated from modified-live vaccine viruses likely administered upon arrival.

Financial Support: This research was supported by funding from the Agricultural Development Fund (ADF), Beef Cattle Research Council (BCRC) and Genome Canada.

Notes:

118 - Enriching without culture: target-enriched metagenomics allows for strain-level characterization of *M. haemolytica*

Enrique Doster[1], Lee J. Pinnell[1], Cory Wolfe[1], Noelle R. Noyes[3], Robert Valeris-Chacin[1], William B. Crosby[2], Michael L. Clawson[4], Amelia R. Woolums[2], Paul S. Morley[1]

[1]VERO Program, Texas A&M University, [2]Mississippi State University, [3]University of Minnesota, [4]US Meat Animal Research Center, USDA-ARS. Enriquedoster@tamu.edu

Session: Bovine Respiratory Disease 1, 2025-01-20, 11:15 - 11:30

Objective: *Mannheimia haemolytica* (Mh) is a key pathogen associated with Bovine Respiratory Disease (BRD). Previously, investigation of genetic variation that may affect microbial ecology and disease epidemiology was reliant upon the ability to culture Mh and perform whole genome sequencing. The goal of this study was to investigate target-enriched (TE) shotgun sequencing as a culture-independent method for detection and strain-level characterization of Mh in metagenomic microbial communities.

Methods: We developed a custom Agilent SureSelect bait system for targeted metagenomic sequencing of Mh and refined the strain-level classification of metagenomic reads. To address the challenges created by incomplete strain annotations in public databases, we constructed a custom database of 2,137 Mh genomes from GenBank, clustering them into phylogenetically related sequence variants (PSVs) based on whole-genome sequence similarity. We systematically evaluated classification specificity by testing the number of PSVs clusters that could be generated before losing precision. Mock samples containing sequences from an increasing number of PSVs were used to assess classification accuracy, optimizing both precision and recall. 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 further evaluate our workflow, we analyzed TE sequencing from 39 nasal samples from feedlot cattle, categorized into five strata based on Mh relative abundance (>30%, 10-30%, 1-10%, 0.1-1%, and 0%) as determined through 16S rRNA amplicon sequencing. Differences in beta diversity of PSVs were tested using PERMANOVA and PERMDISP tests.

Results: This customized TE sequencing and data analysis workflow provided a sensitive and specific method for strain-level characterization of Mh without culture of isolates. Mh was highly enriched in TE sequencing, yielding an average of 90% (range 50-93%) of non-host reads classified as Mh at the species level. Further, analysis of genomic variants allowed the identification of 8 unique Mh PSVs within the test data set, including in samples where Mannheimia was not identified using 16S sequencing, culture, and PCR. Samples in the “zero” Mh strata had significantly different PSV composition than samples in the “low”, “medium”, and “highest” strata ($p < 0.05$), however, significant differences in the dispersion of variance only allow for comparison to the “medium” strata. Overall, these data suggest that a small number of dominant PSVs drive Mh population dynamics, with increased prevalence of low-abundance PSVs present in samples with a lower abundance of Mh.

Conclusions: This study presents a novel metagenomic approach for strain-level characterization of Mannheimia haemolytica, offering new insights into the complex dynamics of bovine respiratory microbial communities. By removing reliance on accurate metadata and adopting a data-driven method, this approach leverages the increasing availability of genome sequences to improve strain-level resolution. The identification of a multiple PSVs in our samples underscores the genetic fluidity of Mannheimia haemolytica and highlights the need for further investigation into their biological significance and potential impact on BRD epidemiology. Importantly, this approach can be adapted to target other key pathogens, making it a versatile tool for studying microbial populations across diverse ecosystems and diseases.

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:

119 - Development and optimization of targeted enriched metagenomics protocol to obtain strain-level data for *Mycoplasma bovis* in respiratory samples from cattle

Kushal R. Lamsal[1], Christopher Panaretos[2], Ethan P. Dudley[1], Lee J. Pinnell[1], Matthew A. Scott[1], John T. Richeson[3], Hatem H. Kittana[4], Paul S. Morley[1], Robert Valeris-Chacin[1]

[1]VERO Program, Texas A&M Univeristy, [2]Veterinary Integrative Biosciences, Texas A&M University, [3]Department of Agricultural Sciences, West Texas A&M University, [4]Veterinary Diagnostic Laboratory, Kansas State University. sal@tamu.edu

Session: Bovine Respiratory Disease 1, 2025-01-20, 11:30 - 11:45

Objective: Bovine respiratory diseases (BRD) is a major health challenge to the beef cattle industry. *Mycoplasma bovis* has been linked to chronic cases of BRD. Advances in molecular techniques may provide further insights into *M. bovis* ecology, removing bias associated with culture technique. Therefore, the objective of this study was to develop targeted enriched metagenomics protocol (TE) for *M. bovis* in samples from the respiratory tract of beef cattle. Specifically, we used combination of field strains and ATCC strain to spike and create a mock ecology of *M. bovis* to quantify the limit of detection of TE.

Methods: Nasopharyngeal swabs collected from crossbred steers with average body weight (BW = 247±28) housed at West Texas A&M University Research Feedlot underwent DNA isolation using the PowerSoil Pro (Qiagen) kit, and were tested for *M. bovis* via digital qPCR. Samples confirmed negative in triplicate were aliquoted into 120 TE reactions for validation. These 120 reactions were spiked with 12 *M. bovis* strains consisting of 11 field strain (analyzed through whole genome sequencing) and ATCC 25523 in 6 groups: 2, 4, 6, 8, 10, or 12 strains pooled at the same concentration. The bait set for the TE protocol were designed using syotti and manufactured by Agilent. Based on the results we to be used at half molarity strength across all the reactions. Two rounds of TE (double-capture) and a pre-library preparation enrichment were used. DNA libraries were submitted to North Texas Genome Center (NTGS) for sequencing in an Illumina NovaSeq platform. Reads obtained from sequencing were aligned to the standard kraken2 database to calculate the number and percentage of reads on target i.e., classified as *M. bovis*. These reads were extracted using Kraken tools and were pseudoaligned to an index of a set of *M. bovis* genomes using Themisto. Subsequently, relative abundances of Phylogenetic Sequence Variants (PSV's), determined using Mash distances (0.0001), were inferred from the pseudoalignment output via mSWEEP. The limit of detection of the TE protocol were calculated as the highest number of different pooled strains with a probability of 0.95 of complete recovery.

Results: We observed a statistically significant decrease in the concentration of DNA with the molarity of baits in our final library, a decrease in the classification of our targeted genome at the species level, but an increase in the PSV level. The greatest effect on the PSV beta diversity was observed on the quarter molarity; thus, half concentration could be used to reduce the total cost of the TE protocol without compromising the classification at the strain level. We determined the limit of detection of TE protocol for M. bovis to be 8 strains per sample.

Conclusions: These findings will be helpful in tracking *M. bovis* in cattle populations and study its within-host ecology. Discernment of how *M. bovis* strains cooperate during BRD can be used in designing new preventive and therapeutical interventions to decrease the burden of BRD in beef cattle.

Financial Support: Funding for this research was provided by Texas AgriLife Research Animal Health and Disease Capacity Funding (FY22-23)

Notes:

120 - Bovine herpesvirus-1, *Mycoplasmopsis bovis* co-infection rapidly enhances lung pathogenesis at early timepoints post-infection

Bryan S. Kaplan[1], Carly R. Kanipe[1], Kaitlyn S. Davila[1], Jenny Rasmussen[1], Randy E. Sacco[1]

[1]National Animal Disease Center, USDA-ARS, bryan.kaplan@usda.gov

Session: Bovine Respiratory Disease 1, 2025-01-20, 11:45 - 12:00

Objective: Bovine respiratory disease (BRD), a major cause of economic losses to multiple industries, is a multifactorial disease complex with polymicrobial infections intricately linked to disease etiology. *Mycoplasmopsis bovis* (*M. bovis*) is a small, wall-less bacterial pathogen associated with BRD and mastitis. Co-infection with viruses, like Bovine herpesvirus-1 (BoHV-1), can exacerbate *M. bovis* infection leading to severe pneumonia and dissemination to sites outside of the respiratory tract. The objectives of this study were to characterize the tissue pathology and viral and bacterial replication kinetics associated with early infection.

Methods: Thirty Holstein calves with no history of prior BoHV-1 or *M. bovis* infection, were split into four groups and housed in separate rooms of an ABSL-2 containment facility: control (n = 6), BoHV-1 infected (n = 8), *M. bovis* (n = 8), and BoHV-1 + *M. bovis* infection (n = 8). Infected calves were inoculated via nebulization with BoHV-1 Cooper strain followed by *M. bovis* KRB5 four days post infection (DPI). Nasal swabs were collected and assessed for the presence of virus and bacteria every 3-4 following *M. bovis* infection. Half of the animals in each group were euthanized during one of two periods: 8-10 DPI and 12-14 DPI for post-mortem analysis. Tissue swabs and samples were collected for pathogen quantification and histopathologic analysis, respectively.

Results: BoHV-1 + *M. bovis* co-infection resulted in increased inflammation and lung lesion formation compared to single BoHV-1 or *M. bovis* infections characterized by marked lesions present in all lung lobes with noted caseonecrotic infiltrates. BoHV-1 + *M. bovis* co-infection increased bacterial loads in the lung, 10^6 CFU/mL compared to 10^4 CFU/mL in *M. bovis* only. Additionally, co-infection was associated with the persistence of high *M. bovis* counts in all lung lobes on 13/14 DPI in 3 of 4 calves. Both the frequency and titers of *M. bovis* positive nasal, tracheal, and middle ear swabs were increased in BoHV-1 + *M. bovis* co-infection compared to *M. bovis* only infection. BoHV-1 titers were similar between BoHV-1 only and BoHV-1 + *M. bovis* groups.

Conclusions: BoHV-1 + *M. bovis* co-infection resulted in increased bacterial shedding, persistence, and dissemination compared to *M. bovis* only during the first 14 DPI. Together these results suggest co-infection rapidly enhances *M. bovis* colonization of the host and replication in multiple tissues and/or diminishes the ability of the host immune system to mount an effective response against the secondary *Mycoplasma* infection.

Notes:

121 - A simple and fast reverse genetics system for porcine reproductive and respiratory syndrome virus and generation of transcription-reprogrammed virus

Junyu Tang[1], Dongwan Yoo[1]

[1]Department of Pathobiology, University of Illinois at Urbana-Champaign. junyut2@illinois.edu

Session: Porcine Reproductive and Respiratory Syndrome, 2025-01-20, 10:30 - 10:45

Objective: Porcine reproductive and respiratory syndrome virus (PRRSV) is an RNA virus that significantly impacts the swine industry. Modified live vaccines exist but they pose a high risk of genomic RNA recombination with field viruses. Transcription regulatory sequences (TRSs) located at the 5' leader (TRS-L) and upstream of each gene (TRS-B) are essential elements for discontinuous transcription and viral replication. We hypothesize that reprogramming the TRS network will cause a lethal incompatibility when RNA recombination occurs with field viruses, allowing the construction of recombination-resistant PRRSV as a future vaccine candidate. Current infectious clone systems for PRRSV are cumbersome for viral genome manipulation and unstable in *E. coli* during clonal propagation. We aimed to develop a simple and fast reverse genetics system devoid of bacterial propagation, and to use this system to construct TRS-reprogrammed recombination-resistant PRRSV.**Methods:** PRRSV-2 strains NVSL 97-7895 and P129 were used to develop an alternative reverse genetics system. The full-length viral genome was divided into overlapping fragments: F1-F4 for NVSL 97-7895 and F1-F5 for P129. The 5' F1 fragment was coupled with the CMV promoter, and the 3' most fragment was linked to the SV40 polyadenylation signal. Each fragment was PCR-amplified and all overlapping fragments representing the entire 15 kb viral genome were co-transfected into BHK-21 cells. After 2 days, cell culture supernatants were transferred to alveolar macrophages, and the production of infectious virus was confirmed via cytopathic effects, RT-PCR, immunostaining, and Western blot analysis.**Results:** The F1 through F5 fragments were successfully amplified to overlap with the upstream and downstream fragments by approximately 100 nucleotides. When PRRSV-permissive Cl3 cells were infected with the 'passage 2' culture supernatant, cytopathic effects were evident. Viral genome replication was determined by RT-PCR for ORF2, and viral transcription was determined by detecting subgenomic mRNA for the N protein. Infectious virus production was further validated by Western blot for N. The results confirmed that the new reverse genetics system was functional, and we designated it the linear overlapping infectious polymerase amplicon (LOIPA) system. Subsequently, we remodeled the TRS-L and TRS-B transcription networks by analyzing the RNA secondary structure adjacent to the TRSs. Specific mutations were introduced sequentially into TRS-L and individual TRS-Bs, resulting in the generation of a recombination-resistant PRRSV.**Conclusions:** A new reverse genetics system, LOIPA, was developed for PRRSV as an alternative to full-length infectious cDNA clones. This system is simple, rapid, easy to construct, without the need for bacterial propagation, making it stable and free from spontaneous deletions or gene rearrangements that may occur in *E. coli* . Using the new system, RNA recombination-resistant PRRSV was generated, which may serve as a potential vaccine candidate.

Financial Support: Supported by AFRI Competitive Grants no. 2023-67015-39710 from the USDA-NIFA

Notes:

122 - Effect of dietary vitamin D3 on PRRSV disease severity and immune response in nursery pigs

KG. Keen[1], E. van Heugten[2], P. Sitthicharoenchai[1], S. Hernandez[1], V. Bamrung[1], S. Hough[3], M. Rahe[1]

[1]Department of Population Health and Pathobiology, NC State University, [2]Department of Animal Science, NC State University, [3]DSM-Firmenich. kgkeen@ncsu.edu

Session: Porcine Reproductive and Respiratory Syndrome, 2025-01-20, 10:45 - 11:00

Objective: Recent studies in multiple species have reported anti-inflammatory effects of vitamin D3 characterized by reduced IFN-y and increased IL-10 production. Moreover, dietary supplementation with a metabolite of vitamin D3, 25-hydroxyvitamin D3 (25-OH D3), has been shown to modulate immune function in weaning pigs. Porcine reproductive and respiratory syndrome virus (PRRSV) is the most devastating pathogen of swine production in the US, causing interstitial pneumonia and severe systemic inflammation. The objective of this study was to evaluate the effect of dietary vitamin D3 on PRRSV disease outcomes.

Methods: Three-week-old pigs were designated to one of four treatment groups: marginal dietary vitamin D3+no challenge (Negative), marginal dietary vitamin D3 + PRRSV challenge (Low), industry dietary vitamin D3 + PRRSV challenge (Standard), or industry dietary vitamin D3 with 25-OH D3 supplementation + PRRSV challenge (Supplemented). Following dietary acclimation, PRRSV challenged groups were inoculated with a lineage 1 PRRSV-2 virus (L1C.5) at 5x105 TCID50/pig. Throughout the study, samples were collected to characterize clinical disease, viral load, and adaptive immune responses. PRRSV-specific serum antibodies were detected using an IDEXX ELISA kit and neutralizing antibodies were assessed with a fluorescent focus neutralization assay. At 14 days post-challenge, all pigs were euthanized, and necropsies were performed to collect lung tissue for gross and microscopic pathological examination.

Results: Significant differences in serum 25-OH D3 were observed between groups two weeks following diet assignment, with serum concentrations decreasing in challenged groups following PRRSV inoculation. PRRSV-specific serum IgM, IgG, and neutralizing antibodies positively correlated to dietary vitamin D3 treatment. Histological evaluation revealed pigs in the Low group had higher necrotic macrophage scores compared to those in the Standard or Supplemented groups. IFN-y production in PBMCs did not significantly differ between groups. However, evaluation of IL-10 producing cells in the blood and T cell analysis of the lungs is ongoing. No prominent differences in body weight, fever, gross pathology, or viremia were observed between the challenged groups.

Conclusions: Present findings suggest increased dietary vitamin D3 improves the antibody response to PRRSV infection with ongoing sample analysis to further characterize the T cell response.

Financial Support: This research was supported by DSM-Firmenich.

Notes:

123 - PRRSV-2 immune biobank for vaccine efficacy prediction

Lanre Sulaiman[1], John Byrne[1], Ben H. Hause[2], Juliana Ferreira[1], Glen Almond[1], Tobias Käser[3], Gustavo Machado[1], Elisa Crisci[1]

[1]Department of Population Health and Pathobiology, North Carolina State University, [2]Cambridge Technologies, [3]Department of Biomedical Sciences and Pathobiology, University of Veterinary Medicine Vienna. ecrisci@ncsu.edu

Session: Porcine Reproductive and Respiratory Syndrome, 2025-01-20, 11:00 - 11:15

Objective: The high mutation rate of PRRSV represents a big challenge and raises an important question for swine producers: Which vaccine can best protect my herd against an emerging strain? Currently no technology can adequately answer that question. To overcome this issue, we have established at the North Carolina State Veterinary College a vaccine efficacy prediction system that consist of an immune biobank (cells and serum) from pigs which received five PRRSV-2 modified live vaccines (MLV) that are commercially available in US. The immune biobank enables to evaluate in vitro which vaccine induces the strongest immune response against a circulating PRRSV-2 strain. The project aims to improve precision animal management for PRRSV-2 in North Carolina by decreasing the impact of the disease with a proactive outbreak mitigation approach.

Methods: Four-weeks old pigs were divided into six groups of eight pigs each (four females and four males). Five groups were immunized with the different MLV vaccines, and one group was used as control. Collection of PBMCs and serum was performed at 28 days post immunization. The immune biobank in vitro testing is based on neutralizing antibodies (nAb) levels, interferon-gamma producing cells responses (ELISpot assay), and cell proliferation assay evaluated by flow cytometry.

Results: The immune biobank was tested against four North Carolina (NC) PRRSV-2 lineage 1A isolates (NC18-9-7, NC20-1, NC23-11), L1C isolate (NC134), two prototype viruses (VR2332, NADC-20) and five MLV strains: differences in vaccine interferon-gamma responses were observed against NC18-9-7, NC23-11, NC20-1 and VR2332, whereas all vaccines showed similar responses against NC134 and NADC-20. Sera from vaccinated animals showed different nAb titers against NADC-20 and VR2332, but no nAb titers were present against NC134 and NC18-9-7 strains. Additionally, a whole genome sequence homology approach was used to evaluate the evolutionary divergences between vaccine strains and field isolates tested in the immune biobank. An amino acid sequence homology between field isolates and vaccine strains will be evaluated for GP2, GP3, GP4 and GP5.

Conclusions: The immune biobank can help determine the most promising vaccination regimen against the tested PRRSV-2 strains. The vaccination regimen will be recommended to North Carolina swine veterinarians for precision management implementation in the field.

Financial Support: This work is supported by the Agriculture and Food Research Initiative Inter-Disciplinary Engagement in Animal Systems, project award no. 2022-68014-37266, from the U.S. Department of Agriculture's National Institute of Food and Agriculture.

Notes:

124 - Influence of gestational age on vertical transmission of PRRSV2

Dayeon Jeon[1], Sarah Innis[1], Alyssa Smith[1], Kaylyn Rudy[1], Isabel Turner[1], Ryan Cabot[1], J. Alex Pasternak[1]

[1]Department of Animal Sciences, Purdue University. jpastern@purdue.edu

Session: Porcine Reproductive and Respiratory Syndrome, 2025-01-20, 11:15 - 11:30

Objective: Porcine reproductive and respiratory virus (PRRSV) crosses the normally restrictive epitheliochorial placenta and proceeds to disrupt fetal development, increasing fetal mortality and the occurrence of spontaneous abortion. Collectively, this results in an estimated annual cost to the American breeding herd in excess of 330M. Preventing vertical transmission would significantly limit this impact, but the mechanism by which the virus crosses the placenta has yet to be determined. To better understand the mechanism underlying vertical transmission, our larger project is focused on understanding the developmental correlates of fetal susceptibility. Prior studies with PRRSV1 or mild strains of PRRSV2 indicate complete fetal resistance during mid gestation, with vertical transmission beginning after gestation day (GD) 72 with proportional infection rates rising thereafter. However more recent studies call into question this temporal restriction and suggest transplacental fetal infection by highly pathogenic strains may occur as early as GD 60.

Methods: To investigate the effect of gestational age three batches of eight pregnant gilts were obtained from a commercial PRRSV free nucleus herd (N=24 total). On GD 50, 60, 70 and 80 (+/- 1 day) relative to first AI, all gilts were inoculated with PRRSV2 strain NVSL-97 7895 via a combination of intranasal and intramuscular delivery. Gilts were monitored daily for clinical signs, and maternal blood samples and rectal temps collected on 0-, 7- and 21-days post inoculation (DPI). All gilts were humanely euthanized at 21 DPI (GD 71, 81, 91, 101). Fetal preservation status and basic phenotypic parameters were evaluated for each fetus. Fetal blood samples were collected from the axillary artery, and samples of the placenta, fetal thymus, heart and kidney collected for additional analysis.

Results: Maternal rectal temp was significantly elevated on 7 DPI but returned to baseline by 21DPI, but this response was not influenced by GD. Viremia was confirmed in all dams at 7 days post inoculation, with mild to moderate clinical signs observed in less than 1/3 of the animals. Fetal viability was 79.7, 82.1, 88.5 and 62.3% on GD 50, 60, 70 and 80 respectively (21 days post maternal inoculation). No meconium staining was observed in litters from day 50, but 0.9, 2.6 and 4.9 % of fetuses were meconium stained at each respective time point thereafter. A novel form of fetal preservation, characterized by whole body edema and cutaneous transparency and apparently poor ossification of the limbs was observed in 10.6% and 8.5% of fetuses from dams inoculated on GD 50 and 60. Substantial viral load was detected in serum from viable fetuses collected at all four time points. Contrary to expectation the highest median viral load in fetal serum was observed following maternal infection at day 50 of gestation.

Conclusions: Collectively our results indicate that PRRSV2 strain NVSL-97 7895 can cross the placenta and productively infected the fetus prior to day 71 where it induces significant fetal mortality. Information in this area will be critical in developing strategies to reduce or eliminate fetal PRRSV infection, and thereby increase profitability within the swine breeding herd.

Financial Support: This project was supported by Agriculture and Food Research Initiative Competitive Grant no. 2023-67015-39080 from the USDA National Institute of Food and Agriculture.

Notes:

125 - Decoding the spatial patterns of PRRS induced cell death

Rachael Weiderman[1], Franco Matias-Ferreyra[1], Patricia Assato[1], Anna Fritzson[1], Shristi Ghimire[1], Jurgen Richt, Laura Miller

[1]Department of Diagnostic Medicine and Pathobiology, Kansas State University. warhurst@ksu.edu

Session: Porcine Reproductive and Respiratory Syndrome, 2025-01-20, 11:30 - 11:45

Objective: Porcine Respiratory and Reproductive Syndrome (PRRS) is the most economically damaging illness for the swine industry, costing over 1 billion per year in lost production in the United States. Our research aims to provide a greater understanding of PRRS host-immune response by identifying genes involved in the host response to infection and determining how infection with PRRS alters the host immune response pathways. We aim to answer previously unanswered questions about infected and bystander cells in the intrinsic and extrinsic apoptotic pathways during PRRS infection.

Methods: A lung tissue sample from an adolescent swine infected with PRRS was analyzed through spatial molecular imaging, providing quantification of 1,000 RNAs through in situ hybridization chemistry at single-cell and sub-cellular resolution. Spatial imaging and data were analyzed using Python and R code packages to provide analysis and deeper understanding of gene expression across the tissue sample in the infected state.

Results: The spatial molecular imaging and spatial informatics data provided 71 transcripts per cell of new information regarding gene expression in PRRS infection. Gene activity involved in the intrinsic and extrinsic apoptosis pathways, including ATF-3, p53, Caspase-8, and Caspase-3 is measured and analyzed to show the connection between PRRS infection and induction of apoptosis.

Conclusions: Previously, it has been unanswered whether PRRS infection directly induces apoptosis and apoptotic gene expression. Spatial transcriptomics provides a new platform to answer questions that have previously not been answered about viruses and immune response pathways by analyzing infected and bystander cells in a positional and regional context.

Financial Support: This research was supported [in part] by the intramural research program of the U.S. Department of Agriculture, National Institute of Food and Agriculture, Hatch-Multistate project 7008266.

Notes:

126 - A longitudinal trial of fecal microbiota transplant therapy to weaned pigs during post-weaning viral co-infection

Yale Deng[1], Pratiksha Khanal[2], Aubrey Deavours[2], Ada G. Cino-Ozuna[3], Megan C. Niederwerder[2, 4], Noelle R. Noyes[1]

[1]Department of Veterinary Population Medicine, University of Minnesota, [2]Department of Diagnostic Medicine/Pathobiology, Kansas State University, [3]Toxicologic Pathology Associates, [4]Swine Health Information Center. deng0291@umn.edu

Session: Porcine Reproductive and Respiratory Syndrome, 2025-01-20, 11:45 - 12:00

Objective: The primary objective was to investigate the efficacy of fecal microbiome transplant (FMT) as a therapy to mitigate the impacts of viral co-infection in post-weaned pigs. A secondary objective was to describe temporal changes in the fecal microbiome of pigs exposed to FMT therapy versus a placebo.

Methods: Weaned piglets (N = 100) were matched by litter and sex and randomly assigned to one of two treatment groups: a control group (CTRL) administered oral sterile saline, and a treatment group administered oral FMT therapy (FMT) daily for 7 consecutive days. Then, all pigs were challenged with porcine reproductive and respiratory syndrome virus (PRRSV) and porcine circovirus type 2 (PCV2). Blood samples and individual per-rectum fecal samples were collected weekly over the 6-week post-infection trial. Pigs weight, morbidity and mortality data were recorded throughout the trial. Immune responses and viral load in blood samples were measured post-challenge. FMT therapy materials and fecal samples were subjected to DNA extraction and 16S rRNA V3-V4 amplicon sequencing.

Results: FMT treatment showed no effect on pig body weight and survival post viral coinfection. FMT group displayed a lower count of primary clinical concerns, such as abscess, attitude and diarrhea (n=150) than CTRL group (n=280) in surviving pigs, and worse fecal condition (> 75% had diarrhea) and body conditions (on average = 2) than CTRL (fecal condition, > 40% had diarrhea; body condition, on average = 3). Most pigs were PRRSV and PCV2 antibody positive after 7 dpi, the virus gene copies in blood samples were similar between the two groups for PCV2, but FMT had a significant higher PRRSV copies than CTRL. The fecal microbiome samples were grouped into four clusters: a cluster of -7 dpi samples (just-weaned), a cluster of 0 dpi (post-treatment), a cluster containing 7-35 dpi samples (post-infection), and a cluster of 42 dpi samples. Similarly, both richness and Shannon diversity indices increased from -7 to 0 dpi; remained stable during 7-35 dpi; and then decreased at 42 dpi. Differential abundance analysis suggested that Terrisporobacter, Turicibacter, Clostridium sensus stricto 1 and Romboutsia showed higher abundance in FMT vs CTRL on 0 dpi as well as 0 vs -7 dpi. These four genera were also found high in abundance in the FMT donor materials. Moreover, potentially beneficial and detrimental microbes were identified by comparing their abundance between the two groups. Significant differences were observed between the microbiomes of FMT versus CTRL pigs, but PERMANOVA testing portioned less than 2% of the total microbiome variation to treatment group.

Conclusions: After 7 days of daily oral inoculation, FMT treatment made no difference in pig body weight and survival, but reduced primary clinical concerns in surviving pigs and increased PRRSV copies in the blood samples. Four genera were found in high abundance in the FMT material and resulted in increased relative abundance in the fecal samples of treated pigs. After viral coinfection, the fecal microbiome of weaned piglets was significantly modified by FMT treatment, but with a limited effect size.

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:

127 - Using challenge models to identify pathogenicity traits of novel Avian Pathogenic *E. coli* (APEC) serogroups

K. Runcharoon[1], J.I. Lima[1], Y.Y. Tsai[1], C.M. Logue[1]

[1]Department of Population Health, University of Georgia. kr71624@uga.edu

Session: Bacteriology 2, 2025-01-20, 10:30 - 10:45

Objective: Avian Pathogenic *Escherichia coli* (APEC) causes colibacillosis leading to systemic or localized infections in poultry. Three serogroups O1, O2, and O78 have been reported as the most often linked with disease. However, our data suggests multiple serogroups may be responsible for colibacillosis cases in Georgia poultry. The pathogenicity of these novel APEC serogroups in poultry is currently unknown. Therefore, this study aims to investigate the pathogenicity of 10 APEC strains from 9 different serogroups using three different animal challenge models.

Methods: Nine different APEC serogroups (10 strains) were selected and challenged in three animal models: 12-day-old chicken embryos, 1-day-old chicks, and 3-week-old SPF-layer chickens. For the embryo challenge, 10 embryos per group were injected with 300-500 CFU/mL of the challenge strain via allantoic fluid at 12 days of age (d.o.a). Eggs were candled daily, and deaths were recorded until the embryos reached 18 d.o.a. Isolates causing embryo death of >29%, 10%-29% and <10% considered as, virulent, moderately virulent, and avirulent isolates, respectively. In the chicks challenge assay, each group of 10 birds was inoculated subcutaneously with 0.1 mL (10^7 CFU). *E. coli* isolates that killed >50%, 10%-50%, and 0-10% of chicks considered virulent, moderately virulent, and avirulent, respectively. Mortality, pathogenicity score (PS), and lethality score (LS) were observed and compared to those of the control strain (APEC 380 - O18). For older birds, 3 week SPF-layers were challenged with 10^8 CFU/mL of bacteria via the intratracheal (I.T) route to mimic natural infection. Mortality and clinical signs were observed for 5 days, after which the remaining birds were euthanized by carbon dioxide, and lesions and bacterial counts were evaluated.

Results: For the embryo lethality assay, all strains were found to be highly virulent causing over 28% mortality within 5 days post-infection (d.p.i). Notably, two serogroups (O161 and O45) caused 100% mortality. In the chick challenge, only O15, O91, and O88 had lower PS scores compared to the control strain ($p<0.001$). APEC O25 and Onovel 12 caused 100% mortality within 5 d.p.i. In 3 week SPF-layers, APEC O91 was found to cause 80% mortality after 1 d.p.i. Moreover, APEC O115 and O86 were identified as moderately virulent strains.

Conclusions: The novel APEC serogroups exhibited varying levels of pathogenicity and high virulence in embryos, chicks, and chickens. Interestingly, the virulence of these strains varied across different challenge models, suggesting that the route of infection and the bird's immune system may influence disease development. Collectively, these APEC serogroups can significantly impact poultry health, especially in younger birds, warranting further investigation and the development of new mitigation strategies.

Financial Support: This research was supported by the National Institute of Food and Agriculture under Award Number #: 2022-67015-36878

Notes:

128 - *M. hyopneumoniae* infects and disrupts the air-liquid cultured porcine-derived primary respiratory epithelial cells

A.F. Castillo-Espinoza[1], R.K. Nelli[1], J.C. Mora-Diaz[1], R. Rauh[2], N.C. Twu[1], L. Gimenez-Lirola[1]

[1]Department of Veterinary Diagnostic and Production Animal Medicine, Iowa State University, [2]Tetracore. afc@iastate.edu

Session: Bacteriology 2, 2025-01-20, 10:45 - 11:00

Objective: *Mycoplasma hyopneumoniae* (Mhp) is a fastidious respiratory pathogen that colonizes porcine ciliated airway cells, disrupting mucociliary clearance and contributing to chronic respiratory disease. Understanding early interactions at the infection site is critical for developing interventions. This study aimed to evaluate the suitability of air-liquid interface primary porcine respiratory epithelial cells (ALI-PRECs) as an in vitro model for studying Mhp infection dynamics and to assess the effect of Friis medium, traditionally used to culture Mhp, on ALI-PRECs.

Methods: Tracheal epithelial cells were isolated from three six-week-old CD/CD pigs, seeded onto collagen-coated transwell inserts, and cultured under ALI conditions for four weeks to form a pseudostratified, ciliated epithelium. ALI-PRECs from one pig were inoculated with Mhp strain 232 at doses of 10^5, 10^6, 10^7 CCU/mL, or a mock control, with exposure times of either 2 or 5 h. After inoculation, cultures were incubated for 24, 48, and 72 h post-inoculation (hpi) at 37°C and 5% CO2. Infection dynamics was extended to 144 hpi in subsequent experiments using 2 h exposure with a dose of 10^7 CCU/ml. Cytopathic effects (CPE), ciliary motility, Mhp detection by immunofluorescence (IFA), and quantitation of Mhp DNA by qPCR were assessed in both epithelia and subnatants. FIJI Image J software was used to quantify FITC-labeled Mhp P46 membrane protein and DAPI-stained cell nuclei numbers. Cells from four symmetric micrographs per transwell were quantified and normalized to mock-inoculated controls. A three-way ANOVA and a two-way ANOVA with a Tukey test were applied to assess the individual effects of dose, time, and exposure time, and the interaction between variables.

Results: Mhp infection led to characteristic CPE, including epithelial cell rounding, clustering, detachment, and reduced ciliary activity by 24 hpi. These effects were dose- and time-dependent, with statistical reductions in cell numbers at higher Mhp doses ($p<0.05$). Mhp P46 protein was detected by 24 hpi, with peak fluorescence at 48 hpi and gradual decline up to 144 hpi. Co-localization of Mhp P46 in ciliated regions confirmed the pathogen's affinity for ciliated cells, essential for mucociliary clearance. Mhp DNA was detected in all inoculated ALI-PRECs by 24 hpi, regardless of dose, but was only found in the subnatants at the highest Mhp dose (107 CCU/ml) after 24 hpi (for 5 h exposure) or 48 hpi (for 2 h exposure). This suggests that significant epithelial disruption is required for Mhp to breach the epithelial barrier. A significant reduction in Cq values ($p<0.05$) was observed in subnatants at 144 hpi, indicating progressive epithelial damage. Despite epithelial disruption, ciliary movement persisted throughout the infection period. Importantly, no histological or functional changes were observed in the Friis medium-only control group. Mock-inoculated cultures remained healthy, with no evidence of Mhp infection by IFA or qPCR.

Conclusions: ALI-PRECs provide a robust, physiologically relevant model for studying Mhp-host interactions, including mucociliary responses and epithelial integrity. The model confirms that Mhp specifically targets ciliated cells, leading to gradual epithelial disruption. Friis medium does not affect ALI-PRECs, confirming the model's suitability for infection studies.

Financial Support: Boehringer Ingelheim Animal Health.

Notes:

129 - *M. hyopneumoniae* modulates ciliary and adherens junction gene expression in air-liquid porcine respiratory epithelial cells

A.F. Castillo-Espinoza[1], R.K. Nelli[1], J.C. Mora-Diaz[1], R. Rauh[2], B. Reddi[1], A. Saxena[1], N.C. Twu[1], L. Gimenez-Lirola[1]

[1]Department of Veterinary Diagnostic and Production Animal Medicine, Iowa State University, [2]Tetracore. afc@iastate.edu

Session: Bacteriology 2, 2025-01-20, 11:00 - 11:15

Objective: *Mycoplasma hyopneumoniae* (Mhp) is a fastidious bacterium whose pathogenicity lies primarily in its complex interaction with porcine airway ciliated cells. In a previous study, we demonstrated the susceptibility of an air-liquid interface model utilizing porcine-derived primary respiratory cells (ALI-PRECs) to Mhp. Additionally, we described Mhp's ability to cause epithelial disruption and ciliostasis in a dose-, exposure time-, and post-exposure-time-dependent manner. The present study focuses on characterizing the mechanisms of epithelial disruption utilized by Mhp to induce cytopathic damage and ciliostasis in ALI-PRECs.

Methods: Tracheas collected from six-weeks-old CD/CD pigs (n=3) were processed to isolate PRECs. PRECs were seeded into collagen-coated transwells and cultured under ALI conditions for 4 weeks to promote differentiation. Differentiated ALI-PRECs were inoculated with 107 CCU/ml of Mhp strain 232 for 2 h at 37°C and 5% CO2. Following inoculation, ALI-PRECs were monitored every 24 h up to 144 h post-inoculation (hpi). The infection dynamics were determined by monitoring cytopathic effects (CPE), performing immunofluorescence (IFA) to assess FITC-labeled Mhp P46 membrane protein staining, quantifying FITC-fluorescence intensity, and determining cell numbers using cell nuclear staining analyzed with FIJI Image J software. Mhp qPCR was performed on both the pseudostratified epithelia and subnatants of ALI-PRECs. The transcriptional regulation of genes related to ciliary motility (6 genes), cytoarchitecture (2 genes), intercellular junctions (6 genes), and endogenous control genes (7 genes) was evaluated. Relative quantitation was performed using the $\Delta\Delta C_T$ method, and statistical analysis was conducted using GraphPad Prism software v10.2.1.

Results: Following 24 hpi with Mhp, ALI-PRECs exhibited CPE, including progressive loss of epithelial integrity, cell clustering, decreased cell numbers, and occasional ciliostasis. The CPE was more pronounced at later time points, while one biological replicate exhibited partial recovery after 96 hpi, supported nuclear staining ($p<0.05$). The qPCR analysis of Mhp DNA in subnatants over time (24 - 144 hpi) showed a significant trend toward decreasing Cq values ($p<0.05$). The fluorescence intensity of Mhp P46 peaked at 48 hpi and steadily decreased towards 144 hpi in all three biological replicates of ALI-PRECs inoculated with Mhp. Mhp P46 preferentially bound to ciliated cells, and the absence of nuclear staining, combined with Mhp P46 detection, confirmed areas of epithelial discontinuity. Gene expression studies in Mhp-inoculated ALI-PRECs, revealed downregulation of ciliary motility genes (ROPN1L, LRCC51, FOXJ1, LRCC6) at 72 hpi ($p<0.001$), supporting histological data. Interestingly, intercellular junction gene expression (CDH1, CTNNB1, CTNND1) was upregulated at 120 hpi ($p<0.05$), suggesting a mechanism of recovery previously reported in bronchial PRECs and other mycoplasmas.

Conclusions: Mhp adheres to ALI-PRECs, causing cytopathic damage and ciliostasis, with a potential wound healing response in ALI-PRECs that requires further investigation. Our findings underscore variability in ciliary responses, suggesting differences in host susceptibility among pigs.

Financial Support: Boehringer Ingelheim Animal Health.

Notes:

130 - Broiler and layer chickens' responses to host-specific bacteria colonization in early life

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

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

Session: Bacteriology 2, 2025-01-20, 11:15 - 11:30

Objective: In commercial farms, chicks are hatched a clean environment, away from their progenitors and other adult chickens from which they could inherit host-specific bacteria, like Segmented Filamentous Bacteria (SFB) required for their gut maturation. Thus, introducing these bacteria to chicks at hatch is important in industrial settings. In commercial poultry farms, distinct genetic lines of chickens are selected for either improved feed conversion and rapid growth (broilers) or the production of eggs (layers). Due to the varying host-responses to treatments and infections, our research has the objective of evaluating an SFB-based treatment to increase gut maturation in layer and broiler chickens in early life to increase resistance to diseases.

Methods: Day-old broiler and layer chickens were orally treated with layer-derived SFB (D-SFB) (n=15 to 30 birds/group). Non-treated groups were included and housed in separate rooms. Samples were collected either prior to euthanasia (feces) or post-euthanasia (ileum, ceca, and blood). The SFB were tracked overtime in the ileum and feces by qPCR and microscopy for 3-4 weeks. Enterobacteriaceae were enumerated in feces by dilution and plating. The expression of genes associated with gut immune function were measured by RT-qPCR. Resistance to bacteria was tested using in vitro bactericidal assays for blood and ileal tissues. Statistical analyses of the data were performed using the GraphPad Prism software. Multiple t-tests with Holm-Sidák's correction for multiple comparisons was used to detect significance differences between group treatments and times tested.

Results: The SFB-based oral inoculum ensured maximal ileal SFB colonization in layers and broilers in early life and minimized variation in SFB colonization among individuals. In broilers, the colonization of SFB covered the entire length of the ileum. The treatment enhanced intestinal barrier function and homeostasis. Interestingly, in both layers and broilers, there was no increase in IL-17 production indicative of TH17 cell activation like previously shown in mice. This immune activation triggered broad bacterial killing activity on both intestinal and extraintestinal pathogens in vitro and in vivo against bacteria relevant to chickens and humans.

Conclusions: With the antibiotic restrictions in poultry production, strategies to mitigate infections are urgently needed. We developed a live prophylactic for newly hatched chickens to improve animal health and food safety. The ability of layer-derived SFB to cross-colonize in broiler chickens eliminates the need for multiple SFB-based products to be derived from both layers and broilers.

Financial Support: This work was supported by the United States Department of Agriculture (USDA)-NIFA # 20236701539078, USDA- Hatch projects (IOW05700-NC1202 and IOW04202), Kent Corp. to MM.

Notes:

131 - A novel CRISPR-prime editing strategy for single-nucleotide resolution mutations in *Leptospira* spp

Luis Guilherme Virgilio Fernandes[1], Camila Hamond[2], Bienvenido Tibbs-Cortes[1], Ellie Putz[1], Steven Olsen[1], Mitchell Palmer[1]

[1]National Animal Disease Center, USDA-ARS, [2]National Veterinary Services Laboratories, USDA-APHIS. luisgui530@gmail.com

Session: Bacteriology 2, 2025-01-20, 11:30 - 11:45

Objective: Develop, validate, and apply the novel CRISPR prime editing (PE) technology to generate single nucleotide resolution mutations in *Leptospira* spp. Evaluate the virulence phenotype of the mutants in the hamster model of leptospirosis.

Methods: The CRISPR/Cas9 system consists of the expression of endonuclease Cas9, which interacts with single-guide RNA sequences (sgRNA) and a protospacer adjacent motif (PAM) NGG, to create double-strand breaks (DSBs) in the genome. However, DSBs are lethal to *Leptospira*. Alternatively, the CRISPR-PE strategy is capable of deletion, insertion, and base substitutions without introducing DSBs. CRISPR-PE comprises a nickase Cas9 (nCas9) genetically linked with an engineered reverse transcriptase (RT), alongside a modified sgRNA (PEgRNA) containing an extended 3' end in which a primer binding sequence (PBS) and a reverse transcription template (RTT) are present. A plasmid expressing the nCas9 and reverse transcriptase fusion protein was constructed (pMaOriPE). For PEgRNA, the lipL32 promoter was used, followed by a 20-nt protospacer, the nCas9 handle, a 3’ extension comprising a 13-nt RTT sequence and a 13-nt PBS. RTT sequences were designed to disrupt the NGG motif and create frameshift mutations. Plasmids were delivered to several species and serovars of *Leptospira* spp. by conjugation with *E. coli* β2163. nCas9-RT and target protein expression were evaluated by immunoblotting. Mutations were confirmed by sequencing. The virulence of mutant phenotypes was evaluated in the hamster model of leptospirosis.

Results: CRISPR-PE was first validated in the saprophyte L. biflexa model: delivery of plasmids by conjugation targeting either 1 or 2 nucleotide deletions or insertions in the β-galactosidase gene resulted in colonies containing knock-out (KO) mutant cells confirmed by sequencing. Application of CRISPR-PE with different serovars of *L. interrogans* and *L. borgpetersenii* resulted in an efficient KO mutant recovery targeting lipL32. LipL32 mutants were still virulent in the hamster model of infection. After 2 in vitro passages without selective pressure, plasmid could be eliminated from the population, resulting is markerless mutants. Since LipL32 is a conserved, immunodominant protein of pathogenic Leptospira and expressed during infection, KO mutants for this gene could be used for the development of DIVA bacterins for veterinary use.

Conclusions: CRISPR prime editing technique was successfully applied in Leptospira, allowing the generation of knockout mutants in several pathogenic species, with mutations comprising just a single nucleotide resolution. Notably, we generated a mutant in the *Leptospira borgpetersenii* background, a prevalent pathogenic species of humans and cattle. Our application of this method opens new avenues for studying pathogenic mechanisms of Leptospira and the identification of virulence factors across multiple species. These methods can also be used to facilitate the generation of marker-less knockout strains for updated and improved bacterin and/or live vaccines.

Financial Support: This research was supported by USDA and APHIS Research Participation Program administered by the Oak Ridge Institute for Science and Education (ORISE) through an interagency agreement between the U.S. Department of Energy (DOE) and the U.S. Department of Agriculture (USDA).

Notes:

132 - Sequencing of the chicken segmented filamentous bacteria genome reveals differences between host-species

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

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

Session: Bacteriology 2, 2025-01-20, 11:45 - 12:00

Objective: Chickens raised for meat (broilers) and egg (layers) production are genetically distinct and respond differently to infections and treatments. Commercial chicks are hatched in the absence of their progenitors who may transfer key host-specific bacteria like Segmented Filamentous Bacteria (SFB). Our team has developed a SFB-based treatment for newly hatched chicks and demonstrated its ability to enhance gut maturation and resistance to infections in early life. Most studies on SFB genome sequencing have been completed in humans and rodents. However, because of the host-specificity of SFB, studies on chicken SFB are needed. The objective of this study was to fully sequence and analyze the genome of chicken SFB (C-SFB) and investigate the strain variability between those of broilers, layers, and other host-species.

Methods: SFB were collected from two week-old broiler and layer chickens acquired from commercial facilities via mucosal scrapings of the distal ileum. Construction of the C-SFB genome was accomplished through Hi-C and Oxford Nanopore sequencing. The comparison with human, turkey, and rodent SFB was done utilizing Anvi'o's pangenomic analysis software. The metabolic network of C-SFB was analyzed and compared to rodent SFB and model organisms using Pathway Tools MetaCyc software. Differences between C-SFB acquired from broilers and layers were assessed through Sanger sequencing of certain effector proteins and further multiple sequence alignment.

Results: The metagenomic construction of the entire genome showed that the C-SFB genome is 1,612,002 base pairs in size. Compared to recently sequenced SFB genomes from mice, rats, humans, and turkeys, C-SFB showed differences among SFB from different hosts. At the average nucleotide identity (ANI) level, C-SFB showed an approximately 97% identity with that of turkey SFB, suggesting a close evolutionary relationship within avian hosts. The average amino acid identity (AAI) level demonstrated an approximately 70% relationship with that of mammalian SFB. Further analysis of the metabolic networks associated with C-SFB illustrated several complete pathways in biosynthesis, transport of nutrients, and utilization and degradation of metabolites. The C-SFB metabolic network lacks several pathways in multiple key networks. However, several pathways identified in the C-SFB genome suggest the production of short chain fatty acids (SCFA), an important secondary metabolite in gut homeostasis. Finally, investigation of specific effector proteins sequenced from broiler and layer chickens revealed that strain variability occurs between C-SFB analyzed from different hosts in the same species.

Conclusions: Through fully sequencing the C-SFB genome, we have illustrated its evolutionary split from the other sequenced SFB from mammalian hosts and its similarity to SFB obtained from other avian species. Understanding the differences between SFB isolated from different host-species will help us better understand how these host-specific bacteria promote immune maturation and protection from disease in the chicken gut. As layer-derived SFB can readily colonize broiler chickens, understanding the strain-specific interactions of SFB at a molecular level reveal key insights into how these bacteria drive immune maturation in both broilers and layers.

Financial Support: This work was supported by Kent Corp, the United States Department of Agriculture (USDA)-NIFA #20236701539078, USDA- Hatch projects (IOW05700-NC1202 and IOW04202) to MM.

Notes:

133 - Challenges to conducting epidemiologic research regarding companion animal welfare

Jannet M. Scarlett[1]

[1]College of Veterinary Medicine, Cornell University. jms15@cornell.edu

Session: AVEPM - Schwabe Symposium, 2025-01-20, 2:15 - 3:00

The focus of this presentation is on pet homelessness, one of numerous threats facing companion animal welfare in the U.S. An estimated 6.5 million dogs and cats entered animal shelters and rescues throughout the country in 2023, 690,000 were euthanized and 60-100 million cats lived their lives on the streets. This, despite the expenditure of approximately $147 billion in the same year on pets. Not surprisingly, epidemiologic studies have contributed to understanding of the welfare of homeless pets, addressing a broad range of issues (e.g., the magnitude and scope, potential causes and care of sheltered and free-roaming populations) - all in a quest to find optimal preventive, control and management strategies aimed at improving pet welfare. Sources of data to address these issues include 1) former and prospective owners, 2) government- and privately- owned animal shelters and rescues, 3) programs (e.g., TNR) serving free-roaming populations and 4) targeted research studies.One challenge facing researchers of pet homelessness is the variability inherent to data from animal shelters. Unfortunately, the growth of animal sheltering was haphazard, hampered by scarce resources and variable responses to the growing number of pets (owned and homeless) after WW II. No one organization led the response to homeless pets, and communities addressed the issue using various approaches on various timelines. Therefore, today there is no one (or even a few) organizations that manage shelters in a region, let alone across the country. There is no federal legislation mandating data collection in animal shelters. Even in the small number of states mandating collection of data (primarily intake and outcome), enforcement of laws is often of low priority. This has resulted in no centralized sampling frame of shelters or collection of data from them. Agreement on the most important data to collect and definition of data fields is not standardized, despite efforts to address these. Despite huge advancements in technology facilitating data collection, some shelters lack the resources even for computers and software. Many shelters with computers and software lack expertise to manage their data or manage only a small fraction of available information. Social pressures to appear to “save” a large proportion of incoming animals (despite inadequate resources to do so) have led some shelters to manipulate definitions and report biased, “favorable” data or avoid reporting at all. Competition for donor and grant dollars is fierce. Despite these limitations, leadership in the animal sheltering community has long recognized the value of valid data. In 2016, efforts to establish a national database to measure progress in pet welfare, inspire collaboration, and reduce pet homelessness resulted in the collection of standardized intake and outcome data by Shelter Animals Count (SAC). Currently, approximately ¼ of U.S. shelters send data, and researchers are encouraged to submit proposals to utilize these data.Overall, difficulties in obtaining valid data, small numbers of well-trained, interested researchers, lack of federal and limited foundation funding, and academic pressures to obtain large grants have all contributed to slow research progress towards preventing, controlling and managing pet homelessness.

Notes:

134 - Illuminating the welfare implications of free-roaming unowned cats via epidemiological approaches

Margaret R Slater

mslater@cvm.tamu.edu

Session: AVEPM - Schwabe Symposium, 2025-01-20, 3:00 - 3:30

Free-roaming unowned cats (FRUCs) go by many names but most have contact with humans. In this presentation, I'll outline where epidemiologists have essential, life-saving roles to play in the FRUC world, highlight some examples and gaps, and argue why epidemiologists need to be familiar with the larger picture in which these cats' journey.In more urbanized settings, it is almost impossible to find a FRUC who doesn't have someone feeding or otherwise engaging with them. And many times, these carers are quite attached even though they don't believe they own the cats. The line between owned and unowned is very permeable for cats especially in industrialized countries. FRUCs may have been in a home and owned just days ago and gotten lost or been abandoned or they may be multiple generations distant from an indoor home. Yet they are still domestic animals. FRUCs are a result of human activities or lack thereof giving humans responsibilities towards them.Epidemiologists have three main potential roles in the welfare of these cats. The first arises from concerns about public health and wildlife. Each of these spheres is enormous but they are unified by their crucial implications for policy and law and need for location specific research which is largely absent. In public health, zoonotic disease transmission and bites are the most frequent concern, yet data on actual risk and effectiveness of potential control measures are rare. Predation on native wildlife by FRUCs is high profile in the public media. Transmission of diseases and competition between cats and other animals are two additional wildlife concerns with little substantiation. Rational data-driven discourse is critical to develop and implement viable solutions.The second area relates to modeling: generating data for or conducting population dynamics and management research and then developing practical guidance with uptake by the intended audiences. Birth and death rates, immigration and emigration, are the foundations for this modeling and also our roots in descriptive epidemiology. Implications and development of evidence-driven ordinances and guidelines as well as local grassroots and animal welfare activities should flow from these endeavors.The third role for epidemiologists is in recognizing and evaluating the consequences of the welfare and health of the cats themselves. This focus is related to the development and use of valid and reliable instruments to measure cat welfare and to gain insights into the human perceptions and activities surrounding cat welfare. Assessment of common clinical presentations in these populations and success of treatments in returning these cats to their outdoor homes as well as the costs and benefits of investing in treatments (for the cats and human residents) need creative and valid examination.Epidemiologists study health and disease in populations! In the veterinary world, we need to keep on doing that for free-roaming unowned cats. It not only helps the cats and the many citizens who care about them but also, I argue, enriches and betters the communities in which they live.

Notes:

135 - Antimicrobial resistance in the different stages of commercial poultry production environment

Pankaj Gaonkar[1], Alinne Santana-Pereira[1], Alyssa Lambert[1], Reed Golden[1], Courtney Higgins[1], Yagya Adhikari[2], Matthew Bailey[2], Kenneth Macklin[3], Laura 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: Antimicrobial resistance 2, 2025-01-20, 2:00 - 2:15

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 to determine the AMR level in the environment of different stages of commercial poultry farms.

Methods: Commercial poultry farms in different stages of production and practicing restricted antimicrobial use were included in this study. Antimicrobial use (AMU) data were collected, along with litter samples from inside the poultry house and soil samples from outside the poultry house. Carcass rinses were collected from the processing plant from the 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. Metagenomics was performed on litter and soil 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. Litter samples had AMR to majority of antimicrobial classes, especially higher in broiler followed by breeder and pullet. However, there were exceptions: tetracycline and beta-lactam were comparable in breeder and broiler litter, while sulfonamide and antiseptic were higher in breeder. Overall, soil had lower AMR and stayed constant among different farm types, with few exceptions: MLSB ARGs were higher in the broiler, sulfonamide ARGs higher in pullet and breeder, and bacitracin ARGs higher in the broiler. In the processing plant, the majority of ARGs were restricted to post-pick stage and aminoglycoside ARGs were found in post-chill stage. MGEs were most frequently found in litter and were consistent across the farm types. Alpha diversity was higher in the soil compared to litter. Additionally, distinct microbial composition was observed between litter and soil. Pullet and breeder had almost similar compositions, while broiler had distinct compositions. Firmicutes, Actinobacteria, Proteobacteria, and Bacteroidetes were the most frequently shared phyla between sample and farm types. Furthermore, results at the species level will be explored.

Conclusions: Despite restricted AMU, there is potential for AMR to spread in commercial poultry farm environments and production chains. The historical use of antimicrobials may have resulted in the persistence of ARGs in the environment. The shared microbial communities between inside and outside the poultry house exhibit a potential risk of AMR dissemination. Furthermore, the presence of ARGs in the post-chill stage of the processing plant poses a potential public health risk due to possible contamination of poultry meat. Understanding the environmental aspects of AMR spread will help protect the health of poultry, humans, and the environment.

Notes:

136 - Does antibiotic treatment change the microbial resistome of mares?

Enrique Doster[1], Lee J. Pinnell[1], Stephen Tamm[1], Gregg O. Veneklasen[2], Maggie M. Murphy[1,3], Cory A. Wolfe[1], Kelli N. Beavers[4], Hugh R. Duddy[1], Mark G. Papich[5], Jessica M. Looman[2], Victoria Smalley[2], Sheyanne Pruett[2], Andrew Veneklasen[2], John L. Pipkin[3], Paul S. Morley[1]

[1]VERO Program, Texas A&M University, [2]Timber Creek Veterinary Hospital, Canyon, TX, [3]West Texas A&M University, [4]College of Veterinary Medicine, Oklahoma State University, [5]College of Veterinary Medicine, North Carolina State University. pmorley@tamu.edu

Session: Antimicrobial resistance 2, 2025-01-20, 2:15 - 2:30

Objective: Antimicrobial resistance (AMR) presents a global challenge. Veterinarians and caretakers require access to antimicrobial drugs (AMDs) to treat bacterial infections in horses, but it is crucial to prioritize AMD use practices that minimize potential impact of AMR on horses and the public. To assess the effects of common antimicrobial treatment regimens on microbial communities (microbiome) and AMR genes (resistome) in three biological niches: feces, nasal passage, and vagina.

Methods: In this randomized clinical trial, 40 healthy mares used as embryo transfer recipients were assigned to 5 groups: Group 1 – gentamicin sulfate IM and penicillin G procaine IM; Group 2 – oxytetracycline IV; Group 3 – ceftiofur sodium IM; Group 4 – sulfadiazine-trimethoprime PO; Group 5 – untreated controls. Antibiotic treatments were administered q24hrs for 4 days. Samples were collected on Days 0, 1, 5, 6, 14, 15, 21, and 22 for microbiome and resistome analysis using 16S rRNA gene sequencing and target-enriched shotgun sequencing, respectively. Statistical analyses included Pairwise Wilcoxon rank-sum tests with Benjamini-Hochberg correction for alpha diversity, and ANCOM-BC and pairwise PERMANOVA with Benjamini-Hochberg correction for beta diversity.

Results: All antibiotic treatments significantly impacted the microbiome and resistome compared to untreated controls. Effects were most pronounced immediately post-treatment, with some persisting for 16 days. Impacts varied across biological niches.

Conclusions: Common AMD treatments were associated changes in the microbiome and resistome, with varying magnitude and duration of effects. Microbiome and resistome of horses with bacterial infections may differ from healthy horses and may responding differently to AMD exposures. Comingling of horses by group may also have affected microbiome and resistome composition. Further research should investigate whether these changes influence clinical response to subsequent treatments.

Financial Support: This work was supported by Grayson Jockey Club Foundation

Notes:

137 - Impact of commercial vaccination on the ecology and antimicrobial susceptibility testing of avian *Escherichia coli* in turkey production

Lauren M. White[1], Kari M. Mattison[2], Randall S. Singer[2], Timothy J. Johnson[2]

[1]Dept. of Animal Science, University of Minnesota, [2]Dept. of Veterinary and Biomedical Sciences, University of Minnesota. whit3548@umn.edu

Session: Antimicrobial resistance 2, 2025-01-20, 2:30 - 2:45

Objective: *Escherichia coli* is ubiquitous in the intestinal microflora of animals. Some strains of *E. coli* have enhanced ability to cause disease in poultry, but disease is often a result of the balance between stress levels in the bird and the type of *E. coli* strain present. Avian pathogenic *E. coli* (APEC) refer to strains with shared genetic traits resulting in an increased capacity to cause colibacillosis and have been well characterized. However, less is known about the overall ecology of *E. coli* in poultry production and how ecology changes following mitigation.

Methods: This study examined *E. coli* collected from a field trial following commercially vaccinated barns (n=4) and control barns (n=4) within a single vertically integrated commercial turkey company. The vaccinated group was sprayed with a commercial *E. coli* vaccine following manufacturer's recommendations. Cloacal swabs (n=489), tracheal swabs (n=394), boot sock samples (n=82), and litter samples (n=451) were collected from each barn at different time points, ranging from 0-19 weeks of age. In total, 1,416 *E. coli* isolates were examined from the 8 barns sampled. From each isolate recovered, multiple multiplex PCR assays were used to determine *E. coli* phylogenetic group, status of an isolate as APEC, and status of an isolate as a high-risk APEC clone. Fisher's exact test was run to determine significance between the sample types amongst the age groups for each individual treatment type. Lastly, a subset of 300 tracheal isolates (n=150 per treatment group) were also assessed for antimicrobial susceptibility using broth microdilution. To determine the significance of the MIC results per each isolate from the subset data, a chi-square test was used to identify differences in antimicrobial susceptibility.

Results: Overall, no differences in APEC proportions were found between vaccinated and non-vaccinated flocks. A notable increase in proportions of isolates classified as APEC were observed from the brood through the growout periods. High-risk APEC clones were observed at higher frequency within the vaccinated group when compared to the control group for growout birds, but the overall prevalence of high-risk clones was low. Significant differences were observed in Clermont phylogenetic groups in control versus vaccinated flocks. During the brood period, the vaccinated flocks showed decreases in proportions of isolates belonging to the B1 and C phylogenetic groups, to which the vaccine origin strain belonged. During the growout phase, the vaccinated flocks showed increases in the proportion of isolates belonging to the B2 phylogenetic group. No notable differences were observed for antimicrobial susceptibility profiles between control and vaccinated flocks from tracheal isolates. Relatively high proportions of isolates with decreased susceptibility towards sulfisoxazole and tetracycline were observed, irrespective of treatment group. The prevalence of decreased susceptibility among isolates was low for other antibiotics tested.

Conclusions: This work highlights the importance of considering the overall ecology of *E. coli* as it relates to use of mitigation strategies in poultry production, and suggests that commercial vaccination has an impact on the ecology of *E. coli* even in healthy flocks.

Financial Support: This work is supported by Agricultural and Food Research Initiative (AFRI) grants 2018-68003-27464 and 2020-67015-31678 from the USDA National Institute of Food and Agriculture.

Notes:

138 - Exploring the intestinal colonization, antimicrobial sensitivity, and resistance gene profiles of *Klebsiella* in shelter dogs on Long Island, New York

Rachel M Weninger[1], Reta D Abdi[1]

[1]Department of Biomedical Science, Long Island University. rachel.weninger@my.liu.edu

Session: Antimicrobial resistance 2, 2025-01-20, 2:45 - 3:00

Objective: *Klebsiella* sp. is a global threat to public health due to its high rate of acquisition of antimicrobial resistance (AMR) genes and its pathogenicity to humans and animals. Intestinal colonization is a risk factor for infection and transmission between humans, animals, and the environment, but less studied in shelter dogs waiting for adoption. Therefore, we evaluated the abundance of *Klebsiella* sp. in the intestine of shelter dogs, AMR genes, and their response to antimicrobials by disc diffusion tests.

Methods: Isolation of *Klebsiella* sp. was achieved by culturing feces from 92 shelter dogs of different age, sex, and fertility status (intact vs. spayed/neutered) on *Klebsiella* CHROMOSelect Selective Agar Base. DNA was extracted for each isolate by boiling in Tris-EDTA-NaCl-Triton X-100 buffer followed by chilling and centrifugation. Antimicrobial sensitivity testing against eight antimicrobials (cefpodoxime, cefotaxime, cefotaxime & clavulanic acid, ceftazidime, ceftazidime & clavulanic acid, cefoxitin, tetracycline, and Cefiderocol) was performed for each isolate. We also screened the isolates for presence of beta-lactamase (ESBL) genes (CTX-M, SHV, TEM), tetK, and tetO genes using PCR. Data was analyzed via $\chi 2$ tests to establish significance ($p<0.05$) between demographic information (age, sex, fertility status, and treatment with antibiotics within the last two weeks), presence of *Klebsiella* sp. in dogs' feces, presence of genes, and AMR results by disc diffusion.

Results: Of the 92 shelter dogs tested, 34.8% (32/92) were positive for *Klebsiella* sp. Intestinal colonization by *Klebsiella* in shelter dogs was significantly varied between different age groups ($p=0.039$); but not varied by sex ($p=0.683$), fertility status ($p=0.649$), and treatment with antimicrobials within the last two weeks ($p=0.843$). The disc diffusion tests revealed that 84.4% of the *Klebsiella* sp. species (27/32) were resistant to at least one antibiotic, and the remaining 15.6% (5/32) were pan-susceptible to all antibiotics. Majority of the isolates displayed resistance to tetracycline (62.5%), followed by cefotaxime (59.4%), cefpodoxime (50%), ceftazidime (37.5%), and cefoxitin (31.3%). Cefotaxime & clavulanic acid, ceftazidime & clavulanic acid, and Cefiderocol were the most effective, inhibiting all of 32 isolates (100%). *Klebsiella* of shelter dogs significantly harbored CTX-M ($p<0.000$), tetK ($p<0.000$), and SHV ($p=0.034$) AMR genes. Overall, the studied 32 isolates displayed nine different antimicrobial sensitivity patterns phenotypically and nine different genotypic patterns by their AMR gene profiles.

Conclusions: *Klebsiella* sp. carrying diverse antimicrobial sensitivity profiles, ESBL genes, and tet genes colonized the intestine of shelter dogs waiting for adoption, indicating dogs were a reservoir for AMR *Klebsiella*. All 32 *Klebsiella* sp. isolates from feces of dogs harbored at least two AMR genes, with CTX-M (100%) and tetK (93.8%) being the most common. Ceftazidime & clavulanic acid, cefotaxime & clavulanic acid, and Cefiderocol were the most effective antimicrobials, but these antimicrobials are poorly available for animal use. All shelter dogs tested were adoptable, putting potential adopters at risk; therefore, a coordinated effort between pet adopters, shelter managers, and human and veterinary medical professionals is crucial to stay ahead of the curve and protect people and their pets.

Financial Support: Research is supported by Long Island University.

Notes:

139 - Characterization of ceftiofur-resistant Gram-negative bacteria recovered from dairy cattle

D. Gregory[1], K.A. Vasco[1], S.L. Carbonell[1], R. Mani[2], K. Dodd[2], P. Ruegg[3], S. Manning[1]

[1]Department of Microbiology, Genetics, and Immunology, Michigan State University, [2]Veterinary Diagnostic Laboratory, Michigan State University, [3]Department of Large Animal and Clinical Sciences, Michigan State University. grego261@msu.edu

Session: Antimicrobial resistance 2, 2025-01-20, 3:00 - 3:15

Objective: Increasing frequencies of antibiotic resistance have been linked to overuse of antibiotics in human and veterinary medicine. To understand how the use of cephalosporin, a critically important antibiotic, impacts dairy cattle, we previously enrolled 24 cows at the start of the dry off period; half of the cows were given intramammary ceftiofur as dry-cow therapy and half were given a non-antibiotic teat sealant. Fecal samples were collected weekly for 9 weeks and cultured to isolate and quantify Gram-negative, ceftiofur-resistant (CefR) bacteria. The goal of this follow-up study was to characterize these CefR bacteria and examine cephalosporin-resistance transferability to non-pathogenic *Escherichia coli* .

Methods: The 278 fecal samples obtained from the 24 cows were plated on MacConkey agar with ceftiofur (8ug/mL) to recover CefR bacteria. Among the 67 (24%) samples with any CefR bacteria, up to four morphologically distinct colonies were selected from each plate for characterization by culturing on selective media and MALDI-TOF. Antibiotic susceptibility testing for erythromycin (4ug/mL), tetracycline (16ug/mL), ciprofloxacin (1ug/mL), and kanamycin (64ug/mL) was also performed using the Clinical and Laboratory Standards Institute guidelines. Conjugation assays were conducted for a subset of 56 isolates, and cephalosporin-resistance transfer frequencies were calculated using a rifampicin-resistant *E. coli* K12 recipient.

Results: In all, 139 CefR isolates were recovered. Use of selective media classified most (n=96; 69.1%) of the CefR isolates as *E. coli*, which was confirmed by MALDI-TOF. Among the 43 non-*E. coli* CefR isolates, MALDI-TOF classified 12 (27.9%) as Acinetobacter baumannii, 17 (39.5%) as Achromobacter spp., 9 (20.9%) as Ochrobactrum spp., 4 (9.3%) as Escherichia fergusonii, and 1 (2.3%) as Acinetobacter indicus. Susceptibility testing demonstrated that over a third (n=53; 38.1%) of the 139 isolates were multidrug resistant (MDR) with resistance to ≥3 antibiotic classes (including ceftiofur). Conjugation assays revealed transfer of cephalosporin resistance to *E. coli* K12 from 46 of 56 (82.1%) CefR isolates examined to date.

Conclusions: Although the number of CefR isolates recovered from dairy cattle was small, these bacterial hosts serve as reservoirs of resistance determinants that can facilitate the spread of resistance to ceftiofur and other antibiotics within the farm environment. Indeed, most of the isolates examined were capable of transferring cephalosporin resistance horizontally to a non-pathogenic *E. coli* strain. Characterizing the resistant bacterial populations that circulate in food animals and defining their contribution to the spread of resistance, is essential to develop strategies that can combat antimicrobial resistance.

Financial Support: Support was provided by USDA 2019-67017-29112.

Notes:

140 - Integron-encoded antimicrobial resistance genes in *Salmonella* Typhimurium isolated from dairy cattle

Sami U.K. Bahadur[1], Nora J. Nealon[1, 2], Roberta Magnuson[1], Josh Daniels[1], Mo Salman[1], Sangeeta Rao[1]

[1]College of Veterinary Medicine and Biomedical Sciences, Colorado State University, [2]College of Veterinary Medicine, The Ohio State University. sami.bahadur@colostate.edu

Session: Antimicrobial resistance 2, 2025-01-20, 3:15 - 3:30

Objective: Antimicrobial resistance (AMR) has emerged as one of the top global public health threats, with *Salmonella enterica* serovar Typhimurium (S. Typhimurium) being a key pathogen linked to foodborne illnesses and the transmission of antibiotic-resistant strains. Integrons, which capture and disseminate antimicrobial resistance genes (ARGs), play a critical role in the horizontal transfer of resistance genes, contributing significantly to the spread of AMR among bacterial populations. The objective of this study is to identify the genetic locations of integrons and antimicrobial resistance (AMR) genes in *Salmonella* Typhimurium isolates from dairy cattle, emphasizing on the role of integrons in AMR gene carriage.

Methods: A total of 33 *Salmonella* Typhimurium isolates were collected and confirmed from two Veterinary Diagnostics Labs across the United States. Polymerase chain reaction (PCR) was used to amplify the conserved 5' and 3' regions of class I integrons, followed by sequencing. DNA was extracted, quantified, barcoded, and sequenced using the Illumina MiSeq platform. Trimmed sequences were assembled with the Tadpole assembler in Geneious Prime, and plasmids were identified using the PlasmidFinder tool on the CGE webpage, with validation against NCBI sequences. Location of Integrons was determined by aligning sequences with contigs and identified plasmids, while antimicrobial resistance (AMR) genes were identified through BLAST against the MEGARes 3.0 database and their locations confirmed by their alignment with the isolate, plasmid, and integron sequences. Statistical analysis was performed using Fisher's exact test.

Results: A total of seven plasmid types were identified among the *Salmonella* Typhimurium isolates: IncFII(S) in 14 isolates (42.42%), IncFIB(S) in 13 isolates (39.39%), IncC in 8 isolates (24.24%), Inc1-I(Alpha) in 3 isolates (9.09%), and ColpVC, Col(pAHAD28), and Col8282, each present in 1 isolate. Among the 15 isolates with a 1000 bp integron, 12 of the integrons were located on the IncFIB(S) plasmid, while 2 were on the IncC plasmid and 1 on the chromosome; 1200 bp integron from all 12 isolates were located on chromosomes ($p < 0.05$). Furthermore, significant associations were detected between integrons and resistance genes sul1, aadA2, blaCARB02, and floR ($p < 0.01$), while no significant association was detected with the blaCMY02 gene ($p = 0.29$). The locations of specific AMR genes included aadA2 located on both IncFIB(S) and IncC plasmids; blaCARB-2 on the chromosome; blaCMY-2 on IncC plasmid and chromosome; floR on the chromosome; qacEdelta1 on IncFIB(S) IncC, and chromosome; and tet(A) and tet(G) all on the chromosome.

Conclusions: These findings indicate that Integrons have role in the presence and location of AMR genes in *Salmonella* Typhimurium isolates from dairy cattle. This study highlights the importance of monitoring integrons in understanding the dynamics of AMR, which is crucial for developing effective strategies to combat the issue in foodborne pathogens and to safeguard public health.

Notes:

141 - Novel antibiotic alternative approaches for controlling *Salmonella* infections in vitro

Bibek Lamichhane[1], Yosra A. Helmy[1]

[1]University of Kentucky. bla260@uky.edu

Session: Disease therapeutics, 2025-01-20, 2:00 - 2:15

Objective: *Salmonella* is one of the common causes of foodborne gastroenteritis in animals and humans. Poultry and poultry products serve as primary sources of infections in humans. *Salmonella* infections in animals generally occur due to the consumption of contaminated food and water. Currently, *Salmonella* infections in both animals and humans are treated using antibiotics such as ciprofloxacin and enrofloxacin. However, increasing antimicrobial-resistant *Salmonella* has necessitated the development of alternative therapeutic strategies such as probiotics. The objective of this study is to evaluate the effect of the next-generation probiotics (NGPs) against S. Typhimurium in vitro. This study aims to develop NGPs as viable antibiotic-alternative therapeutics for controlling S. Typhimurium infections in both animals and humans.

Methods: We screened 38 different probiotic strains for their effect in the growth of S. Typhimurium using an agar-well diffusion assay. Probiotics that demonstrated the biggest zone of inhibition of S. Typhimurium's growth were subjected to further development in vitro. These probiotics were evaluated for their effect in inhibiting S. Typhimurium's growth in liquid media using co-culture assay. The probiotics-supernatants were further evaluated for their ability to inhibit biofilm formation and pre-formed biofilms of *Salmonella*. They were also tested for their effect on adhesion, invasion, and intracellular survival of S. Typhimurium in human intestinal cells (Ht-29 MTX cell lines). Furthermore, we also evaluated the effect of each probiotic strain on the expression of virulence-associated genes of S. Typhimurium using RT-PCR. Each of the experiments were repeated at least twice, and the results were evaluated using two-way ANOVA for multiple comparison using Tukey analysis.

Results: All the probiotic strains effectively inhibited the growth of S. Typhimurium on agar well diffusion assay with different efficacies, however, the best 7 probiotics that possessed the biggest zone of inhibition of S. Typhimurium were selected for further development in vitro. All the selected candidates significantly inhibited ($p<0.05$) the growth of the bacteria when cocultured in liquid media. They also inhibited the growth of other *Salmonella* strains. Three out of the seven selected candidates inhibited up to 100% of S. Typhimurium's biofilm formation and pre-formed biofilms. Interestingly, all the selected candidates significantly ($p<0.05$) inhibited adhesion, invasion, and intracellular survival of S. Typhimurium in Ht-29 MTX cell lines. Additionally, all seven NGP candidates significantly downregulated the genes associated with the expression of virulence factors such as biofilm formation, quorum sensing, motility, and invasion.

Conclusions: Our findings highlight NGPs as promising antibiotic alternatives to control *Salmonella* infections. In the future, we will continue the in vitro evaluations and we will focus on understanding how these probiotics mediate their actions on the cells. Our result will facilitate the establishment of probiotics as alternatives to antibiotics for controlling *Salmonella* infections in animals and humans.

Financial Support: This research was funded by the National Center for Advancing Translational Sciences, National Institutes of Health (grant number KL2TR001996).

Notes:

142 - Novel quorum sensing inhibitors as potential therapeutics for the control of *Salmonella* infections

Yosra A. Helmy[1], Bibek Lamichhane[1], Ajran Kabir[1], Khaled A. Shaaban[2], Larissa V. Ponomareva[2], Jon S. Thorson[2]

[1]Department of Veterinary Science, University of Kentucky, [2]Department of Pharmaceutical Sciences, University of Kentucky. yosra.helmy@uky.edu

Session: Disease therapeutics, 2025-01-20, 2:15 - 2:30

Objective: *Salmonella* Typhimurium is a major foodborne pathogen transmitted through contaminated food and water. While poultry and poultry products are the primary sources of infection, other animals also contribute significantly to the transmission of this pathogen to humans. The emergence of multidrug-resistant (MDR) *Salmonella* strains has created an urgent need to develop alternative treatment strategies. Quorum sensing (QS) is a cell-to-cell communication process that allows the bacteria to detect and respond to their cell population density and regulate its virulence. This communication occurs via signaling molecules known as autoinducers 2 (AI-2). Small molecules (SMs) can potentially act as AI-2 inhibitors, leading to quorum sensing inhibition. This study aims to screen small molecule QS inhibitors and evaluate their effect on virulence, and biofilm formation of *Salmonella* in vitro.

Methods: Overall, 1,900 SMs were assessed for their effect on the production of AI-2 and QS in S. Typhimurium. To ascertain the effect of each SM on bacterial growth, bacterial cultures (100µL; OD=0.05) were incubated at 30°C for 6 hours after being treated with 1µL of the SM in 96-well plates (concentration ranging from 10 µM to 0.7µM). SMs demonstrating no significant impact on bacterial growth were subsequently screened via a bioluminescence assay. Cell-free supernatants of treated bacteria were incubated with Vibrio harveyi BB170 to evaluate their effect on AI-2 production. SMs exhibiting the highest inhibitory activity for AI-2 were then tested for their effect on biofilm formation and the expression of virulence, biofilm, and quorum sensing-associated genes using RT-PCR. Three replicates were performed for each drug and each concentration across all assays. To compare the effect of different concentration, one way ANOVA was performed against the control.

Results: Ten small molecules (SMs) demonstrating over 95% inhibition of AI-2 activity without impacting bacterial growth were selected for further assessment. These compounds exhibited significant inhibition of biofilm formation ranging from 95-100%. Additionally, all 10 compounds downregulated the expression of genes linked to quorum sensing, virulence, biofilm development, and motility.

Conclusions: QS inhibitors offer a promising new strategy to combat *Salmonella* infections and mitigate the rising threat of antibiotic resistance in this major foodborne pathogen. To accelerate the in vitro development of these SMs as antibiotic alternatives, the cytotoxic potential will be assessed on human intestinal and chicken macrophage cell lines.

Financial Support: This research is supported by the Center of Biomedical Research Excellence for Translational Chemical Biology (COBRE, NIH P20 GM130456).

Notes:

143 - Treatment of equine thorax wounds with mesenchymal stromal cell secretome

Megan J. Fahey[1], Rebecca M. Harman[1], Matthew A. Thomas[2], Brenna R. Pugliese[2], Jeanine Peters-Kennedy[3], Michelle L. Delco[2], Gerlinde R. Van de Walle[1]

[1]Department of Microbiology and Immunology, Cornell University, [2]Department of Clinical Sciences, Cornell University, [3]Department of Population Medicine and Diagnostic Sciences, Cornell University. rmh12@cornell.edu

Session: Disease therapeutics, 2025-01-20, 2:30 - 2:45

Objective: We aimed to study the antimicrobial and pro-healing potential of equine mesenchymal stromal cell (MSC) secreted products, the secretome, collected as conditioned media (CM), in a novel preliminary in vivo model of methicillin-resistant Staphylococcus aureus (MRSA)-inoculated equine thorax wounds.

Methods: Six full-thickness cutaneous wounds were created bilaterally on the dorsal thorax of two horses (n = 12 wounds/horse). Wounds on the left thoraces were inoculated with MRSA strain FPR3757 on day 0. All wounds were then treated with either mupirocin ointment (positive control), peripheral blood-derived MSC CM, or vehicle control (negative control) (n = 4 wounds per group) once daily for 3 days. At days 0, 1, 2, 3, 7, 14, 21, and 28, wounds were swabbed to count bacterial colony forming units (CFUs) and photographs were taken to quantify wound scores and sizes. Wound edges were biopsied on days 0, 7, and 28, and scored histologically in a blinded manner.

Results: Inoculated wounds had more bacterial CFUs at day 1 ($p < 0.0001$) and were larger in size at day 28 ($p = 0.0009$) when compared to non-inoculated wounds. MSC CM did not affect CFU numbers in inoculated or non-inoculated wounds. Mupirocin-treated wounds were smaller than MSC CM- and vehicle control-treated wounds at day 28 ($p = 0.003$). MSC CM did not affect the parameters of wound size or gross or microscopic wound scores over time.

Conclusions: MSC CM did not exhibit antimicrobial or pro-healing properties in this preliminary study. The in vivo model of inoculated equine thorax wounds requires further optimization, including using MRSA field strains from equine wounds for experimental infection and treating of the wounds with CM from MSCs isolated from bone marrow or other tissue

Financial Support: This work was funded by USDA-NIFA-AFRI-Foundational grant/award number 2022-67015-36351.

Notes:

144 - Endotoxemia and the pathogenesis of lipolysis dysregulation in periparturient dairy cows

GA Contreras[1], AL Lock[1]

[1]Michigan State University. contre28@msu.edu

Session: Disease pathogenesis, 2025-01-20, 6:00 - 8:00

Objective: Nearly 50% of periparturient dairy cows in the U.S. experience health issues that affect their welfare and cause economic losses. These illnesses often involve metabolic and inflammatory or infectious disease complexes. During this period, intense lipolysis in adipose tissue (AT) provides fatty acids as an energy source to counteract the negative energy balance caused by lactogenesis and limited appetite. In healthy cows, lipolysis induces a temporary inflammatory response marked by anti-inflammatory adipose tissue macrophages (ATM). However, cows at higher disease risk experience chronic inflammation and lipolysis dysregulation, shifting ATM toward pro-inflammatory phenotypes. This shift reduces adipocyte insulin sensitivity, leading to excessive lipolysis. Understanding the mechanisms driving ATM pro-inflammatory polarization and impaired insulin sensitivity in these cows remains a critical research gap. Elevated bacterial endotoxins (ET) during parturition exacerbate this issue by directly inducing lipolysis and promoting pro-inflammatory ATM polarization, disrupting the inflammatory process. We demonstrated that ET triggers lipolysis and impairs insulin responses, but the specific effects on adipocytes and ATM remain unclear. This study explores how ET contributes to lipolysis dysregulation, hypothesizing that ET exposure around parturition activates toll-like receptor signaling, causing an inflammatory response in AT and insulin resistance.

Methods: To develop the hypothesis, we utilized an in vivo model of endotoxemia in mid-lactation cows, an ex vivo model of lipolysis dysregulation in AT explants, and an in vitro model of bovine adipocytes to investigate signaling mechanisms. Additionally, we analyzed blood and AT samples from cows with naturally occurring clinical ketosis within the first 10 days post-calving. Samples from these models and natural cases of clinical ketosis were examined using genomics, proteomics, and lipidomics analyses. In addition, we used functional assays, including ex vivo lipolytic challenges in the presence of insulin, ET, and adrenergic stimulation.

Results: We demonstrated that ET activate both canonical and inflammatory lipolytic pathways and reduce insulin sensitivity in AT in a TLR4-dependent manner, signaling through oxylipins, particularly prostaglandins and their receptors in adipocytes. In a commercial herd, 72.2% of cows with clinical ketosis exhibited endotoxemia, which was associated with higher blood concentrations of dyslipidemia markers, including NEFA and BHB, along with lower glucose and insulin levels. In AT from ketotic cows, endotoxemia was found to reduce Akt phosphorylation, increase the expression of inflammatory gene markers such as CCL2, IL8, IL10, TLR4, and TNF, enhance ERK1/2 phosphorylation, and promote ATM infiltration.

Conclusions: These data provide evidence that during endotoxemia, dairy cows may be more susceptible to lipolysis dysregulation and loss of adipocyte sensitivity to the antilipolytic action of insulin due to inflammatory responses within the organ that include macrophage infiltration. Current studies are determining the mechanisms leading to ATM phenotypic changes and their impact on adipocyte metabolic and immune functions.

Financial Support: This project was supported by Agriculture and Food Research Initiative Competitive Grant no. 2021-67015-34563 from the USDA National Institute of Food and Agriculture, Michigan Alliance for Animal Agriculture (21-154;22-055), Sterner Fund, and Robert and Janet Hafner Fund for Animal Health.

Notes:

145 - Therapeutic efficacy of linalool to reduce *Salmonella* Enteritidis and *Campylobacter jejuni* in market-age broiler chickens

Leya Susan Viju[1], Divya Joseph[1], Kumar Venkitanarayanan[1]

[1]University of Connecticut. leya.viju@uconn.edu

Session: Disease therapeutics, 2025-01-20, 3:00 - 3:15

Objective: *S.* Enteritidis (SE) and *C. jejuni* (CJ) are major foodborne pathogens transmitted through poultry products, contributing to over 70% of foodborne infections and hospitalizations, and 55% of the deaths related to foodborne illnesses in the United States. Chickens are the reservoir host of SE and CJ, with their intestinal colonization constituting the most significant factor causing faecal shedding and meat contamination. Broiler carcasses are reportedly among the meats most contaminated with these two pathogens from processing facilities. Therefore, reducing SE and CJ populations in chickens could potentially reduce the contamination of poultry meat and products. This study investigated the efficacy of infeed supplementation of a GRAS (generally recognized as safe)-status phytochemical, namely linalool as a pre-slaughter dietary strategy to decrease SE and CJ carriage in poultry.

Methods: Day-old commercial broiler chicks (N = 60) were randomly assigned to 6 groups of 10 birds each (n = 10/group) consisting of the control group (challenged with SE/CJ, no linalool), SE treatment group (birds challenged with SE and feed supplemented with 1.5 or 1.8% [vol/wt] linalool) and CJ treatment group (birds challenged with CJ and feed supplemented with 1.5 or 1.8% [vol/wt] linalool). Birds were given ad libitum access to feed and water. On day 25, birds were challenged with a 4-strain mixture of SE or CJ (8 log10 CFU/bird). Two birds from each group were euthanized after 48 hours to check for colonization of SE and CJ in the cecum. Birds were given feed supplemented with linalool (1.5 or 1.8%) for 5 days before slaughter on day 42 (n = 8 birds/group) for determination of SE and CJ populations in the cecum. The experiment included two replications.

Results: Linalool supplementation at 1.5 and 1.8% significantly reduced SE counts in the cecum by 2 log10 CFU/g cecum and 4 log10 CFU/g cecum, respectively ($P < 0.05$). Linalool supplementation at 1.8% significantly decreased CJ counts in the cecum by 5 log10 CFU/g cecum ($P < 0.05$). There were no significant differences in the body weight gain, feed intake and feed conversion ratio of the linalool supplemented groups and control birds ($P > 0.05$).

Conclusions: The results suggest that linalool could effectively be used as a pre-slaughter dietary supplement for reducing SE and CJ counts in broiler chickens without adversely affecting production parameters. This could potentially control transmission of SE and CJ through poultry products.

Financial Support: USDA-AFRI Sustainable Agricultural Systems Grant: 2020-69012-31823

Notes:

146 - Short-term efficacy of enrofloxacin in reducing *Mesomycoplasma ovipneumoniae* infection without sustained clearance in domestic lambs

Sobha Sonar[1], Diane Bimczok[1], B. Tegner Jacobson[1], Stephen Smith[2], Jonathan Sago[2], Nathan Schaff[1], Eli Selong[1], Mckenna Quirk[1], Helen Schwantje[3]

[1]Montana State University, [2]Montana Veterinary Diagnostic Laboratory, [3]Ministry of Forest, Land and Natural Resources, BC, Canada. sobha.sonar@student.montana.edu

Session: Disease therapeutics, 2025-01-20, 3:15 - 3:30

Objective: *Mesomycoplasma ovipneumoniae* (*M. ovi*) is a highly common, facultative respiratory pathogen causing acute pneumonia and reduced productivity in domestic sheep. At present, whether M. ovi infection can be successfully treated with antibiotics is unclear. Our study aimed to assess the treatment effects of the antibiotic-enrofloxacin (Baytril®) in domestic sheep, experimentally infected with M. ovipneumoniae.

Methods: Ten 4-month-old specific-pathogen-free lambs were randomly assigned to two groups, a treatment, and an untreated control group. Lambs were inoculated with fresh nasal wash fluids from *M. ovi*-positive sheep and monitored for four weeks. The treatment group then received enrofloxacin (Baytril®) at a dose of 0.5 mg/kg in 200 mL of PBS as a nasal wash and 5 mg/kg subcutaneously for 5 days, whereas the control group did not receive any treatment. All the lambs were monitored for an additional four weeks, with daily disease scoring, weekly assessment of weight gain, and weekly qPCR analysis of nasal swabs for *M. ovi* colonization to assess treatment efficacy. For the end point analyses, all the lambs were euthanized and swabs samples from the caudal nasal cavity, paranasal sinuses, trachea and bronchi were collected to analyze *M. ovi* colonization. In addition, the lung tissue samples were collected for histopathological evaluation.

Results: Baytril® administration led to a significant decrease in the total lung histopathology score (Mann-Whitney test, p=0.032) in the treated compared to the untreated control group. However, there was no significant difference in the average weight gain and the total disease score of the lambs in the two groups. Interestingly, all treated lambs tested negative for *M. ovi* by qPCR after the first week of the treatment. However, there was a re-emergence of infection in the second week of treatment with 1/5 lambs testing positive at that week and 3/5 lambs testing positive at the end time point (fourth week after treatment) in the treatment group. The *M. ovi* infected lambs in the untreated control group tested *M. ovi* positive throughout the entire experiment. At the experimental end point the tissue sample qPCR analysis revealed a trend of decreased *M. ovi* detection in the bronchi and trachea of the treated lambs compared to the untreated control lambs.

Conclusions: Overall, the study shows that the antibiotic enrofloxacin (Baytril®) can temporarily suppress the *M. ovipneumoniae* infection in the upper respiratory tract of experimentally infected sheep. Importantly, Baytril® treatment was associated with improved health outcomes and reduced lung pathology. The mechanism for the reemergence of *M. ovipneumoniae* in the second week of treatment is the subject of further investigations.

Financial Support: 1. USDA NIFA AFRI Animal Health & Disease Program, grant # 2022-67016-36503. 2. Montana Agricultural Experiment Station (MONB00450). 3. National Institutes of Health Institute of Translational Health Sciences (UL1 TR002319). 4. Predoctoral Fellowship from USDA NIFA, grant # 2023-67011-40356

Notes:

147 - Interferon stimulated genes as predictive morbidity markers in calves following in-utero viral infection

Caroline K. Griffin[1], Andrea S. Lear[1], Morgan L. Adkins[1], Jon Beever[2]

[1]University of Tennessee College of Veterinary Medicine, [2]University of Tennessee Institute of Agriculture Genomics Center. cbenham@vols.utk.edu

Session: Immunology 1, 2025-01-20, 2:00 - 2:15

Objective: During pregnancy, viral infections cause a potent inflammatory response which is linked to adverse pregnancy outcomes with increased risk of fetal morbidity and mortality. Interferon stimulated genes (ISGs) and their proinflammatory products are increased in placental and fetal tissues following in-utero viral infection. An overactive proinflammatory response by the fetal placenta may induce lasting changes to the fetal immune system, resulting in lifelong consequences. Chronic inflammation due to sustained stimulation of the type I interferon (IFN) pathway has been suggested as a mechanism of long-term health consequences from gestational infection with viruses like Zika virus in humans and bovine viral diarrhea virus (BVDV) in cattle. In this study, we compared changes in placental and circulating ISG products in calves exposed to in-utero viral infection and in healthy control calves. The objective was to observe measurable changes in placental ISGs and circulating ISG products in offspring following in-utero viral exposure. We hypothesized that gestational viral infection would result in an increased type I IFN response with a measurable increase in ISG expression in the placenta of infected calves versus healthy controls. We also hypothesized that calves exposed in-utero would have increased circulating ISG products during the neonatal (birth) and calfhood (three months) phases of life.

Methods: Seventeen pregnant, nulliparous beef heifers were enrolled in this study and randomly allocated to groups: 1) control (n=5): pregnant heifers receiving sham inoculation at gestational day (GD) 70 and 200, 2) persistent infection (PI) (n=5): pregnant heifers inoculated with BVDV at GD 70, 3) transient infection (TI) (n=8): pregnant heifers inoculated with BVDV at GD 200. Heifers were allowed to calve naturally under field conditions. Placental tissue was collected after passage of fetal membranes and flash frozen with liquid nitrogen for RNA-Seq with next-generation sequencing (NGS). Whole blood was collected from calves at birth prior to colostrum consumption and again at three months of age prior to vaccination. Serum was separated and stored at -80°C prior to cytokine/chemokine measurement with the MILLIPLEX® Bovine Cytokine/Chemokine Magnetic Bead Panel-1. The effects of treatment, time, and their interaction on cytokine concentrations were analyzed using mixed model analysis for repeated measures and post-hoc multiple comparisons with Tukey's adjustment.

Results: Results indicate that PI and TI calves had significantly decreased concentrations of pro-inflammatory cytokines and ISG products TNF-α ($p<0.01$) and IFN-γ ($p<0.0001$), respectively, at birth compared to CTRL calves. There is a general trend towards lower circulating cytokine and chemokine concentrations at birth in PI and TI calves compared to CTRL calves. The placental RNA library preparation is ongoing with analysis to follow soon.

Conclusions: Findings suggest a dysregulated immune response in PI and TI calves following in-utero viral infection with BVDV. Instead of a chronic pro-inflammatory state, in-utero viral infection may be associated with decreased immune function at birth and into calfhood. This data will be compared to the placental RNA-Seq/NGS analysis for further conclusions. Future research will focus on the mechanisms of immune system dysregulation and chronic inflammatory conditions in offspring secondary to in-utero viral infection.

Financial Support: FY25 USDA Animal Health and Disease Research

Notes:

148 - Bovine humoral immune response to the major piroplasm surface protein of *Theileria orientalis* Ikeda

Reginaldo G. Bastos[1, 2], Cynthia K. Onzere[2], Lowell S. Kappmeyer[1], David H. Herndon[1], Chungwon J. Chung[1, 2]

[1]USDA, [2]Washington State University. reginaldo.bastos@usda.gov

Session: Immunology 1, 2025-01-20, 2:15 - 2:30

Objective: *Theileria orientalis*, an economically significant tick-borne hemoparasite, infects cattle worldwide. The T. orientalis Ikeda genotype, transmitted by *Haemaphysalis longicornis* ticks, is associated with clinical disease characterized by anemia, abortions, and mortality, although subclinical infections prevail. The Ikeda genotype is endemic in Japan, Australia, and New Zealand, and it is currently considered an emerging tick-borne parasite in the US. The lack of effective diagnostics and vaccines poses additional threats to the global cattle industry. The objective of this study was to investigate the kinetics of the bovine humoral immune response to the immunodominant antigen Major Piroplasm Surface Protein (MPSP) of *T. orientalis* Ikeda.

Methods: The kinetics of IgM, total IgG, IgG1, and IgG2 antibody responses to MPSP in six calves experimentally infected with *T. orientalis* Ikeda sporozoites were analyzed using indirect ELISA (iELISA). Antibody response in the animals was monitored for up to 20 weeks post-infection (WPI). The ELISA results were calculated by dividing the mean OD450 of the test serum by the mean OD450 of the pre-infection serum and were presented as ELISA ratio (ER). Sera with an ER ≥ 3 standard deviations of the pre-infection serum were considered positive. The results of ER for IgM, total IgG, IgG1, and IgG2 at specific timepoints post-infection were compared by the t-test, with a p value <0.05 considered statistically significant. Parasite load in peripheral blood was assessed using quantitative PCR targeting the MPSP gene and reported as copies of MPSP per µl of blood.

Results: Levels of anti-MPSP IgM were initially detected at 2 WPI and peaked at 4 WPI; however, even at its peak, the IgM level was significant lower ($P<0.05$) than the level of total anti-MPSP IgG. The IgM levels declined slightly after 4 WPI. Total anti-MPSP IgG peaked and reached a plateau at 6 WPI (ER of 17.3) with levels significantly higher ($P<0.05$) than those of IgM. The levels of total anti-MPSP IgG remained elevated until 20 WPI (ER of 9.6), at which point the experiment was terminated. Similar peak levels of IgG1 and IgG were detected at 6 WPI ($P=0.23$), and these levels remained elevated until the end of the experiment. The peak of parasite load occurred at 6 WPI, reaching an average of 8,566 MPSP gene copies/µl of blood. During the chronic phase of the infection (12-20 WPI), the parasite load ranged from 772 to 24,521 MPSP gene copies/ µl of blood.

Conclusions: Calves developed significant levels of IgM, total IgG, IgG1, and IgG2 against MPSP following experimental infection with *T. orientalis* Ikeda sporozoites. Despite the robust humoral immune response against MPSP, the parasite load in peripheral blood remained elevated during both the acute and chronic phases of the infection.

Notes:

149 - Immunological impacts following an in-utero BVDV exposure in post-weaning calves

Alyssa M. N. Wilson[1], Marc Caldwell[1], Korakrit Poonsuk[2], Nora L. Springer[1], Xiaocun Sun[3], Andrea S. Lear[1]

[1]University of Tennessee, Large Animal Clinical Sciences, [2]Washington State University, College of Veterinary Medicine, [3]University of Tennessee, Office of Innovative Technologies. awils203@vols.utk.edu

Session: Immunology 1, 2025-01-20, 2:30 - 2:45

Objective: Maternal viral infections can have a profound impact on fetal immune development and neonatal susceptibility of disease(s) in humans and livestock. Calves infected in-utero with bovine viral diarrhea virus (BVDV) have dysfunctional immune responses increasing their susceptibility to early life infections such as bovine respiratory disease. The objectives of this study were to 1) Elucidate the possible mechanisms behind the immunological effect of an in-utero BVDV infection on the immune system; 2) Identify any lasting impacts of a late gestation in-utero BVDV infection on the development of bronchopneumonia in claves.

Methods: Weaned calves exposed to BVDV in-utero during the later stages of gestation, resulting in the birth of a transiently infected (TI) calf and healthy controls, were enrolled in the study. Calves were inoculated with, Mannheimia haemolytica (MH), or control media via broncho-alveolar lavage (BAL). A two-by-two study design was used based on in-utero infection status: control calves (not exposed to BVDV in-utero) inoculated with media (CRTL/CRTL, n=4), control calves inoculated with MH (CRTL/MH, n=3), TI calves (exposed to BVDV in-utero) with media inoculation (TI/CRTL, n=3), and TI calves with MH inoculation (TI/MH, n=5). All groups were assigned clinical illness scores daily with thoracic ultrasound scoring (TUS) throughout the study. MH antibody concentrations were analyzed from serum, and saliva samples collected from all groups. Additionally, cytology analysis as well as biomarker concentrations were measured from BAL fluid samples collected.

Results: Results found that thoracic ultrasound scores and rectal temperature correlates with MH infection status and study day. However, the difference between transient infected calves and control calves has yielded no significant correlations when comparing clinical illness and thoracic ultrasound scoring. There was a significant difference between MH infected and non-MH infected calves detected by thoracic ultrasound score on study day 3 (CTRL/CTRL 0 +/-0); CTRL/MH 2.66 +/-2.08) and rectal temperatures on study day 7 (CTRL/CTRL 102.02 +/-0.35); CTRL/MH 104.5 +/-0.65). MCP-1, VEGF-A, white blood cell count (WBC), lymphocytes, and macrophage concentrations in bronchial fluid were found to be significantly different across all infection groups between study days post MH inoculation (p-value < 0.05). However, 8 proinflammatory cytokines and chemokines were found to have numerically lower mean concentrations, yet not statistically significant, on study day 7 in the TI/MH group compared to the CTRL/MH group. This may be indicative of an immune dysfunction among the TI/MH animals. Antibody concentration analysis and results are pending.

Conclusions: Respiratory infection, detected by TUS, was induced - yet no clinical difference in TI and CTRL calves was observed. A numerical trend was found on study day 7 in the TI/MH biomarker concentrations that may show statistical significance with larger sample size and further investigation.

Financial Support: Thank you to our sponsors at the UTIA Genomics Center for the Advancement of Agriculture and the funding of the USDA NIFA Award No. 2022-67015-36502.

Notes:

150 - Exploring differential metabolic responses in immune cells from naturally parasite-resistant sheep

H.G. Teddleton[1], A.D. Wise[2], A.J. Monteith[2], E. Shepherd[1]

[1]Department of Animal Science, University of Tennessee, [2]Department of Microbiology, University of Tennessee. hteddlet@vols.utk.edu

Session: Immunology 1, 2025-01-20, 2:45 - 3:00

Objective: Grazing ruminants are subject to severe pathologies from gastrointestinal nematode Haemonchus contortus, where infection in young or susceptible animals can cause extreme anemia, and in some cases, death. As such, reduced worm burden and increased survivability is predicated on the early signaling of immune responses. Parasite-resistant St. Croix sheep (STC) generate strong T helper type-2 (TH2) responses resulting in a rapid clearance of H. contortus, while parasite-susceptible Suffolk sheep (SUF) have delayed responses. Metabolism impacts immune cell differentiation, activation and function. Therefore, the metabolic environment can influence the ability of its host to respond to pathogens. However, metabolism of immune cell populations in SUF and STC is not known, nor how this relationship can impact overall host responses and its contribution to susceptibility or resistance against helminth infections. Thus, the objective of this study was to measure mitochondrial respiration in context of helminth infection utilizing a model of parasite susceptibility and resistance in sheep.

Methods: To address this, peripheral blood mononuclear cells (PMBCs) were isolated from parasite naive SUF and STC, which were unstimulated to measure baseline mitochondrial respiration. Oxygen consumption (OCR) and extracellular acidification (ECAR) rates were determined via a Seahorse XFe96 analyzer and analyzed using Wave Desktop software (Aligent).

Results: Preliminary data expressed an increase of extracellular acidification rate (ECAR) in STC-derived PBMCs, proportional to baseline glycolysis, which suggested higher basal immune activation in STC-derived PBMCs. Interestingly, SUF-derived PBMCs consumed oxygen (OCR) at a higher rate when compared to STC-derived PBMCs, however both breeds produced the same amount of ATP, which may indicate that STC-derived PBMCs have lower immune metabolic requirements.

Conclusions: While preliminary data did not express statistical differences of baseline mitochondrial respiration between naive SUF- and STC-derived PBMCs, we hypothesize actively parasitized STC-derived PBMCs have increased metabolic respiration compared to SUF-derived PBMCs. Future studies will focus on how helminth infection impacts cellular respiration in STC and SUF PBMCs.

Financial Support: Principal Investigators startup funding from Ag research at the Herbert College of Agriculture at the University of Tennessee, Knoxville.

Notes:

151 - Utilizing zinc to modulate GLUT4 expression in bovine CD4 lymphocytes

Kathryn Willits Kesler[1], Angel Abuelo[1]

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

Session: Immunology 1, 2025-01-20, 3:00 - 3:15

Objective: Immune responses are bioenergetically demanding, and lymphocytes are dependent on metabolic outputs for their functionality. Lymphocytes switch to aerobic glycolysis when activated. This process is a rapid but substrate-inefficient form of metabolism. Glucose has been shown to be a limiting factor for immune cell effector functions. Insulin is a regulator of glucose homeostasis and primarily drives glucose intracellularly through increased translocation of the transporter GLUT4 to the cell membrane thus facilitating glucose uptake. Zinc (Zn) is an established insulinomimetic that potentiates signaling through the insulin receptor by inhibiting the phosphatases that negatively regulate insulin signaling. This suggests that Zn could be an immunometabolic modulator that is harnessed during critical periods such as early lactation where CD4 T-cells have reduced glycolytic capacity. The objective of this project was to determine the extent to which Zn supplementation increases the expression of glucose transporter GLUT4. We hypothesized that Zn increases GLUT4 expression in bovine lymphocytes.

Methods: Whole blood was collected from adult dairy cattle (n=6). Peripheral blood mononuclear cells were isolated using density gradient centrifugation and CD4 T-cells were enriched using magnetic activated cell sorting. Lymphocytes were incubated with ZnCl2 (50, 100, 1000, or 2500uM) for 30 minutes at 37ºC. Media-only controls were used to establish baseline GLUT4 expression and insulin (100nM) was used to increase GLUT4 expression. Cytotoxicity of treatments was monitored using annexin V and PI staining via flow cytometry. Surface GLUT4 expression was determined using flow cytometry. Since the Shapiro-Wilk test indicated non-normality, the data were transformed according to the results of a Box-Cox analysis (lambda= -0.07). Results were analyzed using a mixed model with individual as a random effect and treatment as a fixed effect. Pairwise comparisons were performed using Tukey's honestly significant difference test with an alpha of 0.05.

Results: Zn concentrations ranging from 500 µM to 2,500 µM increased GLUT4 expression in a dose dependent manner. Lymphocytes treated with 500 µM had higher GLUT4 than the 50 µM (P=0.03), insulin (P=0.001), and Zn free control (P<0.001). Cells incubated with 1,000 µM Zn had increased GLUT4 expression relative to 50 µM (P<0.001) and 100µM (P<0.001) Zn as well as the insulin (P<0.001) and control (P<0.001). Cells treated with the 2,500 µM of Zn had greater GLUT4 expression relative to all Zn concentrations lower than 1,000 µM (Control P<0.001; Insulin P<0.001; 50 µM P=0.001; 100 µM P=0.01; 500 µM P=0.02). None of the Zn treatments had evidence of increased cytotoxicity relative to the control. Future work will assess the extent to which increased GLUT4 expression translates into enhanced glycolytic activity in lymphocytes and what lymphocyte effector functions are improved by modulating the glycolytic pathway.

Conclusions: There is evidence that Zn is capable of increasing GLUT4 expression in bovine CD4 lymphocytes. These data opens the door for future research investigating the capacity of Zn to improve the glucose availability and which lymphocyte effector functions can be improved by modulating glycolytic metabolism.

Financial Support: This study was funded by competitive grant USDA-NIFA-2023-67011-40483 from the US Department of Agriculture National Institute of Food and Agriculture.

Notes:

152 - Characterization of regional differences in neonatal calf gut-associated lymphoid tissues using high resolution snRNA-sequencing

Mohamed A. Donia[1], Brian M. Aldridge[2, 3], James F. Lowe[4], Wenli Y. Li[5]

[1]Department of Pathobiology, University of Illinois at Urbana-Champaign, [2]Department of Veterinary Medical Sciences, Clemson University, [3]Department of Biomedical and Translational Sciences, University of Illinois at Urbana-Champaign, [4]Department of Veterinary Clinical Medicine, University of Illinois at Urbana-Champaign, [5]US Dairy Forage Research Center, USDA-ARS. mdonia2@illinois.edu

Session: Immunology 1, 2025-01-20, 3:15 - 3:30

Objective: The intestinal tract harbors the highest density of immune cells of any single organ system and is as a critical portal through which animal management practices modulate host physiological and pathophysiological responses. Although the biogeographical compartmentalization of digestive processes along the alimentary canal is well-characterized, the variation of immunological structure and function across distinct intestinal segments is often overlooked, and the gastrointestinal mucosal immune system is viewed as a single entity. In this study we describe the use of high-resolution single-cell RNA sequencing to the exploration or regional differences in immune cell structure and function in the neonatal bovine intestinal tract.

Methods: A healthy, 3-day old calf was euthanized, and tissues were collected from ileal (PPi) and jejunal (PPi) Peyer's patches, ileal (MLNi) and jejunal (MLNj) mesenteric and pre-femoral (PF) lymph nodes. Single nuclei were extracted followed by library preparation using 10X genomics 3' GEMX and sequencing with a depth of 60,000 reads per nucleus, and 10000 nuclei per sample. Raw reads were mapped to the bovine genome using the Cell Ranger pipeline. Gene counts, cell types, and differential expression were analyzed in R with Seurat package. Immune pathway enrichment was performed using EnrichR, and cell-cell communication was analyzed by the CellChat package.

Results: The results demonstrate that immune cell composition and function of equivalent lymphoid tissues varies by intestinal location. The ileal Peyer's Patches contained the highest proportion of B cells of all immune sites. Cell cycle analysis identified higher proliferative activity in B cells than other immune cell types. Distinct, compartment-specific immune cell gene expression profiles were identified, with each intestinal region displaying a unique transcriptomic signature associated with specific immune pathways. Intercellular communication analysis demonstrated attenuated signaling activity of B cells and T cells in PPi, MLNi, and PPj compared to the MLNj and PF.

Conclusions: This pilot study demonstrates the utility of high-resolution snRNA sequencing in characterizing regional differences in immune cell dynamics along the intestinal tract of the bovine neonate. The study findings underscore the future potential of this powerful molecular tool in evaluating the impact of different management strategies and therapeutic interventions on the development of the gut-associated lymphoid tissue (GALT) and its associated responses to heath challenges.

Financial Support: This study was supported by Carle Illinois College of Medicine HIP funds.

Notes:

153 - Experimental infection and viral pathogenesis of H5N1 in Jersey cattle

Flavio Cargnin Faccin[1], L. Claire Gay[1], Dikshya Regmi[1], Sasha Compton[1], Teresa D Mejias[1], Juliana Calil Brondani[1], Elizabeth W Howerth[2], Daniela S Rajao[1], Roberto A Palomares[1], Daniel R Perez[1]

[1]Department of Population Health, University of Georgia, [2]Department of Pathology, University of Georgia. flaviocargninfaccin@uga.edu

Session: Highly Pathogenic Avian Influenza in Cattle, 2025-01-20, 2:00 - 2:15

Objective: Influenza A viruses (FLUAVs) of the H5N1 subtype have been detected in various species recently, including dairy cows. Since the confirmation of H5N1 in a Texas dairy cow on March 25, 2024, several outbreaks have occurred within the US, raising public health concerns due to the risk of transmission to humans. Previous reports demonstrated that Holstein cows are susceptible to H5N1 infection. To better understand the disease and its impact on dairy cattle, we aimed to investigate the susceptibility of Jersey cows to H5N1 infection and establish a model of H5N1 infection in dairy cows.

Methods: Three Jersey cows were inoculated intranasally and intramammarily with A/Texas/37/2024 (H5N1) at a dose of 106 TCID50/ml. Two quarters (front right and rear left) were inoculated per cow, while the remaining two quarters were left uninfected. Teat swabs, nasal swabs, and milk samples were collected daily to monitor viral loads. On days 5, 6, and 7 post-infection, one cow was humanely euthanized for extensive tissue collection. All swab samples, milk, and tissues were tested for the presence of viral RNA by quantitative RT-PCR.

Results: All three Jersey cows exhibited a significant decrease in milk production following inoculation. As previously reported for Holstein cows, milk samples from infected quarters had a colostrum-like appearance. The cows developed fever and decreased appetite on day 1 post-inoculation. Elevated California Mastitis Test (CMT) scores confirmed mastitis in the infected quarters. External swabs of the infected teats tested positive for viral RNA, and high levels of viral RNA were detected in both internal swabs of the infected teats and milk samples, raising concerns about milk safety. Nasal swabs revealed low levels of viral RNA, suggesting limited viral replication in the respiratory tract of cows infected with H5N1.

Conclusions: This study offers new insights into the pathogenesis of H5N1 influenza virus in Jersey cows, a breed not previously examined for susceptibility to this infection. Our findings demonstrate that Jersey cows are susceptible to H5N1 infection and can serve as a valuable experimental model for further research into disease pathogenesis and the development of effective vaccines.

Financial Support: This work was funded by the Centers of Excellence for Influenza Research and Response (CEIRR), NIAID.

Notes:

154 - Case-control study exploring associations between demographics and HPAI risk in lactating Holstein cows

Alexandre Rico[1], Blaine Nicks[2], Alfonso Lago[3], Noelia Silva-del-Rio[1]

[1]University of California, Davis, [2]Jager Ag, LLC, [3]DairyExperts, INC. aricofernandez@ucdavis.edu

Session: Epidemiology 3, 2025-01-20, 8:30 - 8:45

Objective: Highly Pathogenic Avian Influenza (HPAI) H5N1 (clade 2.3.4.4b) has spillover into dairy cattle populations. Seven months after the initial outbreak, there are still many unknowns about this outbreak. This study aims to identify specific cow-level factors (i.e., days in milk (DIM), milk yield, parity, and reproductive status) and farm-level factors (pen design) that may increase the susceptibility of cattle to H5N1 infection based on on-farm herd records.

Methods: This study was designed as an unmatched cohort-nested case-control. We enrolled 3,281 Holstein lactating cows from a commercial operation with free-stalls and dry lot pens. This herd housed mainly pregnant dairy cows moved from a nearby sister facility. The study herd experienced the peak of the outbreak during the last week of May. Cow information including DIM, milk yield, parity, and reproductive status were obtained from herd health records software using May 1st, 2024, as the date. Among these, cases were defined as cows that had the item "FLU" recorded in the herd health history using August 31st, 2024, as the date. A bivariate descriptive analysis was conducted to explore associations between each demographic characteristic and the HPAI case. Simple logistic regression was performed to assess these associations, reported as OR with 95% CI using R.

Results: Disease incidence was 16.2%. The OR of parity were calculated with 1st parity (n = 1,222) cows as a reference; for 2nd parity (n = 821) OR was 1.81 (95% CI: 1.39, 2.37), for 3rd (n = 611) parity OR was 1.87 (95% CI: 1.40, 2.49) and for ≥3rd parity (n = 627) OR was 2.05 (95% CI: 1.55, 2.72). The OR of DIM were calculated with cows <180 DIM (n = 531) as a reference; for cows with 180-300 DIM (n = 1,993) OR were 1.17 (95% CI: 0.877, 1.58), and for cows with >300 DIM (n = 757) OR was 1.55 (95% CI: 1.13, 2.16). Cows housed in open lot pens (n = 1,155) had a lower OR of 0.598 (95% CI: 0.477, 0.745) compared to those housed in free stalls (n = 2,126). The OR of reproductive status were calculated with non-pregnant cows (n = 307) as a reference; cows with < 90 d (n = 476) OR was 2.51 (95% CI: 1.47, 4.51), cows with 90-180 d (n = 1,681) OR was d 3.01 (95% CI: 1.87, 5.18), and cows with >180 d (n = 817) OR was 3.17 (95% CI: 1.93, 5.54). The OR of milk yield prior the outbreak were calculated using cows producing ≤20 L/d (n = 494) as the reference; for cows producing 20-40 L/d (n = 2,292) OR was 1.00 (95% CI: 0.761, 1.34), for cows producing > 40 L/d (n = 495) OR was 1.21 (95% CI: 0.851, 1.72).

Conclusions: These findings suggest cow and farm level factors may impact HPAI cases on dairy cattle. Understanding intra-herd viral dynamics is critical for making informed decisions and preventing and managing future outbreaks.

Notes:

155 - Distribution of influenza and coronavirus receptors in the respiratory and intestinal tract of beef cattle

N.C. Twu[1], Y.C. Teoh[2], X. Cheng[3], R. Espín-Palazón[3], L. Gimenez-Lirola[1], R. Chowdhury[4, 5], R.K. Nelli[1]

[1]Department of Veterinary Diagnostic and Production Animal Medicine, Iowa State University, [2]Department of Computer Science, Iowa State University, [3]Department of Genetics, Development and Cell Biology, Iowa State University, [4]Department of Chemical and Biological Engineering, Iowa State University, [5]Nanovaccine Institute, Iowa State University. nctwu@iastate.edu

Session: Highly Pathogenic Avian Influenza in Cattle, 2025-01-20, 2:30 - 2:45

Objective: Influenza viruses (IV) and coronaviruses (CoV) are significant pathogens impacting various animal species, including cattle. Influenza C, influenza D, and bovine coronavirus (BCoV) are known to induce respiratory diseases in cattle, with BCoV also affecting the gastrointestinal system. Moreover, cattle have shown susceptibility to the SARS-CoV-2 virus. Recent reports of Influenza A virus (HPAI H5N1) in dairy cattle highlighted the evolving threats these viruses pose to animal and human health. For successful infection, these viruses must bind to specific host cell receptors, such as sialic acids (SA) for influenza viruses and, for example, angiotensin-converting enzyme 2 (ACE2) for some coronaviruses. However, the structural characterization of these receptor-pathogen interactions in beef cattle is thus far lacking. To this end, this study aims to elucidate the distribution patterns of IV and CoV receptors in bovine tissue.

Methods: To assess the IV and CoV specific receptor distribution in the respiratory and intestinal tracts, we utilized formalin-fixed paraffin-embedded tissues (trachea, lung, small intestine, and large intestine) from crossbred Holstein-Angus steers. Lectin histochemistry staining was performed using Sambucus nigra (SNA), Maackia amurensis lectin-I (MAL-I), and Maackia amurensis lectin-II (MAL-II) to identify SA α2,6-Gal, SA α2,3-Galβ (1-4), and SA α2,3-Galβ (1-3) receptors, respectively. Additionally, the expression of N-glycolylneuraminic acid (NeuGc) and coronavirus receptors ACE2, transmembrane serine protease 2 (TMPRSS2), aminopeptidase N (APN), dipeptidyl peptidase 4 (DPP4), and carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1) was evaluated using indirect immunofluorescence assay. The specificity of lectin binding was validated through treatments with or without sialidase A and viral binding assay. Confocal microscopy was employed for imaging, and image analysis was conducted using a novel PixF-image analysis tool (Pixel-based Fluorescence analyzer).

Results: Our analysis reveals that the epithelial lining of both the bovine respiratory and intestinal epithelium predominantly contain SA α2,3-Gal receptors, particularly identified through MAL-II lectin binding. SA α2,6-Gal were mainly localized in glandular regions within these tissues. In the respiratory tract, MAL-II staining diminished progressively from the trachea to the alveoli, with the bronchioles and alveoli displaying similar staining patterns for both MAL-II and SNA. Elevated levels of SA α2,6-Gal expression were observed in the sub-epithelial regions of the respiratory tract and in the lamina propria of the villus and crypt areas of the intestine. NeuGc expression was found to be multifocal, while coronavirus receptors (ACE2, TMPRSS2, APN, DPP4, and CEACAM1) exhibited a consistent distribution across respiratory and intestinal tissues, with CEACAM1 levels being comparatively low.

Conclusions: These findings indicate an abundance of IV and CoV receptors within the bovine respiratory and intestinal tracts, with expression levels varying across tissue types and spatial loci. These insights contribute to a mechanistic understanding of viral entry points for attachment, invasion, and infection in bovine hosts.

Notes:

156 - The impact of highly pathogenic avian influenza H5N1 virus infection in dairy cattle

Felipe Peña-Mosca1, Elisha Frye2, Matthew J MacLachlan2, Ana Rita Rebelo2, Pablo S. Britto de Oliveira2, Michael J. Zurakowski2, Zoe Rose Lieberman2, Lauren Elizabeth Meyer3, Dennis Summers4, Francois Elvinger5, Daryl V. Nydam1, Diego G. Diel2

1Department of Public and Ecosystem Health; Cornell College of Veterinary Medicine, 2Department of Population Medicine and Diagnostic Sciences, Cornell College of Veterinary Medicine, 3Cornell College of Veterinary Medicine, 4Division of Animal Health, Ohio Department of Agriculture, 5Animal Health Diagnostic Center, Cornell College of Veterinary Medicine. dgdiel@cornell.edu

Session: Highly Pathogenic Avian Influenza in Cattle, 2025-01-20, 2:45 - 3:00

Objective: The spillover and spread of HPAI H5N1 virus into dairy cattle U.S. poses major challenges to the industry. Here we examined the effects of an HPAI H5N1 outbreak in dairy cattle, investigating risk factors associated with clinical influenza, its effects on milk production and rumination time, and the duration of the clinical disease, along with herd removal and mortality in affected cows.

Methods: This study was conducted on a free-stall dairy farm in Ohio that milks an average of 3,400 cows and regularly moves animals from another site in Texas. The association between milk production prior to the outbreak, SCC, parity, days in milk (DIM), breed and the risk of clinical influenza was investigated using Cox proportional hazards regression. The relationship between clinical influenza, mortality, and herd removal was investigated using logistic regression. Odds ratios were transformed into relative risks using functions.

Results: A total of 777 cows were diagnosed with clinical influenza, comprising 776 lactating and 1 dry cow, with 24 cases confirmed by polymerase chain reaction. Among cows with clinical signs 53 died with affected cows having an increased risk of death (relative risk [RR]: 6.0 [95% CI: 3.9, 8.9]). Clinically affected cows also presented an increased risk of removal from the herd (RR: 3.9 [95% CI: 3.3, 4.5]) with 245 animals being removed during the 60 day study. We observed an average of 14 days until death and 21 days until removal post-diagnosis. Days in milk and parity were associated with the risk of new clinical influenza (Type III P-value <0.01). Though 182 cows 1-100 DIM were affected those between 100 and 200 DIM had a higher risk (hazard ratio [HR] [95% CI]: 1.4 [1.2, 1.7]), as did cows >200 DIM (HR: 1.879 [1.547, 2.2]), compared to cows between 0 and 100 DIM (referent). Additionally, multiparous cows had an increased risk of clinical influenza compared to primiparous cows (2nd parity: HR 1.8 [1.5, 2.2]; 3rd parity or greater: HR 1.9 [1.5, 2.3]). Breed, baseline milk production, and SCC were not significantly associated with the risk of clinical influenza (P > 0.05). The affected cows experienced a sharp drop in milk production from 35.9 kg/day to a minimum of 11.6 kg/day two days later. Production then stabilized at 20.8-24.0 kg/day, remaining below pre-diagnosis levels throughout the post-clinical phase to the end of the 60d observation period. These patterns were consistent across different lactation stages, parities, and breeds, with cows confirmed by PCR diagnosis showing similar results. Gross cash losses due to lost milk production was estimated at $460 per cow and $350,000 in the herd. Non-adjusted direct gross cash losses due to mortality were approximately $150,000 and premature removal and replacement $340,000.

Conclusions: Our findings indicate that clinical influenza significantly impacts milk production, herd removal, and mortality in affected cows. These adverse effects persisted for at least 60 days after clinical diagnosis, highlighting the need for effective management and preventative strategies to mitigate the impact

Notes:

157 - Experimental infection of calves with swine influenza H3N2 virus

Lei Shi[1], Yuekun Lang[1], Dipali Gupta[1], Sawrab Roy[1], Chao Dai[1], Muhammad Afnan Khalid[1], Richard Webby[2], Juergen Richt[3], Wenjun Ma[1]

[1]University of Missouri, [2]St. Jude Children's Research Hospital, [3]Kansas State University. lsfd9@missouri.edu

Session: Highly Pathogenic Avian Influenza in Cattle, 2025-01-20, 3:00 - 3:15

Objective: Ruminants are not typically considered as the natural hosts for influenza A viruses (IAVs). However, recent and unexpected outbreaks of highly pathogenic H5N1 avian influenza virus among dairy cows in the U.S. have raised significant concerns. To date, research on IAV infection in ruminants remains limited. Here we experimentally infected calves with an A/Swine/Texas/4199-2/98 H3N2 virus (TX98) to investigate their susceptibility and infection dynamics.

Methods: Twelve calves were assigned to two groups: one group of 4 calves was intranasally infected with 108 TCID50 of TX98 once, while the other group of 4 calves was orally infected by feeding them milk containing 108 TCID50 of TX98 for 3 successive days (once per day). Two naïve calves were comingled with infected animals in each group on day 2 post infection to investigate virus transmission. Clinical symptoms and rectal temperatures of each animal were monitored daily. Nasal, oral and rectal swabs were collected from each animal until day 14 post infection to determine viral shedding. Blood samples were taken on days 3, 5, 7, 14, and 21 post infection to determine viremia and seroconversion. Two infected animals from each infection group were euthanized on day 5 post infection to assess viral replication and pathogenicity.

Results: No obvious clinical signs or fever were observed in all experimental calves. Minimal macroscopic lung lesions were found in necropsied calves in both groups on day 5 post infection. In the intranasal infection group, nasal swab samples collected from three out of four infected calves were positive in RT-qPCR on various days post infection, but none of oral and rectal swabs from infected and contact animals were positive. In contrast, only one infected calf showed weak RT-qPCR positivity in a rectal swab sample on one day, and oral and rectal swabs collected from one contact animal were RT-qPCR positive in the oral infection group. All tissue samples from necropsied animals in each group tested negative. Two intranasally infected calves developed hemagglutination inhibition antibodies against TX98 on days 14 and 21 post infection, with antibody titers reaching 25-27. However, orally infected animals and contact calves in each group did not show seroconversion.

Conclusions: H3N2 strain TX98 is able to infect calves. However, the infection is limited as effectively horizontal transmission and noticeable clinical signs have not been observed.

Financial Support: This study is partially supported by the USDA-NIFA 2024-7007674, the Centers of Excellence in Influenza Research and Response (CEIRR), contract number 75N93021C00016 and MU startup fund.

Notes:

158 - Highly pathogenic avian influenza H5N1 virus infection in dairy cattle, serologic antibody detection

Pablo De Oliveira[1], Ana Rebelo[1], Elisha Frye[1], Mohammed Nooruzzaman[1], Annika Diaz[1], Lina Covaleda[1], Diego Diel[1]

[1]Cornell University. psb86@cornell.edu

Session: Highly Pathogenic Avian Influenza in Cattle, 2025-01-20, 3:15 - 3:30

Objective: Since the emergence of highly pathogenic avian influenza (HPAI) H5N1 virus in the US in 2022, it has caused the deaths of millions wild and domestic birds. Notably, in early 2024 HPAI H5N1 virus spilled over into dairy cattle in Texas and has since been reported in several other states. Here we conducted a study to evaluate the antibody responses to HPAI H5N1 virus in dairy cattle. The study aimed at comparing commercial competitive ELISA assays with a virus neutralization assay for HPAI H5N1 virus. Additionally, we aimed at determining the seroprevalence and extent of following an outbreak of HPAI H5N1 virus in a large dairy farm.

Methods: In this study, we tested 1,116 serum samples from dairy cattle: 200 samples collected in 2023 prior to the HPAI H5N1 outbreak, 92 samples collected from animals during clinical outbreaks in early 2024, and 822 samples collected approximately 60 days after an outbreak in a large dairy farm. Three different assays were employed for antibody detection. The assays used included two commercial ELISA enzyme immunoassays (IDEXX Multi-species Influenza A and the ID.Vet Influenza A Multi-species) that assess the presence of antibodies against the nucleocapsid protein (NP). Additionally, we performed the viral neutralization (VN) test using a recombinant HPAI H5N1 virus expressing the miniGFP2 reporter gene developed in our laboratory (rTX2/24-miniGFP2) to detect and quantify neutralizing antibodies. Furthermore, our group currently developing another solid-phase assay bead-based technology (Luminex), to detect and quantify antibodies against HPAI H5 hemagglutinin HA1.

Results: Testing of the 200 serum samples collected pre-HPAI H5N1 spillover into dairy cattle revealed a diagnostic specificity of 100%, and 99.5% for the IDEXX and ID.Vet ELISAs and 99.5% for the virus neutralization test (VNT). Among the samples collected during acute infection, 28 (30%) samples tested positive using the IDEXX ELISA, while 44 (47%) tested positive using the ID.Vet ELISA and 46 (50%) samples tested positive using the VNT. Among the 822 samples collected in 60 days post clinical outbreak, 214 (26%) samples tested positive with the IDEXX ELISA, while 630 (76.6%) were positive with the ID.Vet ELISA. Notably, the VN assay, corroborated the results of the ID.Vet ELISA with 644 (78.3%) samples testing positive.

Conclusions: Our study reveals significant differences in diagnostic sensitivity for antibody detection for HPAI in dairy cattle. The ID.Vet commercial ELISA showed higher sensitivity which was comparable to the VN test. These results also highlight the impact of assay selection on seroprevalence studies and outcomes for HPAI H5N1.

Notes:

159 - Exploring the pathogenesis of newly emerged African swine fever virus in naturally infected pigs from India

Saminathan Mani[1, 2], Karam Pal Singh[1], Sukdeb Nandi[1], Pavulraj Selvaraj[3], Arun Chatla[1], Cole Knuese[4], Jorge E. Osorio[2]

[1]ICAR-Indian Veterinary Research Institute, [2]Global Health Institute, University of Wisconsin-Madison, [3]School of Veterinary Medicine, Louisiana State University, [4]University of Wisconsin-Madison. drswamyvet@gmail.com

Session: Virology 1, 2025-01-20, 2:00 - 2:15

Objective: African swine fever (ASF) is a contagious and emerging viral disease of domestic and wild pigs, caused by ASF virus (ASFV) from Asfarviridae family and associated with 100% mortality. First outbreak of ASFV was reported in India on January 2020 in northeastern states like Arunachal Pradesh and Assam, and since then the disease had spread to various states of India. As of now, no research has investigated on ASFV-induced pathogenesis in Indian pigs. The objective of this present study was to elucidate the ASFV-induced pathogenesis and immune responses during natural epidemic in Indian pigs.

Methods: The study was carried out between July 2022 and March 2024 in the states of Uttar Pradesh and Uttarakhand, India. After the first incursion of ASFV in these states, high morbidity and mortality were recorded. Out of 656 pigs examined, 554 pigs showed clinical signs of ASF, 461 died, and 36 were subjected to post-mortem examination. Detailed gross and histopathology, Th1-type (IFN-α, IFN-β, IFN-γ, IL-2, TNF-α, IL-1β, IL-12p40, and IL-8) and Th2-type (IL-4, IL-10 and IL-6) immune responses, pro- and anti-apoptotic genes by qRT-PCR, ASFV confirmation and genetic analysis were studied.

Results: Most of the ASFV-affected pigs showed clinical signs of pyrexia, anorexia, and congested mucous membranes. Critically-ill and dead animals showed oozing of frothy exudate from nostrils and reddish areas of cutaneous congestion throughout body. Most of the necropsied pigs showed oedema, haemorrhages, and necrosis in lymph nodes (LNs) and tonsils. Thoracic and pericardial cavities contained excessive amounts of dark-red fluid. Lungs showed emphysema, haemorrhages, marbling appearance, and oozing of reddish fluid from cut surfaces. Stomach showed blood-mixed ingesta and haemorrhagic mucosa. Spleen, liver and kidneys were markedly enlarged, haemorrhagic, and dark-brown in colour. Urinary bladder had dark-red urine and petechiae. Brain showed markedly congested meningeal vessels and haemorrhages. Histopathologically, trachea showed oedema and epithelial erosion. Lungs showed haemorrhages, thrombi and interstitial thickening due to oedema and infiltration of inflammatory cells. Alveoli, bronchi and bronchioles were filled with sero-fibrinous exudate. Heart showed haemorrhages, degeneration of muscle fibres, fibrin deposition, and inflammatory cells. Tonsil, LNs and spleen showed lymphoid depletion with necrosis and haemorrhages. Liver and kidneys showed infiltration of mononuclear cells, haemorrhages, and necrosis. ASFV antigen was demonstrated in lymphoid and visceral organs by immunohistochemistry. ASFV was confirmed in blood and lymphoid organs by amplifying B646L gene (encoding p72 protein), E183L gene (encoding p54 protein), and central variable region (CVR) of B602L gene using PCR. Sequencing of these genes showed 100% identity with ASFV genotype II from Mizoram and Assam in India, which might be originated from China and Vietnam. Kinetics of Th1-type and Th2-type cytokines showed significant changes in PBMCs, LNs, spleen, and tonsil, which were correlated with systemic inflammatory response and pathology in affected pigs. Significant upregulation of apoptotic genes of intrinsic pathway and pro-apoptotic genes, and downregulation of anti-apoptotic genes were noticed.

Conclusions: The ASFV genotype II is highly virulent in Indian pigs and these results formed a basis for understanding the molecular mechanisms of ASFV-induced pathogenesis and immune responses.

Financial Support: Authors are highly grateful to Joint Director (CADRAD), Joint Director (Research), and Director, ICAR-Indian Veterinary Research Institute (ICAR-IVRI), Deemed University, Bareilly, Uttar Pradesh, India; Global Health Institute, University of Wisconsin-Madison, USA for providing necessary facilities to carry out this work.

Notes:

160 - Characterization of segment-specific porcine enteroids and susceptibility to porcine epidemic diarrhea virus

Lu Yen[1], Rahul Kumar Nelli[1], Ning-Chieh Twu[1], Juan-Carlos Mora-Diaz[1], Gino Castillo[2], Panchan Sitthicharoenchai[3], Luis G. Gimenez-Lirola[1]

[1]Department of Veterinary Diagnostic and Production Animal Medicine, Iowa State University, [2]Facultad de Ciencia e Ingenieria, Universidad Peruana Cayetano Heredia, [3]Department of Population Health and Pathobiology, North Carolina State University. lyen@iastate.edu

Session: Virology 1, 2025-01-20, 2:15 - 2:30

Objective: The initial stages of porcine epidemic diarrhea virus (PEDV) infection and the corresponding innate immune responses at the intestinal epithelium are not well understood, primarily due to the constraints of conventional cell culture and animal models. This research aimed to develop a porcine enteroid culture system to investigate potential variations in infection susceptibility across different sections of the porcine small intestine (duodenum, jejunum, and ileum).

Methods: Intestinal crypt cells from nursery pigs were cultured in Matrigel to form porcine enteroid in Matrigel cultures (PEMCs). After characterization, these PEMCs were enzymatically dissociated and subcultured on transwell inserts (PETCs) to facilitate apical surface exposure for infection studies. The characterization of PEMCs and PETCs from different intestinal regions involved the assessment of morphology, proliferation, viability, and cellular phenotyping through immunohistochemistry/immunocytochemistry and gene expression analyses. PETCs were then inoculated with 105 TCID50 /mL of a highly pathogenic PEDV non-S INDEL strain and incubated for 24 h. The infection outcomes were evaluated by observing cytopathic effect, PEDV N protein expression via immunofluorescence assay (IFA), and PEDV N-gene detection using quantitative reverse transcription polymerase chain reaction (RT-qPCR).

Results: Morphological and phenotypical assessment reveal no significant differences among PEMCs and PETCs across intestinal segments, indicating a close resemblance to the porcine intestinal epithelium. Although PETCs derived from different segments of the small intestine were susceptible to PEDV infection, those from the jejunum showed a higher PEDV replication rate of the virus, as confirmed by IFA and RT-qPCR.

Conclusions: This segment-specific enteroid culture model offers a reliable platform for virological studies, providing a controlled environment that addresses the limitations of in vivo and traditional cell culture approaches. Establishing standardized culture conditions and thoroughly characterizing the model are crucial for the advancement of enteroid-based infection studies.

Financial Support: This work was supported by the Iowa Livestock Health Advisory Council (ILHAC Award FY20).

Notes:

161 - Japanese encephalitis virus expands genomic heterogeneity during fetal infection in the native amplifying host

P.P. Singh[1, 2], K. Le[1], U. Karniychuk[1, 2]

[1]Department of Veterinary Biosciences, The Ohio State University, [2]School of Public Health, University of Saskatchewan, Canada. zdk427@mail.usask.ca

Session: Virology 1, 2025-01-20, 2:30 - 2:45

Objective: Japanese encephalitis virus (JEV) is a leading cause of viral encephalitis in humans in Southeast Asia, with pigs serving as the major amplifying zoonotic host. JEV poses significant threats to the swine industry, causing abortions in pregnant pigs, boar infertility, and disease in piglets. The geographic range of JEV is expanding, raising concerns about its potential introduction into North America, where large populations of domestic and wild pigs coexist with ubiquitous Culex mosquitoes. Proximity to pig farms is a significant risk factor for human zoonotic JEV infections. However, JEV-pig interactions, particularly interactions with the pregnant host, are not well-studied. In our previous research on fetal Zika virus infection (JEV and Zika are related flaviviruses) we found that in utero-emerged fetal-specific virus variants lead to more aggressive infection phenotypes. Thus, this study tested the hypothesis that JEV infection in pig fetuses, which have immature and developing immunity, results in increased genomic JEV heterogeneity and the emergence of fetal-specific variants.

Methods: Pregnant pigs were injected with JEV at 50 days of gestation, resulting in transplacental and fetal infection. At 28 days post-infection, fetuses were sampled for RNA extraction, followed by the construction of libraries for whole virus genome next-generation sequencing (NGS) to analyze JEV evolution. The well-established intra-host variant analysis from replicate (iVar) bioinformatics pipeline was used to analyze mutations from a sufficient number of maternal and fetal samples. In another experiment, pregnant pigs were injected with JEV at 50 days of gestation. The piglets were delivered at 115-116 days of gestation, and samples were taken at birth for JEV quantification and NGS analysis.

Results: Despite a strong fetal type I interferon response, JEV persisted in the fetal placenta, blood, and brain for at least 28 days. Using short-read (Illumina) and long-read (Oxford Nanopore) NGS, we analyzed Single Nucleotide Variants (SNVs) in maternal and fetal blood. The analysis showed a significantly higher median frequency of SNVs in fetal blood (15.06-42.97%) compared to maternal blood (8.74%). The patterns of newly emerged SNVs (not present in the initial virus inoculum) varied significantly between maternal and fetal blood: the percentage of newly emerged SNVs was much higher in fetuses (25-100%) than in mothers (0-5%). Similarly, in the offspring study, we identified 32 SNVs in maternal samples and 286 SNVs in newborn piglets.

Conclusions: The fetal environment, characterized by developing and immature immunity, is highly conducive to the emergence of new JEV variants. Newborn piglets infected with JEV in utero may carry an extended JEV heterogeneity after birth, potentially spreading new variants within herds. It is crucial to determine whether these in utero-emerged JEV variants lead to more aggressive infection phenotypes, posing increased risks to swine herd health and zoonotic spillover.

Financial Support: Department of Veterinary Biosciences, College of Veterinary Medicine, The Ohio State University, Columbus. School of Public Health, University of Saskatchewan, Canada.

Notes:

162 - Nextclade dataset for the rapid classification of Porcine Circovirus 2

Michael Zeller[1], Jennifer Chang[2], Rabsa Naseer[3], Giovani Trevisan[3], Phillip Gauger[3], Pablo Pineyro[3], Rodger Main[1]

[1]Veterinary Diagnostic Laboratory, Iowa State University, [2]Vaccine and Infectious Disease Division, Fred Hutchinson Cancer Institute, [3]Department of Veterinary Diagnostic & Production Animal Medicine, Iowa State University. mazeller@iastate.edu

Session: Virology 1, 2025-01-20, 2:45 - 3:00

Objective: Porcine circovirus 2 (PCV2) is the causative agent of porcine circovirus-associated disease (PCVAD). The virus is globally distributed and has a significant economic impact in North America due to either sporadic outbreak or vaccination costs. PCV2 is a single-stranded DNA virus with two open reading frames (ORFs): ORF1 coding for replicase and ORF2 coding for the capsid. The ORF2 gene is approximately 702 nucleotides and by convention used for genotypic classification and is routinely sequenced by the Iowa State University Veterinary Diagnostic Laboratory (ISU VDL). At least eight genotypes are currently recognized for PCV2, PCV2a-e, though only PCV2a, b, d and e have been detected by the ISU VDL. The substitution rate for PCV2 has been estimated to be between 8.4 × 10-4 to 1.2 × 10-3 nucleotide substitutions/site/year, or between 1.2 - 1.7 nucleotide mutations within the ORF2 per year. This suggests long term stability of the virus's genotypes. The objective of this research is to provide convenient rapid classification of PCV2 ORF2 sequences.

Methods: PCV2 ORF2 sequences representing all prior published lineages were collected from GenBank. Lineages were assigned using a curated reference set representing all prior published lineages from PCV2a to PCV2h. Classified sequences were divided by lineage and a representative subset was selected by evenly sampling taxa along a phylogenetic tree, generating a reference tree of approximately 300 sequences. Sequence names were formatted to contain information about the GenBank accession, year of collection, and lineage designation. An additional metadata file was generated containing similar information. The reference sequences were run through the Augur pipeline provided by the Nextstrain team to create a prototypical PCV2 Nextclade dataset. The prototype dataset was rooted to the PCV2a genotype sequence, MF139075.1.

Results: A Nextclade-compatible PCV2 dataset was created to classify lineages a through h. The Nextclade dataset provides standardized references for classifying user-submitted sequences, such that user sequences are grafted onto a consistent reference tree. The implementation on Nextclade provides an ease-of-use platform wrapping the reference set and the method into a single tool. User-submitted sequences are aligned and grafted onto a scaffold tree through Nextclade without requiring additional knowledge from the user. Nextclade provides additional utility by providing information that may be of research interest, such as additional information on amino acid mutations of new sequences.

Conclusions: The PCV2 Nextclade dataset offers rapid fine-scale classification to PCV2 ORF2 sequences, allowing for more precise monitoring of viral diversity in circulation in swine. Additional work is still needed to refine the dataset, specifically finding a more appropriate root. The prototype of this tool is publicly accessible (https://clades.nextstrain.org/?dataset-url=https://github.com/mazeller/nextclade_test/tree/main/pcv2).

Financial Support: We would like to acknowledge the Iowa State University Veterinary Diagnostic Laboratory for the funding of this research.

Notes:

163 - The SERPIN function of Senecavirus A 2C protein and its regulation of the viral 3C protease

Annika N. Diaz[1], Maureen H.V. Fernandes[1], Diego G. Diel[1]

[1]Cornell University. and62@cornell.edu

Session: Virology 1, 2025-01-20, 3:00 - 3:15

Objective: Senecavirus A (SVA) is an emerging viral pathogen which causes vesicular disease in swine that is clinically indistinguishable from foot and mouth disease virus (FMDV) lesions. SVA can establish persistent infections in animals and threatens swine production systems due to the need for costly and disruptive differential diagnostic procedures. Bioinformatic analysis of the genome of SVA revealed a putative SERPIN reactive center loop (RCL) motif, within the 2C encoding region of the genome. Serine protease inhibitors (SERPINs) are a ubiquitous family of serine and cysteine protease inhibitor proteins found in all kingdoms of life and some viruses. We aimed to characterize the putative RCL of SVA by 1) determining the role of the protein motif in viral protein dynamics, 2) pinpointing key residues involved in SERPIN-like activity, and 3) assessing the conservation of 2C SERPIN-like activity among other picornaviruses.

Methods: We sought to characterize the potential SERPIN:target relationship of SVA 2C and the viral encoded cysteine protease, 3Cpro by co-transfection of 2C and 3C encoding plasmids. Western blot and semi-quantitative densitometric analysis was preformed to determine changes in protein levels. To determine key residues within the putative 2C RCL, targeted site-directed mutagenesis of the SVA 2C RCL was performed, generating 11 mutants spanning the RCL. The same mutations were also introduced into an SVA-SD-15-26 infectious clone to determine the role of these mutations on the virus. Growth curves and plaque assays were performed in swine turbinate cells to characterize each virus. Co-expression and densitometric analysis was performed for EMCV, FMDV, PV, EV-A71, EV-D68, and HAV 2C and 3C proteins.

Results: Starting at 8 hours post transfection, detection of SVA 3Cpro via western blot analysis showed a significant decrease ($p = 0.022$) when co-expressed with SVA 2C, as compared to individual expression. Two residues within the RCL motif (G180A and F209A), when mutated, resulted in significantly lower degradation of 3C during co-expression, indicating their importance in the SERPIN-like function of 2C. Several mutations within the RCL resulted in non-viable viruses, while G175A, Q188N, Q194A, and Q194H were able to be rescued. Interestingly, the Q188N virus exhibited significantly altered growth kinetics and a plaque deficiency, suggesting an important role of this residue in the virus life cycle. As for the co-expression of heterologous picornavirus species 2C and 3C, densitometric analysis of the expression levels showed EMCV 3C ($p= 0.0171$), FMDV 3C ($p= 0.0030$), PV 2C ($p= 0.0134$) were significantly degraded during co-expression, while HAV 3C showed a significant ($p= 0.0030$) increase in detection during co-expression.

Conclusions: From these findings, we hypothesize the SERPIN function of SVA 2C is involved in viral self-regulation by antagonizing the activity of SVA 3C to mediate appropriate progression of infection. Notably, studies with 2C proteins of other picornaviruses confirmed SERPIN activity on the viruses' 3C proteases, which supports our data assessing 2C and 3C protein dynamics of several picornaviruses during co-expression. Thus, suggesting self-regulation of the viral protease through the SERPIN function of 2C may be critical for the life cycle of picornaviruses.

Financial Support: Funding for this project was provided by the Cornell Alfred P. Sloan Foundation (G-2019-11435), the NSF GRFP (DGE - 2139899), and USDA-NIFA (Award #2024-67015-42737).

Notes:

164 - A comparative study of structural protein compositions to assemble virus-like particles of Seneca Valley virus

Hanjun Kim[1, 2], Limin Li[1, 3], Baoqing Guo[1], Kyoung-Jin Yoon[1]

[1]Department of Veterinary Diagnostic and Production Animal Medicine, Iowa State University, [2]USDA-ARS, [3]College of Veterinary Medicine, Hebei Agricultural University. hkim16@iastate.edu

Session: Virology 1, 2025-01-20, 3:15 - 3:30

Objective: Seneca Valley Virus (SVV), a member of the Picornaviridae family, causes vesicular disease in pigs, which is clinically similar to foot-and-mouth disease (FMD) and other swine vesicular diseases. As such, effective intervention and mitigation require an accurate diagnosis. Vaccination can assist that effort in certain circumstances. The virus-like particle (VLP)-based immunogen is one of the vaccine platforms to consider. While four structural proteins (VP1, VP2, VP3, and VP4) of SVV have been identified in the virion, the role of each SVV structural protein in virus assembly remains unclear. This study was to investigate the optimal conditions for VLP assembly by comparing various compositions of these structural proteins.

Methods: Viral RNA was extracted from a contemporary SVV strain isolated from a swine vesicular outbreak in the United States. The structural proteins VP0, VP1, VP2, VP3, and VP4 were amplified using polymerase chain reaction (PCR). The resulting amplicons were cloned into a commercial expression vector, pETite N-His SUMO (Lucigen). After confirming the nucleotide sequences of the cloned vectors, the constructs were individually transfected into an *Escherichia coli* strain BL21 (DE3) for protein expression. Protein expression was induced using isopropyl β-D-1-thiogalactopyranoside (IPTG). The His-SUMO fusion tags were cleaved with SUMO protease to produce the final water-soluble proteins. The successful expression and purification of these proteins were confirmed using Western immunoblot analysis with either anti-SVV rabbit serum or an anti-HIS tag antibody. To evaluate the assembly of VLPs, mixtures of the expressed proteins—either VP0, VP1, and VP3 or VP1, VP2, VP3, and VP4—were subjected to purification via a density gradient centrifugation. The presence of VLPs was confirmed through transmission electron microscopy (TEM).

Results: The PCR amplification produced specific products corresponding to the expected sizes of each protein. Subsequent cloning and sequencing verified the integrity of the constructs. Following IPTG induction, Western immunoblot analysis confirmed the successful expression of the His-SUMO fusion proteins, which were then cleaved to yield the individual viral proteins. The mixed proteins were collected after density gradient centrifugation. TEM analysis revealed the presence of icosahedral structures consistent with the morphology of SVV in the mixture of VP0, VP1, and VP3, thereby confirming the successful assembly of VLPs from these expressed proteins. However, VLPs were not observed in the mixture containing VP1, VP2, VP3, and VP4.

Conclusions: This study demonstrates that VLPs can be formed by mixing VP0, VP1, and VP3 without further cleavage of VP0 into VP2 and VP4, suggesting VP0 may be essential in assembling SVV-like particles. Since VLP can be a basis for a safe vaccine and therapeutic strategy against SVV, further investigations are necessary to identify an effective and stable composition of the assembled VLPs for potential applications in vaccine development.

Financial Support: The study was supported by a grant via the USDA Cooperative Agreement (58-5030-6-081) and the ISUVDL R&D Fund.

Notes:

165 - The use of mixed methods to explore the complex issue of companion animal relinquishment to and adoption from animal shelters

Alexandra Protopopova[1]

[1]University of British Columbia. a.protopopova@ubc.ca

Session: AVEPM - Schwabe Symposium, 2025-01-20, 4:15 - 4:45

Companion animal relinquishment to and adoption from shelters reflects complex human-animal dynamics that are shaped by individual and societal factors. Examining how bonds are formed and broken between companion animals and their guardians requires a multifaceted research approach. Although shelter data provides valuable insights, especially in identifying predictors of adoption and linking animal flow to broader societal issues, these data come with inherent limitations. Reliability and validity concerns, as well as the narrow representation of only a fraction of community animals, constrain the broader applicability of shelter data alone. To address these gaps, our research integrates mixed methods, combining shelter and non-shelter datasets, survey methodologies, and qualitative analyses, to create a more comprehensive understanding of companion animal ownership patterns. This approach enables a fuller exploration of the factors influencing the relinquishment and adoption process, offering new perspectives on the role of societal influences in animal care and management.

Financial Support: Natural Sciences and Engineering Research Council of Canada; British Columbia Society for the Prevention of Cruelty to Animals

Notes:

166 - Epidemiological insights: Classic tools for modern animal shelter and community animal health research

Rachel E. Kriesler[1]

[1]Midwestern University College of Veterinary Medicine. rkreis@midwestern.edu

Session: AVEPM - Schwabe Symposium, 2025-01-20, 4:45 - 5:15

Observational epidemiology remains a cornerstone of veterinary research, offering robust methodologies for investigating health outcomes across diverse animal populations. Traditional epidemiologic tools provide insights for evidence-based decision-making in the animal shelter environment, where resources are constrained and populations are dynamic. This presentation highlights the application of classic study designs in animal shelter and community animal health research.Case-control and cohort studies, both retrospective and prospective, are particularly well-suited to addressing questions in the animal shelter and community environment. Case-control studies allow for the efficient evaluation of rare outcomes, such as perioperative mortality in specific subpopulations, by comparing affected cases with matched controls to identify risk factors. Retrospective cohort studies leverage existing data to explore associations across large populations. Prospective cohort studies, when possible, offer an additional dimension by enabling the collection of tailored data over time, providing a clearer temporal relationship between exposures and outcomes. These classic designs can uncover actionable insights in environments characterized by logistical constraints and high animal turnover. Classic statistical approaches to observational studies, such as logistic regression for case-control studies, offer powerful yet relatively accessible tools for novice or nontraditional researchers. For many well-established methodologies, user-friendly statistical software and online resources are readily available to shelter and community animal health researchers. For example, simple logistic regression can be performed in Excel, which is commonly available, or R, which is freely available. While R's command line interface can be intimidating to novice or nontraditional researchers, multiple software packages offer a graphical user interface Two studies serve as an example. In the first, a retrospective cohort study of perioperative mortality in high-volume surgical sterilization clinics utilized over 1.2 million records to identify critical risk factors and clinic-level variability. In the second, a nested case-control study investigated mortality in rabbits undergoing elective procedures in a shelter setting. In both studies, risk factors were identified using multivariable logistic regression. These findings can guide clinical decision-making regarding patient selection and protocols. More examples can be found at the Journal of Shelter Medicine and Community Animal Health.While advancements in computational methods and statistical modeling expand the horizons of veterinary epidemiology, there is significant value in the foundational principles of study design and fundamental statistical approaches. The strengths of classic tools can be leveraged to navigate the complexities of modern animal sheltering and identify targets for interventions that improve animal health and welfare.

Notes:

167 - Characterizing pig farmer practices in Trinidad and Tobago in the context of African swine fever surveillance

Teola Noel[1], Jose Pablo Gómez-Vázquez[2], Carlos Gonzalez Crespo[2], Jose Henrique Hildebrand Grisi Filho[2], Beatriz Martínez López[2], Christopher Oura[1], Anil Persad[1], Nirvana Dodo[1], Cheryl-Ann Wharwood[3], Lana Gyan[3], Joanne Caruth[4], Annika Gordon[4], Karla Georg[1]

[1]The University of the West Indies, Trinidad and Tobago, [2]Center for Animal Disease Modeling and Surveillance, University California Davis, [3]Ministry of Agriculture, Land and Fisheries, Government of Trinidad and Tobago, [4]Division of Food Security, Natural Resources, The Environment and Sustainable Development, Tobago House of Assembly. Teola.Noel@sta.uwi.edu

Session: African Swine Fever, 2025-01-20, 4:15 - 4:30

Objective: Spread of transboundary swine diseases like African swine fever (ASF) continues to be a challenge to the swine industry as this is often accompanied by poor farm biosecurity, as well as unregulated operations of farms and industries. The Caribbean region remains under threat of ASF spread after its introduction into the Dominican Republic and Haiti in 2021. This study aims to evaluate biosecurity and management practices of pig farms in T&T as it relates to the risk of entry of ASF.

Methods: Semi-structured interviews with a questionnaire were conducted with pig farmers to collect information on farm demographics and biosecurity practices. Using R statistical software, Multiple Correspondence Analysis (MCA) and Hierarchical Clustering on Principal components (HCPC) was done to explore the distribution of responses. The principle of parsimony and domain expertise was used to select a combination of variables that provides higher variance with the least dimensions.

Results: A total of 90 responses was collected, 46 from Tobago and 44 from Trinidad. 12.9% of the respondents practiced swill feeding. The variables used in the MCA included island, farm size, biosecurity practices and trade, with 40% of the variance being explained by the first 3 dimensions.Discussion: The HCPC detected 4 distinct clusters. Clusters 2 and 3 presented characteristics more commonly associated with commercial farrow-to-finish and reproduction-only systems, respectively. Clusters 1 and 4 presented characteristics more commonly associated with more informal production systems.

Conclusions: This study has provided an in-depth analysis of management practices on pig farms across T&T. The information gathered is being used to inform risk assessments and models of pig production dynamics in the country. This will aid in the identification of farms with higher risk of ASF introduction and will provide a better understanding of how ASFV would spread between farms if introduced.

Financial Support: This work is financially supported by PROCINORTE-IICA.

Notes:

168 - A dynamic network evaluation of human, animal, and truck movement data across a swine farm system

Tara Prezioso[1], Alicia Boakes[2], Jeff Wrathall[2], Suman Bhowmick[1], Rebecca Lee Smith[1]

[1]University of Illinois Urbana Champaign, [2]Farm Health Guardian. tarap3@illinois.edu

Session: African Swine Fever, 2025-01-20, 4:30 - 4:45

Objective: It is an established fact that a healthy North American swine industry is important to agriculture, related industries, the country's gross domestic product, and international relations and trade. Movement networks are used to identify disease spread risks and design response plans. Many network evaluations in North America focus only on animal or truck movements. Very few include human movements. We previously examined a static network made up of three North American swine farms and found that when human movements are included, the network properties change, leading to a change in inference. We now examine the same data in a dynamic setting and evaluate how addition of the human movement changes the dynamic network structure as well as affect disease transmission. Hypothesis: The dynamic network including the human movement will more closely resemble the observed outbreaks than the dynamic network without the human movement.

Methods: Farm visit data was collected from a private database over the period from December 22nd, 2021, to April 29th, 2024, 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. PEDv outbreak data was collected during the same time frame for one of the management companies. Two dynamic networks were built using the network and networkDynamic packages in R. One included all the movement data, and one included just the animal and truck movement data. Each time a truck entered a truck wash, it was assumed cleaned and free of disease, and was provided a new vehicle ID. Each level in the network represents a break timepoint (35d) of PEDv disease transmission. Transmission models are compared to the outbreak data.

Results: The final full dynamic network included 28261 unique edges (or 200913 total trips), 182 properties, 17 different property types and 10 unique vehicle types. Measures of network structure include density at each timepoint, the indegree and outdegree of each node at each timepoint. Outcomes are recorded in visualizations and tables because there is no longer one network to report, but one network with multiple layers.

Conclusions: We hypothesize monitoring human movement is just as, or more important, than monitoring animal movement alone.

Financial Support: This research was supported by funding from the University of Illinois Urbana-Champaign Department of Pathobiology.

Notes:

169 - An innovative and effective CRISPR based anti-viral treatment in pigs infected with African Swine Fever Virus

Naveen Verma[1], Dylan Keiser[1], Roky Mohammad[1], Craig Mosman[1], Deric Holden[1], Alison Mahony[1], Douglas Gladue[1]

[1]Seek Labs. Doug@seeklabs.com

Session: African Swine Fever, 2025-01-20, 4:45 - 5:00

Objective: To develop a sequence specific treatment for African swine fever (ASF) is a devastating hemorrhagic disease with the causative agent being a large and complex double stranded DNA virus. In addition to devastating impacts on food resources from the loss of production animals, effects on global economies were widely felt after the 2018 ASF outbreak in China, including trade restrictions and world-wide shortages of swine derived Heparin and other swine products. Currently there are no treatments or commercial vaccines for ASF other than one vaccine that is selectively effective against Genotype II being used only in Vietnam. With the current pandemic strain circulating in Asia and Europe causing death in nearly 100% of infected swine, there is a critical and urgent need for effective anti-viral treatments for ASF. Infected farms have relied on culling of all animals followed by deep cleaning and subsequent repopulation to stop the spread of this highly infectious and lethal pathogen.

Methods: CRISPR/Cas systems have evolutionarily evolved to be an effective method to protect prokaryotes against viruses by detecting and destroying the DNA or RNA genomes of invading bacteriophages. Using our CRISPR platform we designed and developed a CRISPR-Cas9 and multiplexed guide RNAs system to target conserved ASFV genomic sequences as an effective therapeutic for ASF. This innovative approach is designed to specifically target and cleave multiple viral nucleotide sequences reducing viral replication and limiting disease burden in infected hosted. By targeting multiple conserved sites in the ASFV genome the CRISPR treatment is not genotype specific and could potentially be used to universally target all current circulating strains of ASFV.

Results: Here we report that following intramuscular (IM) injection of a lethal dose of ASF, swine treated with our anti-ASFV CRISPR therapeutic had reduced viral loads, were less symptomatic with a majority (57%) demonstrating complete clearance of the virus and achieving full recovery from ASF infection. Interestingly, all surviving animals developed robust antibody-based adaptive immunity from the initial exposure to ASF virus, and when rechallenged with a lethal dose of ASF, were fully protected and survived the trial.

Conclusions: Trail outcomes indicate that our CRISPR-Cas9 targeted treatment for ASFV could broadly treat all circulating strains of ASFV, and be given after the initial farm outbreak to prevent the culling of infected animals or farms. Moreover, this innovative approach could be quickly adapted to disrupt other livestock diseases by simply swapping the guide RNAs to the specific viral pathogen with computationally prioritized guide RNAs.

Notes:

170 - Molecular characterization reveals novel African swine fever virus recombinant between genotypes I and II

K. Lee[1], T.T.H. Vu[2], M. Yeom[1], V.D. Nguyen[3], T.T. Than[3], V.Y. Nguyen[2], D. Jeong[4], A. Ambagala[5], V.P. Le[3], D. Song[1]

[1]Department of Virology, Seoul National University, [2]Institute of Veterinary Science and Technology, Vietnam Union of Science and Technology Association, [3]College of Veterinary Medicine, Vietnam National University of Agriculture, [4]Korea Research Institute of Bioscience and Biotechnology, [5]Canadian Food Inspection Agency, National Centre for Foreign Animal Disease. km.lee@snu.ac.kr

Session: African Swine Fever, 2025-01-20, 5:00 - 5:15

Objective: To characterize emerging recombinant African swine fever virus (ASFV) strains of genotypes I and II identified in northern Vietnam in 2023 through whole-genome sequencing and comparative genomic analysis, aiming to understand their genetic composition and evolutionary relationships.

Methods: Seven ASFV-positive whole blood samples were collected from unvaccinated pigs showing acute ASF symptoms in six northern Vietnam provinces in September 2023. Virus isolation was performed using porcine alveolar macrophages, followed by hemadsorption testing. Whole-genome sequencing was conducted on isolates showing recombinant characteristics. Genome-wide recombination analysis was performed using Recombination Detection Program 4. Comparative genomic analysis identified single nucleotide polymorphisms (SNPs) and insertion/deletions (INDELs). The central variable region (CVR) of the B602L gene and the I73R-I329L intergenic region were examined for genetic diversity. Phylogenetic analysis determined the evolutionary relationships of the recombinant strains.

Results: Recombinant ASFV strains were detected in Bac Giang, Phu Tho, and Vinh Phuc provinces. These isolates showed hemadsorption positivity despite having genotype I based on the B646L gene, confirming their recombinant nature. Genome-wide analysis revealed 19 recombination breakpoints, consistent with Chinese recombinant ASFV strains. Vietnamese isolates shared 99.86-99.98% nucleotide identity with Chinese recombinant strains from 2021-2022, forming a distinct monophyletic group. Comparative genomic analysis identified 50 SNPs and INDELs among recombinant ASFV strains, with 39 variations distinguishing Vietnamese from Chinese strains. Unique genetic markers in C962R, I329L, and MGF 505-11L genes were found in Vietnamese recombinants, while mutations in C122R and NP1450L differentiated all recombinants from parental genotypes. The central variable region (CVR) of the B602L gene showed diverse patterns among Vietnamese isolates, with variations in tetrameric repeats. The I73R-I329L intergenic region analysis placed these new recombinant isolates in the IGR2 group, consistent with most previously reported genotype II ASFV strains from East and Southeast Asia.

Conclusions: This study provides insights into the genetic characteristics and evolution of recombinant ASFV strains in Vietnam. The high genetic relatedness between Chinese and Vietnamese recombinant ASFV genomes suggests a common evolutionary origin and highlights the risk of transboundary transmission. The identified genetic markers offer valuable tools for detection and characterization of these recombinant strains. The observed genetic diversity poses challenges for existing diagnostic methods and vaccine development. Future research should focus on the functional implications of these genetic variations and their potential impact on ASFV pathogenicity and transmissibility. Comprehensive surveillance is crucial to assess the prevalence and spread of these recombinant strains in Vietnam and neighboring countries, informing future control measures and preparedness strategies.

Financial Support: Korea Institute of Planning and Evaluation for Technology in Food, Agriculture and Forestryn(IPET), through High-Risk Animal infectious Disease Control Technology Development Program, funded by Ministry of Agriculture, Food and Rural Affairs (MAFRA) (No.RS-2024-00399940) and Vietnam Union of Science and Technology Association, Vietnam

Notes:

171 - Comparison of immunogenicity among membrane-bound, secreted, and intracellular forms of viral capsid proteins

Fangfeng Yuan[1], Junru Cui[1], Jane Qin[2], Ju Hyeong Jeon[2], Renhuan Xu[2], Jianzhu Chen[1]

[1]Massachusetts Institute of Technology, [2]Advanced RNA Vaccine Technologies, Inc. fy9@mit.edu

Session: African Swine Fever, 2025-01-20, 5:15 - 5:30

Objective: Humoral and cellular responses constitute the key branches of vaccine-induced protective immunity. It is of high interest to develop subunit vaccines against African Swine Fever Virus (ASFV) due to disease threat to swine industry and safety issue of live vaccines. Viral capsid proteins are widely explored for subunit vaccine development but are often hampered by their complexity of production and low immunogenicity. The objective of this study is to apply protein engineering of ASFV capsid for inducing optimal immune response.

Methods: Here, we engineered the ASFV capsid protein for altered cellular localizations, namely membrane-bound and secreted forms. The membrane-anchoring strategy employed contains a long linker for not impeding structural conformations.

Results: In vitro characterization shows that the engineered capsid proteins readily form multimeric structures and notably a single amino acid mutation fully abolishes glycosylation of engineered proteins. Coupled with lipid nanoparticle-mRNA delivery technology, the membrane-bound capsid proteins elicit significantly higher antigen-specific B cell and T cell responses in mice than the secreted and natively intracellular forms. Importantly, the membrane-bound antigen induces robust antigen-specific, long-lived bone marrow plasma cell response while the follicular T helper cell response in germinal center remains unchanged as compared to the intracellular antigen.

Conclusions: Overall, these results show that the membrane-anchoring strategy of viral capsid protein could potentiate both B cell and T cell immunities, thus may facilitate the development of effective subunit vaccine against ASFV and other non-enveloped viruses, such as Poliovirus.

Financial Support: This project was supported by New Hope Group Singapore and Agriculture and Food Research Initiative Competitive Grant no. 2024-67012-42721 from the USDA National Institute of Food and Agriculture.

Notes:

172 - Intensive population control for transboundary animal disease control influences space-use; case with wild pigs (*Sus scrofa*)

Kayleigh Chalkowski[1], Nathan P. Snow[1], Bruce R. Leland[2], Kurt C. Vercauteren[1], Ryan S. Miller[3], Kim M. Pepin[1]

[1]USDA-APHIS-WS, National Wildlife Research Center, [2]USDA-APHIS-WS, [3]USDA-APHIS-VS, Center for Epidemiology and Animal Health. kzc0061@auburn.edu

Session: African Swine Fever, 2025-01-20, 5:30 - 5:45

Objective: Combating new transboundary animal disease introductions with an emergency response requires quickly limiting the spread of the disease with intensive culling of wild animals at a continuous, landscape scale. African swine fever virus (ASFv) has been spreading globally and is one ongoing TAD threat to the United States. Our objective was to compare movement behaviors (home range size and displacement) before, during, and after landscape-scale, intensive removal activities.

Methods: We conducted the study in north-central TX on a study site divided into four zones (aerial, trap, toxicant, and a control area), totaling 225.1 km2. Between October 2022 and June 2023, we collared 122 adult wild pigs, and removed 610 wild pigs (256 aerial, 296 trapping, 58 toxicant). We estimated home range sizes of wild pigs using autocorrelated kernel density estimates (akde) for each removal period (before, during, after), and each removal type (aerial, trap, toxicant). Displacement was estimated between periods using weekly median net squared displacements (mNSD). Differences in home range area and mNSD was compared between periods using gamma-distributed generalized linear mixed models. For each removal type, models were compared using AIC (deltaAIC <2).

Results: There was a significant interaction between toxicant treatment and removal period, such that home range of pigs subjected to toxicant treatment was reduced compared to increased home ranges across time in the control set. Change in home range size of pigs subjected to trapping or aerial removals were not significantly different from control individuals. There were significant interactions of sex and trapping activities on mNSD compared to controls. Females subjected to trapping removals were range resident while female control individuals increased mNSD. Median net squared displacement of males subjected to trapping removals significantly increased compared to control males. For toxicant treatments, surviving individuals displaced significantly less during toxicant treatment compared to controls, but increased displacement following toxicant treatment.

Conclusions: Behavioral responses of wild pigs subjected to landscape-scale continuous control varied between different removal strategies. Increased displacements seen in males subjected to trapping activities could exacerbate TAD spread. Further research to understand fine-scale drivers of behavioral responses of wild pigs to continuous, intensive removals are needed.

Financial Support: NIFA

Notes:

173 - AUDIO: An app to aid antimicrobial use monitoring on dairy farms

Katherine J. Koebel[1], Ece Bulut[1], Daryl V. Nydam[2], Michael B. Capel[3], Renata Ivanek[1]

[1]Dept. of Population Medicine & Diagnostic Sciences, Cornell University, [2]Dept. of Public & Ecosystem Health, Cornell University, [3]Perry Veterinary Clinic. kjk239@cornell.edu

Session: Antimicrobial use, 2025-01-20, 4:15 - 4:30

Objective: Antimicrobial use (AMU) in livestock carries implications for all 3 arms of One Health: humans, animals, and the environment. Quantifying AMU is challenging on dairy farms, where treatment protocols, disease pressures, case definitions, and animal inventory vary widely across operations. Tools are needed so AMU may be monitored. We previously defined 16 antimicrobial use indicators (AUI): standardized and scalable metrics that allow for numerical assessment of AMU on farms. Our objective was to develop a user-friendly application that allows dairy stakeholders to calculate AUI for their operations so these values may be monitored.**Methods:** Antibiotic Use in Dairy Investigated Online (AUDIO) is a web application developed in the R language package Shiny. AUDIO utilizes a 3-template input system in which animal treatment records and site-specific information are downloaded from the farm's management software, inserted into AUDIO's comma-separated value (.CSV) templates, and reuploaded. 15 count-, dose- and mass-based AUI are instantly calculated. These values may be subset based on animal production category, antibiotic class, or treatment indication for more intensive analyses. Additionally, we recruited a cohort of 5 New York dairy farms for pilot testing and end-user feedback. **Results:** Interviews with farm managers will be conducted in the following weeks. These interviews will provide information to guide improvements to the app as well as investigate farmer attitudes towards AMU monitoring. Additionally, the AUI values from the initial cohort will inform our work into setting benchmark values in future versions of AUDIO. Options for future development include the ability to subset based on time (i.e. monthly or quarterly), and incorporation of artificial intelligence to streamline the data preparation process. **Conclusions:** AUDIO is a promising tool for dairy stakeholders to track AMU on their operations. By monitoring trends in AUI, managers can better identify opportunities for more judicious use of antimicrobials. In aiding the practice of prudent antimicrobial stewardship, AUDIO will contribute to the health of animals, people, and our environment.

Financial Support: USDA National Institute of Food and Agriculture (NIFA), Hatch Grant Accession #7000433. Partial support was received from the CDC One Health AMR Program.

Notes:

174 - Environmental antimicrobial resistance threats to pollinators

Kiranmayee Bhimavarapu[1], Pankaj Gaonkar[1], Alinne L. R. Santana-Pereira[1], Geoffrey Williams[2], Camila Rodrigues[2], Andre L.B.R. da Silva[2], Laura Huber[1]

[1]College of Veterinary Medicine, Auburn University, [2]College of Agriculture, Auburn University. kzb0148@auburn.edu

Session: Antimicrobial use, 2025-01-20, 4:30 - 4:45

Objective: Antimicrobial use in food animals contributed to the emergence of Antimicrobial Resistance (AMR) in the environment. Honey and pollen have been shown to be reliable bioindicators of environmental AMR. However, the interconnectedness of antimicrobial use in animals, AMR environmental contamination and the impact on pollinator health is unknown. This study aims to infer the mechanisms of AMR spread from the environment to 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 semi-field experiment, two high tunnel greenhouses were divided into 3 subsets. Yellow squash was planted and fertilized with either fresh or composted poultry litter. Apis mellifera honeybee colonies were introduced when squash plants flowered. Soil, pollen and honeybee samples were collected 5 times within a period of 56 days. The relative concentration of antimicrobial genes (ARGs), and mobile genetic elements (MGEs) in all samples were compared between groups using qPCR. Bees were screened for the presence of pathogenic and opportunistic organisms. The metagenome of soil and bee samples from 3 time points were analyzed.

Results: Preliminary analysis revealed higher levels of ARGs and MGEs in fresh litter when compared with compost using qPCR. Metagenomic analysis showed that foraging bees exposed to the fresh litter environment displayed a significantly different (PERMANOVA, $p = 0.001$) microbiome than those exposed to composted litter. Most notably, honeybees exposed to fresh litter had over 70% relative abundance of Bartonella sp (common but not core bacteria) while the genus had abundance lower than 10% in bees exposed to composted litter. Isolation efforts corroborated the metagenomic observations, showing a drop in the number of isolated G. apicola (gut health biomarker) and a trend towards increase in opportunistic pathogens S. marcescens and *E. coli* after 21 days of exposure indicating a possible dysbiosis event. ARGs richness also increased in soil and bees exposed to fresh litter than those exposed to compost. Despite soil and foraging bees having drastically different resistome profiles (PERMANOVA, $p = 0.001$), soil and bees shared a substantial amount of ARGs. They shared 144 type-specific resistant genes, MLSb, polymyxin, bacitracin, and tetracycline being the most shared genes between soil and the bees. Both fresh and composted litter increased the diversity of ARGs in the soil and in the honeybee microbiome, but raw litter fertilizer did so at a higher rate.

Conclusions: Fertilizing crops with either fresh poultry litter or compost contaminates the environment with AMR. Foraging bees in contact with these fertilized crops experience impact in their overall microbiome, and consequently, an increase in opportunistic organisms that could cause disease and mortality. The overlap between soil and bee ARGs indicates horizontal gene transfer across environments. Our future studies will investigate the direct transmission of ARGs from fertilized soil to pollen and honeybees through whole genome sequencing of shared organisms. We will also explore the direct effect of antibiotic residues on AMR emergence and spread.

Financial Support: This research is funded by the Animal Health & Disease Research Funds, Research and Graduate studies, Auburn University

Notes:

175 - Efficacy of antimicrobial drugs in the prevention and control of protozoal and bacterial calf diarrhea: A scoping review

Christian Bernal-Córdoba[1], Rúbia Branco-Lopes[2], Caroline Minjee-Lee[1], Diana Pérez-Solano[3], Erik Fausak[1], Richard Pereira[1], Noelia Silva-del-Rio[1]

[1]University of Califonia, Davis, [2]Division of Agriculture and Natural Resources, University of California, [3]University of Wisconsin, Madison. crbernalcordoba@ucdavis.edu

Session: Antimicrobial use, 2025-01-20, 4:45 - 5:00

Objective: This scoping review aimed to identify scientific literature evaluating the efficacy of antimicrobial drugs (AMDs) in preventing and controlling protozoal and bacterial calf diarrhea. Secondary objectives were to characterize health assessment methods and explore the feasibility of conducting meta-analyses on AMD efficacy in calf diarrhea.

Methods: This review followed a registered protocol. Four electronic databases were searched, with the initial search conducted in 2019 and the latest update in 2023. Eligibility criteria included controlled trials that evaluated AMDs for diarrhea prevention and control in calves ≤6 months and assessed AMD efficacy through health outcomes and/or fecal pathogen shedding.

Results: The search yielded 4,486 references. After removing duplicates (n = 1,273), two reviewers screened titles (n = 3,259), abstracts (n = 341), and full texts (n = 106). Forty-three articles encompassing 62 eligible trials were included. Trials primarily occurred in Europe (48.4%) and North America (43.5%), with sample sizes ranging from 8 to 513 calves. The predominant etiological agents associated with diarrhea were Eimeria spp. (41.9%) and *Cryptosporidium* spp. (40.3%), followed by *Salmonella* spp. (8.1%), *Escherichia coli* (3.2%), and undifferentiated diarrhea (6.5%). Diclazuril, toltrazuril, and lasalocid were the most commonly assessed AMDs for diarrhea associated with *Eimeria* spp. Halofuginone, chlortetracycline, and oxytetracycline were the most frequently evaluated AMDs for diarrhea associated with *Cryptosporidium* spp., *Salmonella* spp., and *E. coli*, respectively. Fecal consistency (93.5%) and stool blood (48.4%) were the most consistently assessed health outcomes. There was high variability and often incomplete reporting of health assessment methods.

Conclusions: Future trials evaluating AMD efficacy should follow reporting and "good clinical practice" guidelines, refine diarrhea causation assessments, evaluate AMD efficacy in different management settings, and incorporate interventions that adhere to label recommendations. The results support the feasibility of conducting meta-analyses to evaluate the efficacy of AMDs in diarrhea associated with *Cryptosporidium* spp. (halofuginone) and *Eimeria* spp. (diclazuril, toltrazuril, and lasalocid).

Financial Support: The California Department of Food and Agriculture (CDFA) Antimicrobial Use Stewardship Program partially provided financial support for this research (the sponsor had no role in either protocol or review).

Notes:

176 - Understanding the barriers and drivers influencing treatment decisions in large dairy farms in Ohio and California

Rafael Portillo-Gonzalez[1], Adriana Garzon[2], Ting-Yu Cheng[1], Devon J. Wilson[3], Richard Pereira[2], Greg Habing[1]

[1]The Ohio State University, [2]University of California, Davis, [3]Private practitioner. portillo-gonzalez.1@osu.edu

Session: Antimicrobial use, 2025-01-20, 5:00 - 5:15

Objective: This study aimed to identify the barriers and drivers influencing on-farm treatment practices and to understand the reasons that prompt farmworkers to initiate, change, or cease antimicrobial therapy.

Methods: This study was part of a quasi-experimental field trial but only the farms that received AMS training were invited for this component of the study. Fourteen trained farmworkers were individually interviewed using a semi-structured questionnaire for approximately 35 minutes. The interviews were audio-recorded, translated (Spanish version), transcribed, and evaluated using thematic analysis.

Results: The qualitative analysis indicated the formation of six themes with 1 to 3 subthemes each. Farmworkers identified animal welfare and job success as the primary drivers for continuing their education on responsible antimicrobial use and acting as on-farm antimicrobial stewards. However, they faced significant challenges, including knowledge deficiencies, time/supply constraints, and farm administration/management, that hindered the adoption of AMS practices. Additionally, farmworkers described that recognizing multiple illness signs, experiencing treatment failures, and culling cows due to poor production and reproductive performance were the main reasons for starting, changing, or stopping on-farm antimicrobial treatments, respectively.

Conclusions: Creating a work environment where farmworkers feel valued, supported, and empowered is essential to maintaining high standards of animal care. Tailored education and increased awareness of prudent antimicrobial use are needed among dairy farmworkers in Ohio and California. Finally, farmworkers cited several signs of illness, treatment failure, and reduced productivity as key reasons for adjusting antimicrobial treatment therapy.

Financial Support: The authors recognize the USDA (Washington, DC) for funding this study through grant #2018-68003-27466. This research was conducted in partnership with the University of California, Davis.

Notes:

177 - Assessment of dual-platform technology for bone regeneration and local antibiotic delivery in a goat osteomyelitis model

Gabriela Bastos[1], Tatiana D. Dias[1], David E. Anderson[1], Pierre Y. Mulon[1], Silke Hecht[2], Elizabeth Croy[1], Lori Terrones[1]

[1]Large Animal Clinical Science, University of Tennessee, [2]Department of Small Animal Clinical Sciences, University of Tennessee. gbastos@vols.utk.edu

Session: Antimicrobial use, 2025-01-20, 5:15 - 5:30

Objective: Osteomyelitis (OM) poses significant medical challenges. Diagnosis is often difficult and requires multiple diagnostic modalities and a deep understanding of the disease by the clinician. As a result, recognition of infection can be delayed, which may prolong hospitalization. The cost of treatment increases medical expenses by an average of $17,000 per case, representing a significant economic burden. This makes local drug delivery devices a promising technology for the prevention and treatment of OM. This study introduces a dual-platform device designed to promote bone healing and deliver antibiotics to prevent OM, addressing the limitations of most currently available commercial osteogenic implants, which lack antimicrobial properties. Our hypothesis was that an established bone tissue regeneration scaffold could deliver antibiotics sufficient to eliminate bacteria at the site of bone injury.

Methods: Adult, female, Boer-cross goats were randomly allocated into four treatment groups: 1) SA: Vancomycin-loaded scaffolds (n=5); 2) SB: Staphylococcus aureus-contaminated scaffolds (n=6); 3) SBA: SA+B (n=6); 4) NS: Native scaffolds (n=6). Scaffolds, with or without vancomycin and with or without bacteria, were implanted in a 2 cm segmental defect in the right tibia of each goat. Radiographs of the tibias were taken biweekly over a period of 3 months. Subsequently, the animals were humanely euthanized, and computed tomography (CT) of the tibias was completed, along with microbiological assessments of the scaffolds and surrounding soft tissue. Each radiograph and CT image were scored by a blinded radiologist for OM using a 0-5 scale. Group score data was evaluated using mixed model analysis for repeated measures with group as the between-subject factor and days as the within-subject effect.

Results: All goats in the SB group were removed from the study within the first 6 weeks due to OM, and 1 goat from the NS group was removed in week 10. Two goats from the NS group experienced contamination, with one developing OM. The SA group showed better overall outcomes compared to the NS group, with no cases of contamination. Radiographic OM scores differed significantly across groups: SA and NS had similar outcomes, SB had high scores indicating aggressive OM progression, and SBA had less severe and delayed onset of OM. CT findings aligned with radiographic OM scores, and both radiographs and CT provided consistent assessments of healing with no significant differences between the methods.

Conclusions: Our results suggest that vancomycin-loaded scaffolds may offer a distinct advantage in contaminated surgeries compared to scaffolds without antibiotics. They delay the onset and reduce the severity of OM, providing surgeons with the time needed to prevent or treat developing OM before more severe complications arise. This underscores the importance of antibiotic integration in the treatment of osteomyelitis.

Financial Support: Funding source: DOD JWMRP 81XWH1920014

Notes:

178 - Associations with selective antimicrobial use practices in adult dairy cattle

Ting-Yu Cheng[1], Molly Piela[1], Jessica A. Pempek[2], Kathryn L. Proudfoot[3], David L. Renaud[4], Greg G. Habing[1]

[1]Department of Veterinary Preventive Medicine, The Ohio State University, [2]Livestock Behavior Research Unit, USDA-ARS, [3]Department of Health Management, University of Prince Edward Island, [4]Department of Population Medicine, University of Guelph. chengtingyu722@gmail.com

Session: Antimicrobial use, 2025-01-20, 5:30 - 5:45

Objective: A cross-sectional study was conducted to characterize and evaluate dairy producers' attitudes and practices on antimicrobial use (AMU) and antimicrobial resistance (AMR).

Methods: U.S. dairy producers (n = 1,000) in five states (Florida, Michigan, Ohio, Vermont, and Wisconsin) were selected using stratified random sampling. The questionnaire included questions probing 1) producers' demographics and farm characteristics, 2) producers' attitudes toward AMU and AMR, 3) on-farm treatment protocols and recording, and 4) treatment responses to metritis vignettes (hypothetical cases). Three vignettes described clinical signs typically observed in cows with mild, moderate, and severe metritis. Producers' level of agreement for 14 statements regarding general AMU and AMR were descriptively summarized. Producers that indicated they would normally treat all three vignettes with antimicrobials were considered using antimicrobials non-selectively, whereas those only treated moderate and/or severe vignettes were selective users. Producers' level of agreement toward 14 statements regarding AMU and AMR were descriptively analyzed. The association between producers' demographics, farm characteristics, and on-farm treatment protocols and selective AMU and attitudes toward AMU and AMR was first screened by directed acyclic graphs (DAGs). Variables of interest and potential confounders were included in the multivariable models, while those with variance inflation factor ≥ 10 were excluded to avoid multicollinearity. Four multivariable models were constructed for investigating the association between 1) producers' demographics, 2) farm type, 3) veterinarian visit frequency, and 4) robustness of metritis treatment protocol and the selective AMU practices, controlling for potential confounders. Three multivariable models were built investigating the association between 1) producers' demographics, 2) farm type, and 3) veterinarian visit frequency and producers' attitudes toward AMU/AMR controlling for potential confounders. Significant associations were declared at $p < 0.05$.

Results: By May 2021, 315 usable responses (33.1%) were returned by U.S. dairy producers. Over 75% of producers had had discussion with their herd veterinarians about AMU and believed they could explain the concept of AMR to their neighbors. Approximately one third of producers disagreed that 1) antimicrobials were being overused in the dairy industry, 2) on-farm AMU could cause AMR in humans, and 3) selective dry-cow therapy was more appropriate than blanket therapy. Multivariable models reported that producers went to college were 12.5 times more likely to use antimicrobials selectively for metritis treatment ($p = 0.03$), were more confident to explain AMR to their neighbors ($p = 0.01$), and believed reducing AMU would decrease animal welfare ($p = 0.04$) than those who did not.

Conclusions: Farm characteristic and producers' demographic were associated with selective AMU practices on U.S. dairy farms. We found that producers who went to college were more likely to implement selective AMU for metritis treatment, more knowledgeable for AMR, and more concerned with the negative impact on animal welfare by reducing AMU. These qualities can inform additional research to better understand how to implement best practices on farms.

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

Notes:

179 - Immunogenicity of innate nanoparticles in lambs and efficacy against Respiratory Syncytial Virus infection

Fabian Diaz[1], Elizabeth Grego[1], Balaji Narasimhan[1], Jodi McGill[1]

[1]Iowa State University. fediaz1@iastate.edu

Session: Immunology 2, 2025-01-20, 4:15 - 4:30

Objective: Despite the use of antimicrobials and vaccines, Bovine respiratory disease (BRD) remains a highly prevalent disease in the cattle industry, with bovine Respiratory Syncytial Virus (bRSV) 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 agonists have immunostimulant properties on bovine epithelial and myeloid cells and protect mice against a human RSV challenge. Here, we evaluated whether PANPs are immunogenic in lambs and explored their efficacy in protecting lambs against bRSV disease.

Methods: PANPs were generated by mixing polyanhydride copolymers (5:95 CPTEG:SA, 20:80 CPH:SA and 20:80 CPTEG:CPH) and loading them with CL413 (TLR2/7 agonist) or PAM3CSK4 (TLR1/2 agonist). Two-to-three-week-old lambs were stratified by body weight and sex and given 500 μg of NPs via nasal instillation (n=6 each group) or vehicle (sterile saline, untreated, n=4). Nasal swabs were collected 3, 7, and 14 days after, and airway cells and lung tissue were collected during necropsy. In a second experiment, two-to-three-week-old lambs were stratified by body weight and sex and given 500 μg of NPs via nasal instillation (n=8-12 each group) or vehicle (sterile saline, untreated, n=10) on day zero, then infected with bRSV on day 7. Nasal swabs were collected before and after infection, and respiratory scores were recorded daily after infection. Animals were euthanized six days after infection to evaluate gross pathology and collect lung tissue to determine viral copies, and airway cells to determine immune cell infiltration. Nasal swabs were used to evaluate the transcription of cytokines through RT-PCR and determine viral shedding. Gene expression, cell infiltration, and IL1-β levels were analyzed using one-way ANOVA, and clinical scores using two-way ANOVA with repeated measures.

Results: While most PANPs did not have a significant effect on IL-6, IL-8, and IL-10 transcription in the upper respiratory tract on day 3 after administration, the 5:95 CPTEG:SA PANP encapsulating CL413 increased IL-10 transcription ($p=0.002$) by day 7 after administration. Transcription of these cytokines was not upregulated in airway cells after 14 days; however, the percentage of airway neutrophils and macrophages was modified by PANP treatment, suggesting differential cell recruitment. Airway cells from the 5:95 CPTEG:SA CL413 nanoparticle-treated lambs showed reduced IL1-β secretion upon ex vivo LPS restimulation ($p=0.0033$). After bRSV infection, untreated lambs showed mild respiratory signs and higher airway neutrophil infiltration compared to healthy lambs, but respiratory scores were reduced in PANP-treated groups ($p<0.05$ for CL413 PANPs). Viral titers and shedding are currently being determined.

Conclusions: These results suggest that intranasal PANP delivery in lambs can modify the immune cell environment in the airways and its ability to respond to subsequent inflammatory stimuli. Innate nanoparticle treatment was associated with reduced clinical signs during bRSV infection, suggesting the potential to prevent RSV disease in ruminants. Future studies will address their mechanisms of action, optimize dosing schedules, and their potential use against RSV in cattle to prevent RSV and BRD

Financial Support: Agriculture and Food Research Initiative Competitive Grant no. 2021-07002 (USDA NIFA). Iowa State University Vice President for Research

Notes:

180 - Modifications of mRNA encoding a monoclonal antibody against *Rhodococcus equi* for nebulizing foals

Noah Cohen[1], Daryll Vanover[2], Athos de Oliveira[3], Karin Borba[1], Jae Yeon Joo[2], Rebecca Legere[1], Amelia Woolums[4], Michael Criscitiello[1], Angela Bordin[1], Philip Santangelo[2], Jeroen Pollet[3]

[1]Texas A&M University, [2]Emory University, [3]Baylor College of Medicine, [4]Mississippi State University. ncohen@tamu.edu

Session: Immunology 2, 2025-01-20, 4:30 - 4:45

Objective: *Rhodococcus equi* causes severe pneumonia in foals worldwide. The standard method for preventing *R. equi* foal pneumonia is transfusion of hyperimmune plasma (HIP). Transfusion of HIP is expensive, labor- and time-intensive, and carries risks for foals including circulatory volume overload. We have nebulized mRNA encoding a monoclonal antibody (mAb) targeting the virulence-associated protein A (VapA) of R. equi to produce antibodies in the lungs of foals, but the duration of antibody persistence was <14 days. Thus, our objective was to design mRNA constructs that might provide higher and longer mAb expression.

Methods: Two new strategies were attempted. First, we redesigned and in vitro transcribed (IVT) separate mRNA constructs encoding the heavy (H) and the light (L) chains of the VapA mAb. The H chain sequence included a mammalian signal peptide and a glycosylphosphatidylinositol (GPI) attachment sequence to anchor the mAb to the plasma membrane of transfected cells. The rationale for this anchored design was to provide longer persistence of the mAb in the airway of foals. Second, the Santangelo laboratory designed a single bicistronic mRNA construct expressing both the L and H chains of the mAb. This construct is postulated to decrease cost long term, and possibly be more potent, as every heavy chain is guaranteed to have a light chain. These constructs were used to transfect both Expi293 cells (a human cell line), equine bronchial fibroblasts, and equine fetal lung cells.

Results: Immunostaining confirmed the successful translation of the H and L chain mRNAs, producing VapA mAb antibodies predominantly localized on the external surface of the plasma membrane of both HEK293 cells and equine fibroblasts. The bicistronic mRNA construct resulted in antibody expression and secretion by Expi293 cells; to date, however, expression in equine bronchial fibroblasts or equine fetal lung cells has resulted in intracellular antibody expression in fibroblasts but no secretion of equine antibody by either equine fibroblasts or lung cells. Studies to produce a single bicistronic mRNA encoding L and H that yields a secreted mAb that recognizes VapA are ongoing.

Conclusions: mRNA encoding an anchored mAb against VapA in equine cells is feasible and can be evaluated in vivo for evidence of efficacy to protect foals against infection with *R. equi*. Further work is needed to create a single bicistronic mRNA encoding both L and H that is secreted and suitable for evaluation for efficacy in foals to protect against rhodococcal infection. These constructs might provide foals with protection against rhodococcal pneumonia in way that is safer and more efficacious than plasma transfusion.

Financial Support: This work was supported by a grant from the USDA-NIFA (Project ID: 2022-67015-36335). Additional support was provided by the Link Equine Research Endowment and the Glenn Blodgett Chair in Equine Studies at Texas A&M University.

Notes:

181 - The Role of exosomes in Marek's disease virus-mediated immunosuppression and vaccine responses

Mark S. Parcells[1], Sohee Lee[1], Shannon Modla[1], Arifa Kamal[1], Ken Pendarvis[2], Famatta Perry[1], Phaedra Tavlarides-Hontz[1], Kobia Dwomor[1], Ryan Arsenault[1]

[1]University of Delaware, [2]MZ-Biolabs. Parcells@udel.edu

Session: Immunology 2, 2025-01-20, 4:45 - 5:00

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 pathotypes via size-exclusion chromatography. These were all characterized by TEM, nanotracking analysis (NTA), and proteomic, 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 as well as ex vivo monocyte/macrophages to become activated macrophages and dendritic cells (M1 and M2-polarized) using TPA, chicken GM-CSF, IL-4, IFN-gamma, LPS and IL-13. 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, and have examined the effects of exosomes on innate immune signaling.

Results: This year, we found that (1) purified exosomes injected at 10 days post-vaccination did not appear to affect vaccine efficacy or virulence in MDV-challenged SPF chickens. (2) 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. (3) We have been able to polarize PBMC-derived monocytes to macrophages, dendritic cells and M1/M2-polarized dendritic cells. (4) Exposure of these cells to VEX and TEX was evaluated in terms of its effect on innate immune signaling at 1 and 3 hrs post-treatment with agonists (medium, LPS, Poly I:C and cGAMP).

Conclusions: Our results suggest that by themselves, VEX and TEX do not seem to affect MD vaccine efficacy in the infection model we have evaluated. Changes in innate immune signaling by previous VEX and TEX treatment will be discussed as these results are analyzed.

Financial Support: This research was sponsored by the National Institute for Food and Agriculture, NIFA Award # 2023-67016-40112 awarded to MSP.

Notes:

182 - Probiotic *Lactobacilli* enhances the efficacy of mannose chitosan nanovaccine against *Salmonella* food poisoning in broilers

Sara Dolatyabi[1], Khaled Abdelaziz[2], Raksha Suresh[1], Olaitan Shekoni Comfort[1], Jennifer Schrock[1], Ronna Wood[3], Megan Tenney[1], Sudhir Yadav[1], James Sanko[4], Tonima Rahman[1], Renukaradhya J. Gourapura[1]

[1]Department of Animal Sciences, The Ohio State University, [2]Department of Animal and Veterinary Sciences, Clemson University, [3]The Ohio State College of Veterinary Medicine, [4]Department of Biology, The Ohio State University, USA. dolatyabi.1@buckeyemail.osu.edu

Session: Immunology 2, 2025-01-20, 5:00 - 5:15

Objective: *Salmonella* enterica serovar enteritidis (SE) remains a major source of foodborne illness, with poultry products serving as a significant source of human infections. Widely used current live *Salmonella* vaccines present limitations such as the potential for reversion to virulence and regulatory restrictions on their use in broilers before slaughter. This study aims to evaluate an oral *Salmonella* subunit nanovaccine combined with a probiotic Lactobacilli supplementation, aimed at improving the efficacy of the vaccine by improving the mucosal and systemic immunity in broilers.

Methods: One-hundred-twenty embryonated chicken eggs were obtained from a *Salmonella*-free commercial source. At embryonic day 18, $1x10^6$ CFU of Lactobacillus acidophilus P42 was injected into the amniotic cavity. After hatching chicks received three additional doses of probiotics $1x10^7$ CFU/bird on days 2, 4, and 6 by oral route. A control group received saline and no probiotic. The SE vaccine used in this study was mannose-chitosan nanoparticle formulation entrapped with immunogenic outer membrane proteins (OMP) and flagellin (FLA) and adjuvant whole-cell lysate (WCL) of Mycobacterium smegmatis. The nanoparticle was also surface coated with flagella and mannose to enhance immune targeting. The vaccination regimen involved two oral doses at days 3 and 21, followed by challenge with SE two weeks later. Birds were sacrificed at 4- and 10-days post-challenge. Additionally, a commercial live vaccine was used as a control to our vaccine. Immune responses were evaluated through flow cytometry to analyze recall B-cell and T-cell populations in splenocytes. ELISA was used to quantify antigen-specific secretory IgA or IgY levels in bile, small intestinal wash, cloacal swab, and serum of birds. Bacterial load was measured by analyzing the *Salmonella* colony forming units in the cecal content. Data were statistically analyzed to compare the groups' bacterial load reduction and immune responses.

Results: Bacterial load analysis revealed a 1.2 log reduction in the group receiving the mannose-chitosan nanovaccine with probiotic Lactobacilli (vax+prob) compared to the commercial vaccine group. Flow cytometry data indicated significantly higher B-cell activation in the vax+prob group compared to control groups. Notably, at 10 days post-challenge (DPC 10), the activated B-cell population in the vax+prob group was significantly higher than in the commercial vaccine group, demonstrating a sustained anamnestic antibody response. Cytotoxic T cell population was also significantly higher in the vax+prob group compared to mock-challenge group, and comparable to the commercial vaccine group at DPC 4. At DPC 10, cloacal swab samples had significantly higher OMP-specific sIgA production in the vax+prob group compared to the commercial vaccine group.

Conclusions: The mannose-chitosan based *Salmonella* subunit nanovaccine combined with a probiotic Lactobacilli enhanced the bacterial load reduction and immune responses compared to the commercial vaccine. Combination of the nanovaccine and probiotics led to sustained B-cell and cytotoxic T cell responses, and induced the specific sIgA and IgY production at mucosal and systemic sites. These findings suggest that the *Salmonella* nanovaccine administered in conjunction with a probiotic Lactobacilli offers a promising alternative to a commercial live bacterial vaccine for improving the control of *Salmonella* infection in poultry.

Financial Support: This work was supported by the USDA National Institute of Food and Agriculture USDA-AFRI 2022-67017-36559

Notes:

183 - Mucosal immune responses to avian influenza virus in the Harderian gland of genetically distinct, highly inbred chicken lines

Ying Wang[1], Shenwe Gu[1], Liqi An[1], Yuka Kusunoki[1], Susan J. Lamont[2], Rodrigo A. Gallardo[1], Hans H Cheng[3], Huaijun Zhou[1]

[1]University of California, Davis, [2]Iowa State University, [3]USDA-ARS Avian Disease and Oncology Laboratory. ucywang@ucdavis.edu

Session: Immunology 2, 2025-01-20, 5:15 - 5:30

Objective: Avian influenza (AI) is a significant viral infectious disease that poses substantial economic challenges to the poultry industry. Multiple AI outbreaks, including cases affecting dairy cattle and humans in the U.S. in 2024, emphasize its growing threat to public health. Genetic selection for AI-resistant chicken lines has become an urgent and necessary strategy for the long-term prevention and control of AI outbreaks. The chicken mucosal immune system provides robust defense mechanisms against AI infection through innate and adaptive immune responses. Among the mucosal lymphoid organs, the Harderian gland (HG), which is a part of the upper respiratory tract and densely populated with immune cells, plays a critical role in local mucosal immunity.

Methods: The current study aims to identify key genetic components in the HGs that contribute to genetic resistance against low pathogenic avian influenza (LPAI). We utilized two genetically distinct, highly inbred chicken lines, Fayoumi (relatively AI-resistant) and Leghorn (relatively AI-susceptible), that exhibit significant differences in their resistance to avian influenza virus (AIV). Three-week-old chickens from both lines were inoculated with 107.75 50% egg infective dose (EID50) of LPAIV H6N2 via ocular, nasal, and tracheal routes. Treatment groups included non-infected Leghorn (n=24) and Fayoumi (n=22) as well as infected Leghorn (n=31) and Fayoumi (n=28). Tracheal swabs and tears were collected at 1 and 4 days post-inoculation (dpi) to measure viral titers. Tracheal sections were preserved to measure submucosal thickness and investigate infiltration. HGs were harvested to prepare single-cell (sc) suspensions to analyze macrophage and B-cell populations using the Kruskal-Wallis test with Dunn's multiple comparisons by Prism and scRNAseq at each time point. From each of 4 birds from each group at each time, one HG was snap-frozen to perform bulk ATAC-seq and CUT&RUN targeting H3k27ac, and the other HG was preserved for formalin-fixed, paraffin-embedded tissue sections for performing Xenium in situ spatial gene expression profiling.

Results: Preliminary data showed that at 1 dpi, infected Fayoumis had a higher percentage of macrophages than Leghorns, while Leghorns displayed increased MHC class II expression on macrophages despite their lower macrophage percentages. No significant differences were found in B cell populations, though Leghorns consistently showed higher MHC class II expression on B cells. At 4 dpi, 3 out of 19 infected Leghorns exhibited mild symptoms, while none were observed in Fayoumis. Fayoumis consistently had a higher macrophage percentage than Leghorns, whether infected or not. In Leghorns, MHC class I expression on macrophages was elevated in the infected group compared to non-infected, while Leghorns also exhibited higher MHC class II expression on both macrophages and B cells.

Conclusions: Ongoing scRNA-seq, bulk ATAC-seq, and CUT&RUN assays will identify candidate genes with annotations that may explain the greater AI resistance in Fayoumi chickens. A target gene panel will be developed, and Xenium in situ profiling will offer insights into the molecular mechanisms within the HG that contribute to enhanced resistance. Improving host resilience to AI infection can reduce pathology, enhance vaccine efficacy, minimize economic losses from AI outbreaks, and promote a more sustainable poultry industry.

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:

184 - Duration and characterization of anamnestic responses following RB51 vaccination in bison

Jamison Slate[1, 2], Steven Olsen[3], Mitch Palmer[3], Paola Boggiatto[1, 3]

[1]Iowa State University, [2]ORISE, [3]USDA. jrslate@iastate.edu

Session: Immunology 2, 2025-01-20, 5:30 - 5:45

Objective: Brucellosis is a bacterial zoonotic disease of concern that is both highly transmissible and chronic (causing abortions and stillbirth); brucellosis in cattle is primarily caused by Brucella abortus, which can spread between other ungulate species like American bison (Bison bison) and elk (Cervus canadensis). Treatment for brucellosis in livestock is impractical, although there are efficacious and economical vaccines (S19 & RB51) for cattle that use attenuated strains of B. abortus for disease control. While vaccination efforts between the U.S. Department of Agriculture and livestock industries have virtually eliminated B. abortus in most states, there remains a high-infection rate in the Greater Yellowstone Area (GYA) due to bison and elk populations that act as wildlife reservoirs. Refocusing vaccinations efforts in the GYA has been discussed to control emerging brucellosis from these wildlife species, and previous studies from our lab have successfully used the RB51 vaccine to generate antigen-specific cell-mediated immune responses in captive bison. Therefore, the objective of this follow-up study is to determine how these potentially protective anamnestic responses change over time, and to further characterize RB51-responsive immune cell populations and their effector functions using flow cytometry and enzyme-linked immunosorbent assays (ELISAs).

Methods: In this study cohort of 24 juvenile bison (approximately 9 months old), 16 bison were vaccinated with the commercial RB51 vaccine, and 8 bison were left as non-vaccinated controls. Peripheral blood mononuclear cells (PBMCs) were isolated every 4 weeks up to 24 weeks post-vaccination. The PBMCs were Cell Trace Violet (CTV) stained before being cultured and stimulated (either No Stim, ConA/PMA, RB51, or PPDb) ex vivo for 6 days. Stimulated cell cultures were then treated with a protein transport inhibitor overnight, prior to harvest on day 7. Cultured cells were then washed and labeled with fluorescent antibodies against cell surface markers and intracellular cytokines (ICS) for flow cytometry analysis, while the culture supernatants were stored for later use in commercial ELISA kits for cytokine quantification.

Results: Flow cytometry data showed a gradual increase in RB51-specific immune responses during the first half of the 24-week period, with responses beginning to wane in the latter half of the study. Cell surface markers for CD4, CD8, and gamma-delta T cells combined with ICS staining for interferon gamma (IFN-gamma) and interleukin-17A (IL-17A) allowed for the characterization several effector T cells following homologous antigen (RB51) stimulation. Proliferative responses were greater in vaccinated animals compared to controls following RB51 stimulation. Bovine ELISA kits showed a significant increase in IFN-gamma production from RB51 vaccinated animals beginning at 5 weeks. Interestingly, RB51 vaccinated bison showed significantly more IL-17A production as early as 2 weeks post-vaccination when compared to non-vaccinated controls.

Conclusions: This data underpins the ability of RB51 vaccination to generate anamnestic cell-mediated immune responses in bison. Homologous antigen stimulation increased IFN-gamma production in RB51 vaccinated bison, which previous studies have shown to be important in controlling Brucella infections. Furthermore, for the first time, the data herein also shows the potential of IL-17A mediated immunity following RB51 vaccination in bison.

Financial Support: This project was funded via the USDA through an interagency agreement between Agricultural Research Services (ARS) & the Animal and Plant Health Inspection Service (APHIS). Graduate training was sponsored by the Oak Ridge Institute for Science and Education (ORISE).

Notes:

185 - Characterization and resolution of poly-microbial biofilms in bovine respiratory disease

Thomas J. Inzana[1], Dianjun Cao[1], Bindu Subhadra[1], Yue-Jia Lee[2], Neeti Gandhi[3], Padma Rajagopalan[3], Weiman Weng[1], Nancy Vogelaar[3]

[1]Long Island University, [2]National Taiwan University, [3]Virginia Tech. thomas.inzana@liu.edu

Session: Bovine Respiratory Disease 2, 2025-01-20, 4:15 - 4:30

Objective: Bovine respiratory disease (BRD) is one of the most costly diseases affecting the North American cattle industry. BRD commonly occurs as a chronic infection, which results in a biofilm that enhances bacterial resistance to antibiotics and host defenses. The primary bacterial pathogens associated with BRD are *Histophilus somni, Pasteurella multocida*, and *Mannheimia haemolytica*, and these pathogens can form mono- and poly-microbial biofilms in vitro and in the host. However, there is a substantial gap in our knowledge of how biofilms, particularly poly-microbial biofilms, become established and develop in the host. There is also a need for compounds that can remove the biofilm matrix to enhance the efficacy of antibiotics and host defenses. Our objectives are to: 1) determine if *H. somni* and *M. haemolytica* form a poly-microbial biofilm individually or together with *P. multocida*; 2) use 3D tissue culture modeling of bovine respiratory epithelial and endothelial cells to study how mono- and poly-microbial biofilms become established and develop in vivo; 3) screen an extensive library of compounds for molecules that eliminate the biofilm matrix in vitro and in tissue culture.

Methods: Mono- and poly-microbial biofilms were established in vitro and on 3D bovine tissue culture cells. The biomass of biofilm was assessed by crystal violet staining. Visualization of biofilm architecture was done by fluorescence staining and confocal laser scanning microscopy (CLSM). 3D tissue culture models were developed from bovine pulmonary artery endothelial cells and nasal turbinate epithelial cells and assembled using a collagen matrix. Biofilms were established by simultaneous or sequential addition of log phase bacteria to the cell culture. Compounds from a large library were incubated with established H. somni biofilms prior to biofilm quantification to identify those that could remove at least 75% of the biofilm.

Results: *H. somni, P. multocida*, and *M. haemolytica* formed a poly-microbial biofilm together if *H. somni* initiated biofilm development. *P. multocida* and *M. haemolytica* only formed highly-adherent, prominent mono-species biofilms if decapsulated. *H. somni* and *M. haemolytica* also formed a poly-microbial biofilm on bovine tissue culture cells. *H. somni* formed a prominent biofilm on 3D bovine tissue cultures, and development of the biofilm could be examined over several days. Three compounds were initially identified that could remove most of the *H. somni* biofilm, and were not toxic for the bovine cells at the concentrations tested. Further studies showed that two of the compounds, either individually or together, could remove most of the biofilm formed by *H. somni, P. multocida*, and *M. haemolytica.*

Conclusions: *H. somni* formed the most prominent mono-species biofilm among the three BRD pathogens, but a well-established biofilm could be formed by all three species in vitro, and by at least two species in tissue culture. The application of *H. somni* biofilm formation in a 3D bovine tissue culture system will lead to further *in vivo* studies of biofilm development. Biosafe compounds that can remove bacterial biofilm may enhance the efficacy of antimicrobials and host defenses in controlling BRD and subsequent systemic diseases.

Financial Support: This work was supported by USDA-NIFA grant 2019-67015-74129916, and Long Island University funds to TJI.

Notes:

186 - Impact of six different antimicrobial treatments on the microbiome and resistome of feedlot cattle

Molly McClurg[1], Enrique Doster[1], Lee J. Pinnell[1], Cory Wolfe[1], Rebecca A. Bigelow[2], Robert Valeris-Chacin[1], Matthew A. Scott[1], John T. Richeson[3], Paul S. Morley[1]

[1]VERO Program, Texas A&M University, [2]Kansas State University, [3]West Texas A&M University. mcclurgmc@tamu.edu

Session: Bovine Respiratory Disease 2, 2025-01-20, 4:30 - 4:45

Objective: Metaphylaxis is highly effective in controlling bovine respiratory disease (BRD) in feedlot cattle. While metaphylaxis is a commonly used practice resulting in decreased prevalence of BRD, increased scrutiny about antimicrobial resistance (AMR) has led to greater emphasis on minimizing antimicrobial drug (AMD) treatments when possible. Many commonly used antimicrobial drugs have been evaluated for efficacy in treating sick cattle, but there is limited information about the impacts of AMD treatments on the resistome and microbiome in cattle. This study compared the effects of six commonly used antimicrobials on the resistome and microbiome of 105 cattle over 56 days to identify shifts in resistance gene profiles that could have downstream implications on the spread of AMR.

Methods: Cattle (n=105) were stratified by weight and randomly assigned into seven treatment groups: negative (untreated) control (NC), tulathromycin (TULA), tildipirosin (TILD), oxytetracycline (OXY), ceftiofur (CEFT), florfenicol (FLOR), and enrofloxacin (ENRO). Cattle at low risk for BRD were used for this study to reduce the likelihood of disease processes confounding the impact of drug exposures. All AMDs were administered once according to label instructions. Each pen group (n=15) included ten animals administered their respective AMD on Day 0 and five untreated animals that served as untreated controls within the pen. Nasopharyngeal swabs were collected at six timepoints throughout the study (Days 0, 3, 7, 14, 21, and 56), and fecal samples were collected at three timepoints (Days 0, 21, and 56). Target-enriched shotgun sequencing was used to characterize AMR genes, and 16S rRNA gene sequencing was used to characterize microbial community structures.

Results: Microbial diversity in treated animals decreased from Day 0 to Day 7, increased on Day 14 and 21, and then shifted back to the Day 0 profile by the trial's end on Day 56. The microbiome of sentinel animals remained consistent with that of the negative control group, suggesting there was no impact from contact with treated animals. Pairwise comparisons of Shannon's diversity using the Wilcoxon test in nasopharyngeal samples showed significant differences on days 0, 3, 7, and 14. There were no significant differences on days 21 and 56. Notably, no treatment groups at any time were significantly different from the control group. There were no significant differences in Shannon's diversity of fecal samples by timepoint or treatment group. The relative abundance of resistance genes differed significantly among cattle in the CEFT, ENRO, and OXY groups compared to the TULA, TILD, FLOR, and NC treatment groups.

Conclusions: These results suggest that a single dose of AMDs used for metaphylaxis has a measurable effect on the microbiome and resistome of cattle.

Financial Support: Research supported by Texas A&M AgriLife Research, Texas A&M University

Notes:

187 - Histologic characterization of pulmonary lesions in feedyard cattle mortalities

J. Feng[1], L. F. Feitoza[2], B. J. White[2], B. L. Plattner[1], Abigail Finley[3]

[1]Department of Diagnostic Medicine and Pathobiology, Kansas State University, [2]Beef Cattle Institute, Kansas State University, [3]Shreiber School of Veterinary Medicine, Rowan University. lffeitoza@vet.k-state.edu

Session: Bovine Respiratory Disease 2, 2025-01-20, 4:45 - 5:00

Objetive: This project sought to characterize the subtype and chronicity of pulmonary lesions in feedlot calf pneumonias, to improve our understanding of this economically significant disease.

Methods: Field necropsies (n=178) were performed in commercial Kansas feedlots (2022-2023). Affected lung tissues were examined grossly and photographed, and two representative samples from each animal were collected and routinely processed for histologic evaluation. Thirty-six cases were excluded due to severe postmortem histologic autolysis.

Results: Each sample was classified histopathologically and results from the two samples combined to generate a case-level diagnosis of bronchopneumonia (BP), interstitial pneumonia (IP) or bronchopneumonia with interstitial pneumonia (BIP). A case diagnosis of BP indicated BP was found in one or more samples with no evidence of IP; IP was based on IP on one or more samples with no BP; BIP was based on finding both BP and IP in samples from a case. The remaining cases were grouped as other/non-pneumonia. For each case, pneumonia subtypes were then categorized as acute or chronic based on histologic features; cases with any evidence of chronicity were classified as chronic. The frequency of case diagnosis (n=142): BP (57/142, 40%), BIP (36/142, 25%), IP (33/142, 23%), and other /non-pneumonia (16/142, 11%). Most BP cases were acute (38/57, 67%) while IP and BIP were more commonly chronic (21/33, 64%; 24/36, 66%, respectively).

Conclusions: This work illustrates IP is a frequent histopathologic finding in feedlot mortalities either alone or in conjunction with BP and cases with an IP component tended to have chronic pathology.

Financial Support: Project funded by the Foundation for Food and Agricultural Research Late Day Pulmonary disease Grant ID 22-000564, Beef Cattle Institute at Kansas State University and access to feedyard cattle provided by Innovative Livestock Services.

Notes:

188 - How does vaccination and marketing impact bovine respiratory disease and inflammatory mediator production in beef calves?

Hudson McAllister[1], Sarah Capik[2], Robert Larson[3], Brad White[3], David Amrine[4], Brandi Karisch[5], Kelsey Harvey[6], Jane Parish[5], Amelia Woolums[7], Vinicius Gouvea[8, 9], Alexis Thompson[10], Matthew Scott[1]

[1]Texas A&M University - VERO Program, [2]Tumbleweed Veterinary Services, [3]Kansas State Univeristy Dept. Clinical Sciences, [4]Kansas State University College of Veterinary Medicine, [5]Mississippi State University Dept. Animal and Dairy Sciences, [6]Mississippi State University Prairie Research Unit, [7]Mississippi State University Dept. Pathobiology and Population Medicine, [8]Texas A&M Agrilife Research, [9]Texas A&M University Dept. Animal Science, [10]Texas A&M Veterinary Medical Diagnostic Lab. hmcallister15@tamu.edu

Session: Bovine Respiratory Disease 2, 2025-01-20, 5:00 - 5:15

Objective: Bovine respiratory disease (BRD) continues to plague the beef cattle industry after decades of research. The industry's goal is to prevent BRD through measures like vaccination, weaning and backgrounding, and tailored marketing strategies. However, reported data describing the relationship between these measures and BRD occurrence are surprisingly sparse. This study aims to evaluate how preweaning vaccination and post-weaning marketing decisions influence BRD morbidity and inflammatory mediator production throughout the life of beef calves.

Methods: In this three-year study, in each year 84 bull calves were enrolled in a split-plot randomized controlled trial to quantify the impact of controlled management tactics on health outcomes and performance. Calves were randomly assigned to receive vaccination or not during the cow-calf phase, then further assigned to either direct shipment to a backgrounding facility or an auction market/order buyer facility prior to shipment following abrupt weaning. Calves were evaluated over six timepoints: T1; vaccination; T2, seven days post-vaccination; T3, revaccination and surgical castration; T4, weaning; T5, backgrounding arrival; T6, end of backgrounding. Blood samples were taken at each timepoint for serum evaluation of inflammatory cytokines. At T1, calves were tested via ear notch ELISA for bovine viral diarrhea virus persistent infection and received a multivalent respiratory vaccine (Pyramid 5, Boehringer Ingelheim Animal Health) or 0.9% saline subcutaneously; there were no BVDV-positive calves. At T3, calves were revaccinated identically as T1 and surgically castrated. Treatment groups were maintained with no direct contact. In the 45-day backgrounding phase, cattle were examined daily for signs of BRD and treated based on a standard protocol. Inflammatory cytokines were measured using commercial ELISA kits for bovine IL-1β (ThermoFisher), bovine haptoglobin (Immunology Consultants Laboratory, INC.), and bovine TNF-α (R&D Systems). Serum concentrations were evaluated via generalized linear mixed effect models (GLMMs) estimated via Gaussian distribution with the Kenward-Rodgers adjustment for degrees of freedom; GLMMs were performed with the glmer function in the lme4 package within R v4.1.2. Independent models included serum concentrations (IL1-β, haptoglobin, and TNF-α) as the response variable, evaluating treatment group, day (timepoint), and their interaction as predictor variables, and random intercepts for pasture, animal ID, and year.

Results: There were no significant treatment or time effects on TNF-α in year 1 or year 2. However, IL-1β possessed significant interactions for day, treatment group, and the interaction of day and treatment in year 1. In year 2, IL-1β had a significant interaction of day and treatment. There were no significant treatment or time effects on haptoglobin, however BRD morbidity and mortality was statistically different across years.

Conclusions: This study is one of the first to evaluate the influence of common management strategies on life-long performance and disease in beef cattle. Here, we begin to describe how vaccination during the cow-calf phase of life and post-weaning marketing decisions influence BRD morbidity and mortality, growth and performance, and inflammatory cytokine production that may be used to enhance disease management practices. Significant treatment effects on inflammatory mediators may have been missed due to relatively few sampling time points.

Financial Support: This work is supported by the USDA National Institute of Food and Agriculture (NIFA) Agriculture and Food Research Initiative Competitive Grant No. 2019-67015-29845. Any opinions, conclusions, or recommendations do not necessarily reflect the view of the USDA

189 - Disease severity alters mineral metabolism and gene expression in lesion and non-lesion lung tissues of bovine respiratory disease challenged steers

Emma Rients[1], Stephanie Hansen[1], Jodi McGill[1]

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

Session: Bovine Respiratory Disease 2, 2025-01-20, 5:15 - 5:30

Objective: Bovine respiratory disease (BRD) is a leading cause of morbidity and mortality in the beef industry. Micronutrients such as zinc and vitamin A play an integral role in the response to pathogens. However, there is limited knowledge regarding the metabolism of micronutrients during BRD infection and how disease severity may impact micronutrient needs. This study explored the effects of disease severity on the mineral concentration and gene expression and compared lesioned (LL) and non-lesioned (NLL) lung tissue.

Methods: Thirty dairy x beef crossbred steers (230 ± 12.1 kg) were inoculated with 104 TCID50 BRSV strain 375 on d 0 followed by 9.3 x 109 CFU Mannheimia haemolytica strain D153 on d 7. Necropsies were performed on d -1, 5, 10 and 15 of challenge. Lungs were given gross pathology scores of 0 to 5 where 0 = free of lesions, 1 = 1-5% affected (n = 4), 2 = 5-15% affected (n = 11), 3= 15-30% affected (n=8), 4 = 30-50% affected (n=3) and 5 = more than 50% affected (n=4). Samples of LL and NLL were collected for mineral (Ca, Cu, Fe, Mn, Mg, P, and Zn) concentration and qPCR analysis for genes related to Zn and vitamin A metabolism and immune response. Statistics were analyzed using the Mixed procedure in SAS 9.4 (Cary, NC) as a multivariate paired analysis with fixed effects of lung pathology score (LPath) and tissue type. The slice option was used to determine simple effects of lung pathology score within tissue type.

Results: There was a tendency for an LPath × tissue interaction for Fe concentrations ($P = 0.08$) where NLL had lower Fe concentrations at lower pathology scores (LL simple $P = 0.03$) and NLL was increased in comparison to LL. However, Fe concentrations did not differ significantly within NLL across pathology scores (NLL simple $P = 0.73$). Lesion lung had greater concentrations of Ca, Mn and P compared to NLL (Tissue $P \leq 0.02$). Additionally, lung pathology score influenced Zn and Mg concentrations (LPath $P = 0.04$) where Zn and Mg decreased as LPath severity increased and LL tended to have a greater magnitude of change across LPath (LL simple $P = 0.08$). Gene expression of GLUT2, MMP9, MUC5A/C, RALDH2, STRA6 and ZIP8 was increased in LL (tissue $P \leq 0.03$). Conversely, gene expression of occludin, RBP4, RXRα, ZIP1, ZIP2, ZnT1 and ZnT4 was decreased in LL tissue (tissue $P \leq 0.01$). Expression of AHR, occludin, RXRα, ZIP1 and ZIP2 was affected by LPath score (LPath $P \leq 0.05$). Additionally, AHR, occludin, ZIP1 and ZnT4 expression was affected by LPath score within LL (LL simple $P \leq 0.05$) but not within NLL (NLL simple $P \geq 0.37$).

Conclusions: These finding demonstrate that the severity of BRD is associated with alterations in mineral metabolism, specifically within LL, and there is a change in demand for micronutrients in LL compared to NLL. These results highlight the potential for targeted micronutrient interventions to support the immune response to BRD.

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:

190 - Nasal and bronchoalveolar BRSV IgG-1 transferred from maternal colostrum or a colostrum replacement in dairy calves

O.F. Huertas[1], M.F. Chamorro[1], T. Passler[1], M. Saucedo[1], S. Taylor[1], D. Schwartz[1], J.E. Bayne[1], J. Stockler[1], M. Thoresen[2], A. Woolums[2]

[1]Auburn University, [2]Mississippi State University. ozh0013@auburn.edu

Session: Bovine Respiratory Disease 2, 2025-01-20, 5:30 - 5:45

Objective: Bovine respiratory syncytial virus (BRSV) is an important cause of respiratory disease in young dairy calves worldwide. Clinical protection from maternal colostrum as well as from vaccination with intranasal (IN) modified-live BRSV vaccines is inconsistent. The presence of passively transferred BRSV IgG-1in the respiratory tract of calves could affect the efficacy of IN vaccination. Currently, the initial titers and persistency of BRSV IgG-1 transferred from colostrum into the respiratory tract of neonatal calves is unknown. The objective of this study was to determine the initial level and persistency of BRSV IgG-1 titers transferred from maternal colostrum or a colostrum replacement into the upper and lower respiratory tract of dairy calves.

Methods: Nasal secretion (NS) and bronchoalveolar fluid (BALF) samples were collected from dairy calves that received either 6 L of maternal colostrum (MC; n=18) or a total mass of 300 g of IgG from a commercial colostrum replacement (CR; n=21) within 12 hours of life. Samples were collected at birth, 48 h of life, and at 14, 28, and 90 days of age for determination of BRSV IgG-1 titers by ELISA. Additionally, serum samples were collected from all calves at birth and at 48 hours of life to determine total IgG levels by radial immunodiffusion, respectively. The apparent efficiency of absorption (AEA) of IgG was calculated for the CR group using the following formula: serum IgG concentration at 48 h of age (g/L) × plasma volume (L) ÷ total IgG intake (g).

Results: Titers of NS BRSV IgG-1 were detected at 48 h of life but had decayed completely by 14 days of age in all calves. The median NS BRSV IgG-1 titer at 48 h was greater in MC calves compared with CR calves (50 vs. 25). Preliminary BALF BRSV IgG1 titers persisted longer and were detected in very low levels up to 14 days in MC calves and up to 90 days in CR calves; however, significant differences among MC and CR calves were not observed at any time point. The mean ± SD serum total IgG concentrations at 0 h of life were minimal and not significantly different between groups (P = 0.36). In contrast, the mean ± SD serum total IgG concentrations at 48 h of life were significantly (P = 0.003) greater in MC calves (30.8 ± 4.3 g/L) compared with CR calves (15.4 ± 2.6 g/L). The AEA of IgG for the CR group was 14.7%, which is much lower than AEA reported in previous studies using the same colostrum replacer product in dairy calves.

Conclusions: The short persistency of BRSV IgG-1 titers transferred from colostrum into the upper respiratory tract of dairy calves may not play an important role on interfering with adequate immunization following IN vaccination. The similar respiratory BRSV IgG-1 concentrations and different total serum IgG levels observed in calves from this study reflect the limitations of total serum IgG to predict transfer of adequate mucosal immunity against specific infectious agents.

Financial Support: Department of Clinical Sciences. College of Veterinary Medicine. Auburn University. Department of Pathobiology and Population Medicine. College of Veterinary Medicine. Mississippi State University. Saskatoon Colostrum Company

Notes:

191 - Mass vaccination against avian influenza with novel Modified Live Virus (MLV) vaccines

Daniel Perez[1], Joaquin Caceres[1], Daniela Rajao[1], Brian Jordan[1], Silvia Carnaccini[1], Darrell Kapczynski[2]

[1]University of Georgia, [2]USDA-ARS. dperez1@uga.edu

Session: Vaccinology 1, 2025-01-20, 4:15 - 4:30

Objective: To develop safe and effective modified live virus (MLV) vaccines against H9N2 and H5N1 avian influenza viruses (AIV), amenable to mass vaccination strategies.

Methods: Phylogenetic and antigenic analyses were used to select vaccine strains representative of the antigenic diversity of H9N2 AIV. The studies with H5N1 AIV are an expansion of the original grant application and a representative strain of clade 2.3.4.4b has been chosen. MLV vaccine candidates with rearranged genomes (RAM and RAM42) were generated using reverse genetics approaches. The safety, immunogenicity, and efficacy of the vaccines were evaluated in chickens. Data analyses and graphs are produced using GraphPad Prism software version 10 (GraphPad Software Inc., San Diego, CA, USA). Ordinary one-way or two-way ANOVA as needed are performed to calculate P values followed by Tukey's multiple comparison tests. A P value below 0.05 is considered significant.

Results: RAM MLV vaccine candidates were successfully generated against representative strains of H9N2 AIV and the 2.3.4.4b clade of H5N1 AIV. The vaccines were shown to be safe and immunogenic in chickens using ELISA and hemagglutination inhibition assays. Studies are ongoing to evaluate the efficacy of monovalent and bivalent MLV vaccines against H9N2 and H5N1 AIV in chickens. RAM42 MLV vaccine candidates will be evaluated in the near future.

Conclusions: The MLV vaccine platforms show promise for the development of safe and effective vaccines against H9N2 and H5N1 AIV. The use of MLV vaccines could potentially lead to improved control of AIV in poultry.Note: This abstract is being presented in fulfillment of the requirements for a Project Director of USDA-NIFA funded research, Award Number: 2024-67015-42736.

Financial Support: This research is sponsored by NIFA-USDA Award Number: 2024-67015-42736. Program: Animal Health and Production and Animal Products: Animal Health and Disease. Proposal Title: Mass Vaccination Against H9N2 Avian Influenza With Novel Modified Live Virus Vaccines

Notes:

192 - In silico design and evaluation of a cross-protective, multiepitope vaccine against outbreak-associated *Salmonella*

David J. Bradshaw II[1, 2], Shawn M.D. Bearson[1]

[1]USDA-ARS, [2]Oak Ridge Institute for Science and Education. David.Bradshaw@usda.gov

Session: Vaccinology 1, 2025-01-20, 4:30 - 4:45

Objective: Non-typhoidal *Salmonella* enterica subspecies enterica (NTS) is an important source of foodborne illness and is estimated to cause 1.35 million illnesses, 26,500 hospitalizations, and 420 deaths annually in the United States by the Centers for Disease Control and Prevention (CDC). The Interagency Food Safety Analytics Collaboration estimates that contaminated food animal meat from chickens and turkeys represents 18.6% and 5.5%, respectively, of foodborne NTS infections. Vaccination is a potentially effective intervention to lower NTS loads in food animals, thus reducing food chain transmission. A limitation of currently available commercial vaccines is cross protection against multiple *Salmonella* serovars (>2,600), thereby indicating a need for improved vaccine design. Multiepitope vaccines designed using reverse vaccinology tools can be created with statistically selected, antigenic epitopes and screened/evaluated in silico. This study focused on six clinically- and poultry-related NTS serovars to design a cross-protective, multiepitope vaccine construct (MEVC); the MEVC was evaluated in silico for predicted cross-protection against CDC PulseNet outbreak-associated *Salmonella* isolates.

Methods: Reverse vaccinology tools identified non-LPS and non-flagellar antigenic outer membrane/extracellular/periplasmic chromosomal proteins in the *Salmonella* enterica subsp. enterica serovar Typhimurium str. UK-1 strain (UK-1) genome. These proteins were assessed for immunogenic, antigenic, non-toxic, and hydrophilic epitopes that had homology against six clinically- and poultry-related NTS serovars from serogroups B-E: Typhimurium (B), Infantis (C1), Kentucky (C2), Hadar (C2), Enteritidis (D) and Uganda (E). These epitopes were used to create a MEVC with cytotoxic T-cell (CTL), helper T-cell (HTL) epitopes along with HTL/CTL epitopes predicted to have linear B-cell (LBL) attributes. Presence of the epitopes within PulseNet outbreak-associated genomes was determined with BLAST. Finally, the MEVC was evaluated for physiochemical properties as well as tertiary structure prediction, refinement, and validation.

Results: The reverse vaccinology pipeline resulted in 105 epitopes representing 54 proteins. The nine most antigenic CTL and HTL epitopes along with all LBL epitopes (3 CTL, 6 HTL) were incorporated in a MEVC using epitope-type-associated linkers described in the literature. Each of the selected MEVC epitopes, representing 24 proteins, was compared to sequence assemblies of PulseNet outbreak-associated isolates (n=33,672) representing 142 *Salmonella* serovars; BLAST results showed 19 out of 28 MEVC epitopes had 100% identity and coverage against greater than 99% of the PulseNet outbreak-associated isolates. Following the addition of a *Salmonella* flagellin adjuvant via the EAAK linker, construct evaluation revealed the MEVC to be stable, relatively thermostable, and the predicted tertiary structure model passed defined validation metric thresholds.

Conclusions: A subtractive proteomics and immunoinformatic approach was employed using various reverse vaccinology tools to create and evaluate a *Salmonella* MEVC. Six poultry- and clinically-related NTS serovars were used in the epitope evaluation phase, and the resulting MEVC has predicted cross-protective properties to a variety of *Salmonella* serovars based on sequence homology to outbreak isolates from the PulseNet database. This study shows the utility of reverse vaccinology to identify, assemble, assess, and validate predicted effectiveness of a vaccine design in silico when target organisms are paired with relevant datasets.

Financial Support: We would like to thank the Agricultural Research Service Participation Program (ORISE) for this research opportunity.

Notes:

193 - Enhancing the production of type I interferons to create rationally-defined Marek's disease vaccines

Sonsiray Alvarez-Narvaez[1], Steven Conrad[1], Taejoong Kim[1], Jef Boeke[2], John Dunn[1]

[1]USDA-ARS, [2]NYU Langone. steven.conrad@usda.gov

Session: Vaccinology 1, 2025-01-20, 4:45 - 5:00

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 objective is to ablate MDV genes which frustrate the production of IFN-Is during infection to create vaccine strains with improved protection.

Methods: We purchased five different lentiviruses carrying genes US3, UL46, UL48, Meq, R-LORF-4 and a control GFP gene (under control of a mammalian expression promotor) with puromycin resistance cassette. We infected chicken macrophage-like HD11 cells with each of the lentivirus strains (MOI 10) and exposed infected HD11 to 3.2ug/ml of puromycin for two weeks. Cells that showed resistance to puromycin were expected to have a lentivirus integrated in their genome and therefore expression of inserted MDV gene. After lentivirus insertion we performed a clonal selection and selected five wells that showed only one clonal resistant population and kept expanding them. We have developed a system in which we promote the production of IFN in HD11 cells by transfecting them with a 3µg of a 2kb double-stranded DNA fragment.

Results: We used qPCR to determine the gene expression levels of the IFNω1 gene and identified that the expression of the five MDV genes produced a significant decrease in the expression of IFNω1 compared to the control GFP. To evaluate vaccine efficacy, we used a Meq-deleted MDV clone to produce and rescue three MDV double mutants: (i) Md5-B40ΔmeqΔUL46, Md5-B40ΔmeqΔUL48, and Md5-B40ΔmeqΔR-LORF4. Experiments have been scheduled to evaluate the knockout mutants in chickens.

Conclusions: We have developed and optimized an assay for the identification of MDV immunomodulatory genes based on the activation of avian macrophages expressing target MDV genes via lentivirus integration. We plan to evaluate candidate vaccines 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:

194 - Evaluating the efficacy of mannose conjugated chitosan nanoparticle-based *Salmonella* subunit vaccine in adult layer chickens

Raksha Suresh[1], Shekoni O Comfort[1], Sara Dolatyabi[1], Jennifer Schrock[1], Mithilesh Singh[1], Renukaradhya J Gourapura[1]

[1]Department of Animal Sciences, The Ohio State University. suresh.138@osu.edu

Session: Vaccinology 1, 2025-01-20, 5:00 - 5:15

Objective: *Salmonella* contamination ranks among the leading causes of foodborne illness in humans. The primary source of Salmonellosis is often traced back to contaminated eggs with *Salmonella* Enteritidis. This study was aimed at assessing the efficacy of the mannose chitosan nanoparticle-based S. Enteritidis subunit vaccine containing outer membrane proteins (OMP) and flagella (FLA) [mChitosan (OMP+FLA)/FLA-NP] in adult layer chickens.

Methods: The OMP and FLA were extracted from *S.* Enteritidis and entrapped in mChitosan-NP and the NPs were surface coated with FLA in the vaccine. Three doses of equal amounts of combined OMP and FLA proteins (200 µg, 50 µg, and 10 µg) entrapped in NPs were tested for efficacy in vivo in layer chickens. Control groups include birds receiving only soluble proteins, empty NPs, and mock saline treatment. Birds were vaccinated at 4, 10, 16, and 18 weeks of age and challenged with *S.* Enteritidis $1x10^9$ colony forming units (CFU) per bird at 25 weeks of age. Necropsies were conducted on days 4 and 8 post-challenge with 5-6 birds/group examined at each time point. *Salmonella* loads were assessed in cecum, reproductive tract and eggs and along with the immune responses at local mucosal and systemic sites were carried out.

Results: On day 4 post-challenge, the mChitosan (OMP+FLA)/FLA-NP vaccine with 200 µg proteins showed the greatest reduction in *Salmonella* load in the cecum with 2.5 log reduction compared to the empty mChitosan-NP group, with substantially reduced *S.* Enteritidis positivity rates both in the upper and lower oviducts. The nanoparticle vaccine groups continued to have high levels of specific immune responses at day 8 post-challenge both at the local mucosal and systemic sites of birds. We were unable to detect *Salmonella* in eggs in any of the challenge groups including mock challenge.Statistical differences among groups were determined by one-way ANOVA followed by Tukey's multiple comparison test.

Conclusions: Antibody responses at mucosal and systemic sites supported the bacterial reduction data in the intestines and reproductive tract of adult layer birds administered orally with *Salmonella* subunit mChitosan nanoparticle based vaccine.

Financial Support: This work was supported by the USDA National Institute of Food and Agriculture (USDA-AFRI 2022-67017-36559).

Notes:

195 - Toward a cell-free Marek's disease vaccine

Hafiz Sohaib Zafar[1, 2], Nagendra Prabhu Ponnuraj[1, 2], Keith William Jarosinski[1, 2]

[1]Department of Pathobiology, [2]University of Illinois Urbana Champaign. hszafar2@illinois.edu

Session: Vaccinology 1, 2025-01-20, 5:15 - 5:30

Objective: Marek's disease virus (MDV) is a highly contagious herpesvirus causing Marek's disease (MD) in chickens characterized by tumors, immunosuppression, and paralysis. MD is a significant threat to poultry health worldwide. While modified live virus (MLV) vaccines have been used against MD for over five decades, they have driven MDV to increased virulence despite preventing clinical disease. Most licensed vaccines against MD are administered as cell-associated preparations of attenuated strains of MDV and require liquid nitrogen storage, limiting their accessibility in rural areas. Developing a cell-free vaccine in lyophilized form would greatly benefit the poultry industry worldwide. Our objective is to understand the mechanisms involved in generating infectious cell-free MDV in cell culture to develop more stable, cost-effective MD vaccines. We hypothesize that the conserved herpesvirus protein kinase (CHPK) acts as a master regulator of cell-free virus (CFV) production and influences the subcellular localization of key viral proteins involved in MDV replication and transmission. Specifically, the tegument protein UL47, which shuttles between the nucleus and cytoplasm, is likely regulated by CHPK-mediated post-translational modifications (PTMs) affecting its subcellular localization. Here, we will define the nuclear localization (NLS) and export (NES) sequences.

Methods: Mutagenesis techniques were employed to generate multiple variants from a fluorescently tagged UL47 expression construct (pcUL47mRFP). Subcellular localization assays were conducted to assess the role of putative NLS and NES in UL47 distribution in cells.

Results: Truncation analysis by dividing UL47 into five fragments revealed that the regions containing amino acids 301-450,451-600, and 601-808 exhibited altered subcellular distribution. Furthermore, the deletion of multiple putative NLS motifs significantly shifted the localization of UL47 to the cytoplasm, suggesting a cumulative effect of these signals in nuclear import. Surprisingly and contrary to expectations, the putative NES motif deletion led to pronounced cytoplasmic expression of pUL47.

Conclusions: This study's initial findings challenge the conventional understanding of NLS and NES motifs for UL47 and highlight the complex regulatory mechanisms controlling its subcellular localization. These results also open avenues for further investigation into the molecular complexities of MDV host cell interactions which ultimately help in understanding MDV biology and for the development of novel MDV vaccines and therapeutics.

Financial Support: This work is supported by the "Agriculture and Food Research Initiative (AFRI)" project award no. 2024-67015-42412, from the U.S. Department of Agriculture's National Institute of Food and Agriculture.

Notes:

196 - USDA *Salmonella* vaccine reduces colonization and dissemination of serovar Enteritidis in chickens

Samuel J. Whelan[1], Bradley L. Bearson[2], Maya P.N. Encinosa[1], Durga P. Neupane[1], Shawn M.D. Bearson[1]

[1]USDA-ARS, National Animal Disease Center, [2]USDA-ARS, National Laboratory for Agriculture and the Environment. Samuel.whelan@usda.gov

Session: Vaccinology 1, 2025-01-20, 5:30 - 5:45

Objective: Nontyphoidal *Salmonella* enterica is a leading cause of foodborne illness. Among the most common serotypes globally, *Salmonella* enterica serovar Enteritidis (*S.* Enteritidis) frequently colonizes poultry without causing signs of disease. Recently, a strain of *S.* Enteritidis with decreased susceptibility to ciprofloxacin (DSC), a critical antibiotic prescribed for complicated human infections, has emerged in chickens with the gyrA gene encoding a D87Y modification. In the current study, vaccination with the cross-protective, cross-species USDA BBS 866 *Salmonella* DIVA vaccine was evaluated for reduction of intestinal colonization and systemic dissemination of DSC *S.* Enteritidis in broiler chickens.

Methods: One-day-old broiler chicks purchased from a commercial hatchery tested negative for *Salmonella* and were randomly distributed into two isolation rooms (n=44/room). One room of chicks was vaccinated at one day of age via aerosol spray with the USDA BBS 866 *Salmonella* vaccine and booster vaccinated at two weeks of age via water administration (~3 x 10^8 colony forming units (CFU)/chick); the second room of chicks served as the mock-vaccinated control room and was similarly administered phosphate buffered saline (PBS). At 5 weeks of age, *Salmonella* status was determined for 12 birds/room and all remaining chickens were challenged via oral gavage with 1 x 10^9 CFU of DSC *S.* Enteritidis strain SX514 (FSIS12211648). Chickens were randomly selected at 7- and 14-days post-inoculation (dpi) for euthanasia to collect cecum, spleen, and bone marrow tissues. Qualitative and quantitative bacteriology for *Salmonella* was performed to determine prevalence and colonization load.

Results: At 3-weeks post booster vaccination, the USDA BBS 866 vaccine was detected in the cecal contents from 17% (2/12) of the vaccinated chickens. After challenge, DSC *S.* Enteritidis colonized the cecum (a site of prolonged and high levels of *Salmonella* colonization) as well as the spleen and femoral bone marrow (two sites that indicate systemic dissemination), and vaccination with USDA BBS 866 significantly reduced DSC *S.* Enteritidis intestinal colonization and system dissemination compared to the mock-vaccinated group. Prevalence of DSC *S.* Enteritidis (i.e. +/-) was significantly decreased in the vaccinated chickens for the cecum (14 dpi), spleen (7 and 14 dpi), and bone marrow (7 dpi) compared to the mock-vaccinated chickens. For the bone marrow of mock-vaccinated chickens, 5 out of 13 chickens (7 dpi) and 3 out of 13 chickens (14 dpi) tested *Salmonella*-positive; the bone marrow of all vaccinated chickens at both time points tested negative for *Salmonella*. Quantitative analysis revealed significant reduction in colonization levels of DSC S. Enteritidis in the vaccinated chickens at 7 dpi for the cecum (2.7 log reduction), spleen (2.4 log reduction) and bone marrow (0.4 log reduction) and at 14 dpi for the cecum (1.6 log reduction) and spleen (1.6 log reduction) compared to mock-vaccinated birds.

Conclusions: Vaccination with the USDA BBS 866 *Salmonella* vaccine significantly reduced cecal colonization and splenic dissemination and prevented dissemination to the bone marrow by DSC *S.* Enteritidis in broiler chickens, indicating a promising intervention for preventing food product contamination by DSC *S.* Enteritidis.

Notes:

197 - Systems vaccinology: utilising the "-omics" toolkit to elucidate the mechanisms underlying immune responses

Daniel O'Connor[1]

[1]Department of Paediatrics, University of Oxford. Daniel.OConnor@paediatrics.ox.ac.uk

Session: Animal Vaccinology Research Network Symposium, 2025-01-21, 8:30 - 9:15

Vaccines have revolutionized human and animal health, becoming essential tools in controlling infectious diseases. Despite their success, the precise immunological mechanisms underlying their protective effects remain incompletely understood, and vaccine development has historically relied on empirical approaches. Challenges persist in targeting pathogens that evade current vaccination strategies and in addressing zoonotic diseases with pandemic potential, as starkly highlighted by the COVID-19 crisis.

Recent technological advances offer unprecedented opportunities to dissect the complex immunological processes that drive vaccine responses. Systems vaccinology, leveraging multi-omics approaches, holds immense promise for deepening our understanding of these responses and ushering in a new era of rational vaccine design.

In this talk, I will discuss key findings from multi-omics datasets generated during human immunization studies, highlighting insights that could transform vaccine development. These advances are poised to modernize vaccinology, enhancing our ability to combat infectious diseases and protect the health of both humans and animals worldwide.

Notes:

198 - Genome-led vaccine target discovery for parasitic livestock infections

Gavin Wright[1], Delphine Autheman[1]

[1]University of York. gavin.wright@york.ac.uk

Session: Animal Vaccinology Research Network Symposium, 2025-01-21, 9:15- 10:00

Trypanosomes are protozoan parasites that cause infectious diseases including human African trypanosomiasis (sleeping sickness), and nagana in economically-important livestock animals. An effective vaccine against trypanosomes would be an important control tool, but the parasite has evolved sophisticated immunoprotective mechanisms including antigenic variation that present an apparently insurmountable barrier to vaccination. Using a systematic genome-led reverse vaccinology approach and murine infection models of Trypanosoma infection, we show that protective invariant subunit vaccine antigens can be identified. Vaccination with a single recombinant protein comprising the extracellular region of a conserved cell surface protein induced long-lasting protection. Immunity was passively transferred with immune serum, and recombinant monoclonal antibodies could induce sterile protection and revealed multiple mechanisms of antibody-mediated immunity, including a major role for complement. To translate this research we are developing livestock infection models of both Trypanosoma congolense and Trypanosoma vivax that are suitable for testing subunit vaccines. Our discovery identifies a vaccine candidate for an important parasitic disease that has constrained the socioeconomic development of sub-Saharan African countries and challenges long-held views that vaccinating against trypanosome infections cannot be achieved.

Financial Support: This research received support from the Bill and Melinda Gates Foundation, Wellcome Trust and UK BBSRC.

Notes:

199 - Thermal inactivation spectrum of influenza A H5N1 virus in raw milk

Mohammed Nooruzzaman[1], Lina M. Covaleda[1], Nicole H. Martin[2], Katherine Koebel[1], Renata Ivanek[1], Samuel D. Alcaine[2], Diego G. Diel[1]

[1]Department of Population Medicine and Diagnostic Sciences, Cornell University, [2]Department of Food Science, Cornell University. mn496@cornell.edu

Session: One health / Public health 2, 2025-01-21, 8:30 - 8:45

Objective: The spillover of highly pathogenic avian influenza (HPAI) H5N1 virus to dairy cows and shedding of high amounts of infectious virus in milk raised public health concerns. Here, we evaluated the decay and thermal stability spectrum of HPAI H5N1 virus in raw milk.

Methods: For the decay studies, HPAI H5N1 positive raw milk was incubated at different temperatures (4°C, 20°C, 30°C and 37°C) for 56 days (8 weeks) and viral titers and the thermal death time D-values were estimated. We then heat treated HPAI H5N1 virus positive milk following different thermal conditions including pasteurization (63°C 30 min and 72°C 15 s) and subpasteurization/thermization (50°C 10 min, 60°C 10 min, 63°C 22 s and 69°C 22 s) conditions using a thermocycler and a submerged coil heating system. Virus titers were determined using cell culture and embryonated chicken eggs.

Results: Decay studies revealed long-term stability of HPAI H5N1 in raw milk samples stored at 4°C, with infectious virus being detected for up to 56 days (8 weeks) in these samples. Incubation of raw clinical milk from infected cows at 20°C resulted in inactivation of the virus within 21 days (3 weeks), whereas rapid virus decay was observed at 30°C (6 days, <1 week) and 37°C (2 days) in spiked milk samples. The estimated D-values of HPAI H5N1 at 4°C, 20°C, 30°C and 37°C were 10.74, 2.23, 1.16 and 0.34 days, respectively. Efficient inactivation of the virus was observed in all tested conditions, except for thermization at 50°C 10 min. Utilizing a submerged coil system with temperature ramp up times that resemble commercial pasteurizers, we showed that the virus was rapidly inactivated by pasteurization and most thermization conditions.

Conclusions: Our study provides a comprehensive overview of the thermal inactivation spectrum of HPAI H5N1 virus in raw milk, demonstrating the efficacy of thermal treatment including thermization and pasteurization conditions on inactivation of HPAI H5N1 virus in raw milk.

Financial Support: Financial Support: The work was funded by the New York State Department of Agriculture and Markets (award no. CM04068HM).

Notes:

200 - Regional variability in biosecurity investment choices in the United States: the role of socio-psychological and demographic factors

Asim Zia[1], Richmond Baye[1], Scott Merrill[1], Eric Clark[1], Julie Smith[1]

[1]University of Vermont. rbaye@uvm.edu

Session: One health / Public health 2, 2025-01-21, 8:45 - 9:00

Objective: The United States anticipates severe socio-economic repercussions from an African Swine Fever (ASF) outbreak. Preventing ASF requires robust on-farm biosecurity measures, but human behavior and decisions on farms and at ports of entry may undermine this objective. Current biosecurity policies seek to reduce the impact of disease outbreaks yet because investments in biosecurity are not linked to indemnification, these policies may lead to a perverse incentives of reducing expenditures in biosecurity during an outbreak. Transitioning to a conditional indemnity policy could introduce a cost-sharing mechanism, reducing the financial burden of disease control. We hypothesize that anticipating regret or anticipating benefit will lead respondents to prefer a conditional indemnity policy. Moreover, understanding the importance of biosecurity adoption will switch producers away from the baseline unconditional indemnity policy.

Methods: Before launching the study, we pretested and validated the survey instrument with 20 respondents. We screened participants to ensure they were based in the U.S. and actively involved in the agriculture, food, and natural resources sector. A total of 500 participants were recruited through the online survey platform Prolific. The survey was designed to elicit preferences between the existing unconditional and alternative conditional indemnity policies, as well as to identify the behavioral drivers underlying these choices. To minimize cognitive load, the survey was structured for an average completion time of 6 minutes, with participants receiving $3.50 upon full completion. Incomplete responses were automatically returned to participants for completion. Additionally, attention checks were included to ensure respondents were thoughtfully engaging with the survey. This approach allowed us to achieve a 100% completion rate within one month.

Results: Our findings reveal a strong preference for conditional indemnity policies. Respondents who anticipated regret were more likely to choose the high conditional indemnity policy over the baseline unconditional indemnity policy. Similarly, a higher perceived importance of adopting biosecurity measures was associated with selecting conditional indemnity policies. Additionally, every extra dollar invested in biosecurity correlated with a preference for high conditional indemnity policies. Demographic analysis showed that women and older individuals were more inclined towards conditional indemnity policies. We also observed regional variations: the West and Northeast preferred conditional indemnity policies, while the Midwest favored the baseline unconditional indemnity.

Conclusions: These results suggests that a one-size-fits all solution is not ideal and point to potential shift towards conditional indemnity policies as a way to strengthen biosecurity practices and address disease risk, with varying preference across demographics and regions.

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

Notes:

201 - Comparing environmental and personal air sampling methods for full-length 16S-based microbiome analysis of samples collected in farms, a pilot study

Gerardo R Diaz[1], Juan Mena[1], Mariana Meneguzzi[1], My Yang[1], Montserrat Torremorell[1], Noelle Noyes[1]

[1]Department of Veterinary Population Medicine, University of Minnesota. diazo005@umn.edu

Session: One health / Public health 2, 2025-01-21, 9:00 - 9:15

Objective: The study objective was to compare sample collection methodologies for obtaining surface, airborne particles and personal air samples in livestock production facilities, in order to provide recommendations for the optimal sampling methodology for subsequent microbiome analysis.

Methods: For surface sampling, we sampled pen railings directly using both dry gauze ("DRY") and gauze pre-moistened with viral transport media without antibiotics ("VTM") in two swine farms. For airborne particles sampling in the same swine farms, we placed aluminum foil near the pen railing to capture settled airborne particles over 90 minutes, after which we wiped the foil with both DRY and VTM gauze. For both surface and foil samples, the dry gauze was processed either as-is, or by eluting into 10mL of PBS in the laboratory ("PBS"). For personal air samples, we tested two devices: a 3-stage NIOSH BC251 sampler (air flow rate = 3.5L/min) and an Aerocollect device (air flow rate = 0.2L/min). Personal air samples were collected on a single dairy farm across three daily sessions (3 replicates) by outfitting a single person with both devices and having him walk around the farm for 1 hour. All samples were subjected to total DNA extraction, except for 1 replicate of the Aerocollect sampling. This device lyses cells as part of the collection process, and therefore 1 replicate was not subjected to DNA extraction, in order to compare unextracted and extracted 16S profiles. All samples were subjected to library preparation for the full-length 16S gene, with subsequent long-read sequencing on the Mk1C platform (ONT, UK). Full-length 16S (FL16S) microbiome bioinformatic analysis was performed using the 16S-wf provided within epi2me (ONT, UK).

Results: In pen railing surfaces, we identified an average of 135, 166 and 257 unique bacteria genera using VTM, PBS and DRY samples, respectively. In particles deposited on aluminum foil we identified an average of 109, 175 and 214 unique genera in VTM, PBS and DRY, respectively. The most abundant genera present in swine farm pen railings and settled airborne particles across all 3 gauze types were Clostridium sensu stricto 1, Lactobacillus, Streptococcus and Terrisporobacter. With the exception of three samples, we identified fewer than 4 unique genera in the personal air samples, with Staphylococcus being the most frequently identified. One NIOSH BC251 phase 3 sample (particle size <1 μm), one phase 2 sample (particle size 1-4 μm), and the unextracted Aerocollect sample contained 22, 679 and 47 unique genera, respectively. In the negative control samples that we processed alongside the personal air samples, we identified between 1 and 47 unique genera, with Staphylococcus again being the most frequent.

Conclusions: Our results suggest that while dry gauze may be the most suitable sampling method for microbiome analysis of swine farm surfaces and settled particles, gauze pre-moistened with VTM remains a convenient option, considering its dual purpose for both viral and microbiome analysis. Although our personal air sampling results are inconclusive, they underscore the importance of using negative controls and optimizing DNA extraction protocols to ensure suitable microbiome analysis using full-length 16S methods.

Financial Support: CDC Nat'l Inst For Occup Safety & Health, Award 5 U54OH010170-12-00, "Upper Midwest Agricultural Safety and Health Center"

Notes:

202 - Quantitative risk assessment of human H5N1 infection from consumption of fluid milk

Katherine J. Koebel[1], Ece Bulut[1], Samuel D. Alcaine[2], Aljosa Trmcic[2], Diego G. Diel[1], Renata Ivanek[1]

[1]Dept. of Population Medicine & Diagnostic Sciences, Cornell University, [2]Dept. of Food Science, Cornell University. kjk239@cornell.edu

Session: One health / Public health 2, 2025-01-21, 9:15 - 9:30

Objective: In February 2024, a spillover event from birds to cattle infected a Texas dairy herd with H5N1 clade 2.3.4.4b. This infection has since spread to >170 US herds, causing 4 human infections and raising questions about the safety of domestic fluid milk. To date, all human cases resulted from direct contact with infected cows and the USDA/FDA maintain that pasteurized milk is safe. However, many unknowns remain, including those about raw milk safety. Further investigation is indicated to quantify the public health risk. The purpose of this study was to perform a quantitative risk assessment of human H5N1 infection from consumption of pasteurized and raw fluid milk. Additionally, the risk mitigation effects of two interventions were investigated.

Methods: Two stochastic quantitative risk assessment models were built to represent the US pasteurized and raw fluid milk supply chains. The pasteurized milk model follows milk from harvest on multiple dairies, through pooling and processing at fluid milk plants, retail distribution, home storage, and culminates in consumption of cup (240 mL)-sized servings: thus projecting the annual number of cases at the national level. Conversely, the raw milk model simulates a single herd selling directly to consumers to better represent the US raw milk supply chain, so this model projects risk attributable to a single raw milk purveyor. Values for model parameters were informed by literature review and expert opinion. An exponential dose-response curve was assumed. Whenever possible, data specific to this outbreak were utilized. Stochasticity was introduced in parameters like herd size, prevalence, shedding level, and storage duration. USDA dairy food surveillance data were used for validating the pasteurized milk model. Monte Carlo simulation was performed with 50,000-iteration simulations utilizing Latin hypercube sampling. Sensitivity was assessed with Spearman rank correlation coefficients (ρ). Scenario analysis investigated the effect of two interventions on raw milk risk outputs: bulk tank PCR testing and improved diversion of shedding cows from the pipeline.

Results: In the pasteurized milk model, the projected annual number of human H5N1 cases from consumption of fluid milk in the US was 0 in 99.9% of iterations and 1 in 0.1%. Bulk tank viral loads were highly sensitive to the shedding level of cows (ρ=0.89). Mean contaminated raw milk servings from a simulated operation was 119.3±4.4 (95% CI). Bulk tank PCR testing reduced this to 1.9±0.07, and improved diversion of sick cows reduced it to 112.7±4.1. As new data become available, the models will be updated.

Conclusions: The results of this study demonstrate that the public health risk of H5N1 infection from drinking pasteurized fluid milk to be extremely low. Data scarcity makes assessment of raw milk infection risk more challenging, but it is intuitively higher due to the absence of pasteurization, which has been proven highly effective against H5N1 clade 2.3.4.4b. PCR testing of bulk tanks greatly reduces infection risk, while improved diversion of shedding animals is somewhat efficacious.

Financial Support: USDA National Institute of Food and Agriculture (NIFA), "Artificial Intelligence Institute for Next Generation Food Systems" (AFRI), grant #2020-67021-32855. Partial support was received from the Cornell Institute for Digital Agriculture (CIDA).

Notes:

203 - A One-Health approach in surveilling for emerging respiratory viruses on cattle farms in Kentucky and Indiana

Daniel B. Cummings[1], John T. Groves[2], Alex G. Hagan[3], Judith U. Oguzie[4], Lyudmyla V. Marushchak[4], Thang Nguyen-Tien[4], Ismaila Shittu[4], Claudia M. Trujillo-Vargas[4], Jessica Rodriguez[4], Gregory C. Gray[4]

[1]Heritage Vet Partners, [2]Livestock Veterinary Service, [3]Ironsides Animal Health, [4]University of Texas Medical Branch. dr.hagan@heritage.vet

Session: One health / Public health 2, 2025-01-21, 9:30 - 9:45

Objective: In this prospective, 5-year, One Health study of livestock farms we are surveilling for novel coronaviruses and influenza viruses that may represent spillover events.

Methods: We are collaborating with livestock veterinarians in prospectively studying livestock, farm workers, and the farm environment for evidence of novel respiratory viruses. We are seeking to enroll large livestock farms from multiple states and Mexico. At enrollment and again 4, 8, and 12 months later, we collect questionnaire data from farm workers and farms, and an array of samples: 20 nasal/oral swabs from livestock (up to 75% from animals with signs of respiratory illness), nasopharyngeal swabs and sera from 10 healthy workers, four three-hour bioaerosol samples in areas where livestock and farm workers mix, and other farm samples as guided by veterinarians. Between the four planned farm visits, farm employees use postage-paid sample kits to collect and ship nasal/oral swabs from sick livestock or sick farm workers with signs of respiratory illness. Swab specimens are examined with molecular assays for influenza A, influenza D, and coronaviruses. Interesting samples are further examined with next-generation sequencing (NGS).

Results: From six Kentucky and Indiana beef cattle farms (convenience sample) studied during 2024, we collected 327 samples: 168 cattle nasal swabs, 2 cattle ocular swabs, 3 cow necropsy lung swabs, 3 dead-bird swabs, 37 human nasopharyngeal swabs, 93 farm bioaerosol samples, and 21 other environmental samples (water, car and car tire, and fecal slurry). No specimen was positive for Influenza A. None of the environmental or human specimens were positive. However, 11 (6.5%) of 168 cattle nasal samples (4 with signs of respiratory illness) and two water pen samples had molecular evidence of influenza D. NGS analysis of the influenza D virus strains showed they were similar. Fifty-three (31.5%) of 168 beef cattle nasal swabs and 2 beef cattle ocular swabs had molecular evidence of a coronavirus. Eighteen (34%) of the 53 coronavirus-positive cattle specimens had respiratory signs. Sanger sequences from 29 (54.7%) of the 55 had molecular evidence of bovine coronaviruses. Seven bovine coronavirus strains were studied with NGS, and the 7 genomes were very similar. We also detected and assembled seven additional viruses from beef cattle nasal swabs: bovine rhinitis A virus (100% genome coverage), bovine coronavirus (99.9%), bovine nidovirus (98.4%), enterovirus E virus (97.3%), Praha dicistro-like virus 2 (90.9%), bovine rotavirus (88.9%), bovine rhinitis B virus (82.9%), and flumine dicistrovirus 40 (72.7%). As far as we know the Praha dicistro-like virus 2 and Flumine dicistrovirus 40 have not been previously detected in cattle.

Conclusions: These data demonstrate the potential for using a One Health approach in conducting surveillance for novel respiratory viruses on livestock farms. Such virus surveillance can be of great value to farm biosecurity, animal and farm worker health, and food safety.

Financial Support: The authors would like to acknowledge funding from USDA APHIS. NIFA Award No. 2023-70432-39558

Notes:

204 - Determinants of health and disease at the global livestock-wildlife-human interface: a scoping review

Alaina Macdonald[1], Manuel Perez Maldonado[1], Lauren Grant[1], Claire Jardine[1], E. Jane Parmley[1]

[1]University of Guelph. alaina@uoguelph.ca

Session: One health / Public health 2, 2025-01-21, 9:45 - 10:00

Objective: Infectious diseases are emerging and re-emerging more frequently worldwide, driven by ecological and socioeconomic factors. Many emerging infectious diseases are caused by zoonotic pathogens, and outbreaks often develop in places where livestock and wildlife interact. While current disease surveillance efforts are rapidly undergoing expansion and sophistication, most programs remain reactive in nature, where pathogens are characterized and managed following onset of an outbreak. Thus, surveillance efforts focused on disease detection following outbreaks may be associated with delayed corrective action, prohibitive costs, and human and animal morbidity. We aim to identify factors which could be monitored and included in an early warning surveillance system at the human-wildlife-livestock interface.

Methods: This presentation will describe a scoping review which examines studies from four databases to identify determinants of emerging disease caused by infectious pathogens at the global human-wildlife-livestock interface. Inclusion criteria encompassed primary peer-reviewed articles in English, which analyzed drivers of emerging disease in places where humans, wildlife and livestock interact. Articles were excluded if they were not primary literature or were focused on in vitro pathogen analysis.

Results: Fourteen thousand ninety-nine studies were initially identified. After de-duplication, two independent reviewers screened 7781 abstracts using pre-determined eligibility criteria. Eight hundred eighty-eight studies were then evaluated at the full text level, and 152 were included for data extraction. Most studies (n=101) involved emerging diseases caused by viral pathogens, followed by bacteria (n=41), helminths (n=3) and protozoa (n=3). Most studies focused on a pathogen with zoonotic potential and/or with significant trade and economic consequences. Articles were published from 1986-2023, and most were published within the past five years. The most common pathogens analyzed were highly pathogenic avian influenza virus H5N1 (n=29), followed by Mycobacterium bovis (n=16), and Bacillus anthracis (n=11). Neglected tropical diseases were poorly represented in the literature. Most studies took place in Europe (n=38), Asia (n=36) and Africa (n=34), with fewer in North America (n=17), South America (n=7) or Oceania (n=5). Wild birds were the most frequently studied group of wild species (n=48), followed by wild boar (n=5) and ungulates (n=3). Among livestock species, poultry (n=52), cattle (n=29) and pigs (n=12) were most commonly represented. Reported drivers of disease emergence included: 1) wildlife and livestock population densities, 2) wildlife migratory behaviour and patterns of livestock movement for trade, 3) anthropogenic land use change and inadequate biosecurity practices, 4) ecological drivers including changes in temperature and precipitation, and 5) socioeconomic drivers such as political instability and poverty.

Conclusions: This study contributes to the understanding of factors which precede emerging disease at a high risk global interface. Incorporating measurement and monitoring of these factors in disease surveillance may help future decision makers respond to cues, inform management practices and reinforce biosecurity before disease is detected in humans, wildlife or livestock. Expanding current surveillance efforts to include upstream measures could translate to cost savings and greater resilience to other potential infectious harms.

Notes:

205 - Eugenol nanoemulsion in chicken drinking water reduces *Salmonella* Enteritidis colonization in broiler chickens

Jodie Allen[1], Brindhalakshmi Balasubramanian[1], Ana Leticia De Almeida[1], Omoladunnandi Battles[1], Mackenzie Connors[2], Indu Upadhyaya[3], Abhinav Upadhyay[1]

[1]Department of Animal Science, University of Connecticut, [2]Department of Pathobiology and Veterinary Science, University of Connecticut, [3]Department of Extension, University of Connecticut. jodie.allen@uconn.edu

Session: *Salmonella*, 2025-01-21, 8:30 - 8:45

Objective: Poultry serve as a reservoir host for *Salmonella* Enteritidis (SE), a major poultry-associated foodborne pathogen, and significant contributor to foodborne illnesses globally and in the United States. Consumption of contaminated poultry products is a major source of SE infection in humans. The pathogen colonizes the ceca of broiler chickens in high numbers leading to product contamination during slaughter. Currently, pre-harvest strategies are implemented on farm with minimal antimicrobial success.Eugenol (EG), a Generally Recognized as Safe status compound obtained from clove has been extensively researched for its anti-*Salmonella* efficacy, however, the low water solubility of EG thwarts its application as a potential drinking water supplement for broiler chickens. In this study, the efficacy of Eugenol oil in its nanoemulsion form as an in-water supplement in reducing SE colonization in broiler chickens was investigated.

Methods: Eugenol nanoemulsion (EGNE) was formulated by EG oil, Deionized water (DI water), Gum Arabic and Lecithin (GAL) as emulsifying agents, followed by subjecting the mixture to sonication. A total of 160 Day-old Cornish cross broiler chickens were procured and randomly allotted to 4 groups (10 birds/treatment/timepoint; n=2). Groups included control (fresh tap water), emulsifier control (GAL), EG or EGNE 0.03%, respectively. Broiler chickens were acclimated for 6 days, and on day 7, GAL, EG, or EGNE 0.03% was supplemented in chicken drinking water till day 28. On day 14, birds were inoculated by oral gavage with a 4-strain cocktail of SE (*S.* Enteritidis strain 12, 21, 28, 31; ~9 log CFU/bird). Necropsies were performed on day 21 and 28. Cecal content was collected and enumerated for SE. Weekly body weight gain, feed and water intake were measured. Two independent trials were conducted. Data was analyzed using one-way ANOVA ($p<0.05$).

Results: EGNE had a particle size of ~89 nm, PDI of <0.3 and zeta-potential of ~ -34.45 mV. For SE inoculated birds supplemented fresh tap water (control), ~3.70 and 4.09 log CFU/g SE were recovered in cecal content on day 21 respectively, for Trial 1 and Trial 2. On day 28, ~2.00 and 2.58 log CFU/g SE were recovered in cecal content respectively, for Trial 1 and Trial 2. Emulsifiers (GAL) and EG oil dose 0.03% did not reduce SE colonization in ceca on day 21 and 28 as compared to control ($p>0.05$). In water supplementation of EGNE 0.03% reduced SE colonization on day 21 by ~1.29 and 1.63 log CFU/g respectively for Trial 1 and 2 ($p<0.05$). On day 28, EGNE 0.03% supplementation reduced SE colonization by ~1.38 and 1.65 log CFU/g, respectively, as compared to control ($p<0.05$). No significant difference in body weight gain, feed, and water consumption or FCR were observed in any treatments as compared to control.

Conclusions: Supplementing EGNE in drinking water could potentially be used to control SE colonization in broiler chickens.

Financial Support: This research was supported in part by the USDA-NIFA-AFRI-A1332 program grant #2021-67018-33440: Developing plant-based drinking water supplements for controlling *Salmonella* and Campylobacter jejuni in broiler chickens.

Notes:

206 - Mind the gap: Persistent problems in the world of enteric zoonoses and how data could inform public health action

G. Sean Stapleton[1], Grace Vahey[1], Kate Varela[1], Katharine Benedict[1]

[1]Centers for Disease Control and Prevention. qbs1@cdc.gov

Session: *Salmonella*, 2025-01-21, 8:45 - 9:00

Objective: Enteric diseases linked to contact with animals or their environment are estimated to cause 450,000 illnesses, 5,000 hospitalizations, and 76 deaths in the United States annually. The objective of this presentation is to highlight research that might address gaps in understanding enteric zoonoses that have persistent impact on public health.

Methods: We summarized examples of notable multistate animal contact-associated outbreaks of enteric bacterial infections in people, highlighting gaps in understanding how enteric pathogens persist in animal and environmental reservoirs. These observations were collected through collaborations with animal industry firms during outbreak investigations and reflect practices that may increase the risk of transmission between animals and people.

Results: Annually, CDC and public health officials have investigated multistate outbreaks of *Salmonella* infections linked to backyard poultry. From 2015 through 2024, 9,408 outbreak-associated illnesses, approximately a quarter of which occurred in children <5 years old, were linked to backyard poultry contact. Various strategies can be used to reduce *Salmonella* contamination at backyard poultry hatcheries and points of purchase including stringent biosecurity practices or vaccination of backyard poultry against *Salmonella* strains that have human health consequence. However, it is not known which of these strategies are most effective at preventing the spread of *Salmonella*, nor is it known how commonly each of these practices are implemented across the backyard poultry industry. Despite a federal law and state regulations that ban the sale and distribution of small turtles (shell length <4 inches) as pets, these turtles are sometimes sold illegally online and at stores, flea markets, and roadside stands. People of Hispanic or Latino ethnicity have been disproportionately affected in outbreaks associated with small turtles and represent over a third of cases in outbreaks investigated in 2024. Research to understand the knowledge, attitudes, and practices among populations who purchase small turtles and groups who sell them as pets could help reduce the persistence of this problem.In 2024, half of the patients included in a bearded dragon-associated salmonellosis outbreak were children ≤1 year old. To prevent illnesses linked to indirect animal exposure, risk assessments of *Salmonella* contamination within households that own bearded dragons could indicate common sources of environmental contamination. Furthermore, longitudinal data are needed to characterize the duration and intermittence of *Salmonella* shedding from reptiles.Extensively drug-resistant (XDR) *Campylobacter jejuni* infections in humans have been linked to contact with pet store puppies and continue to be a public health threat. Despite interventions implemented by the puppy breeding industry and public health officials, these infections continue to be identified. Surveillance for XDR *Campylobacter* among puppy breeding and retail establishments could elucidate reservoirs of this pathogen that enable its persistence.

Conclusions: Multistate outbreaks of *Salmonella* infections in people result from contact with various types of animals. These outbreaks are an ongoing problem in the United States and can disproportionately affect young children or people of specific races or ethnicities. Filling gaps in our understanding of how enteric pathogens persist among different animal industries could identify interventions that could benefit public health.

Notes:

207 - Emergence of a *Salmonella* Enteritidis strain with decreased susceptibility to ciprofloxacin in U.S. poultry

Randall Singer[1], Timothy Johnson[1]

[1]University of Minnesota, Department of Veterinary and Biomedical Sciences. rsinger@umn.edu

Session: *Salmonella*, 2025-01-21, 9:00 - 9:15

Objective: The objective of this study was to examine the genomic attributes of an emerging strain of *Salmonella* Enteritidis with decreased susceptibility to the fluoroquinolone antimicrobial ciprofloxacin.

Methods: Whole genome sequences of *Salmonella* Enteritidis isolates collected by the National Antimicrobial Resistance Monitoring System (NARMS) of USDA-FSIS, FDA and CDC were downloaded and analyzed. A total of 812 isolates from FDA retail meat sampling were included. Relationships among isolates were assessed with core genome multilocus sequence typing (cgMLST) and pangenome analyses.

Results: For isolates that were tested for antimicrobial susceptibility by the federal agencies, the emergent strain of *Salmonella* Enteritidis had ciprofloxacin MICs of 0.125 or 0.25 ug/ml, thereby being classified as having decreased susceptibility to ciprofloxacin but not clinical resistance. These isolates formed their own cluster, which was clearly differentiated from historical isolates. The emergent strain first appeared in the FDA retail meat sampling in 2018 and has increased in national distribution since that time. The strain possesses the gyrA D87Y substitution. When the frequency of antimicrobial resistance and virulence genes were compared between the emergent strain and other historical and contemporary strains of *Salmonella* Enteritidis isolated from poultry, no major differences were observed.

Conclusions: Fluoroquinolones are an important antimicrobial used to treat invasive salmonellosis in human medicine. The emergence of a strain of *Salmonella* Enteritidis with decreased susceptibility to ciprofloxacin, which is not clinically resistant, raises concerns about the possibility of this strain mutating to a resistant phenotype. Given that fluoroquinolones have been illegal to use in U.S. poultry production since 2006, other potential selection pressures that aid the dissemination of this strain will need to be investigated. This project highlights an approach for identifying emerging *Salmonella* strains of public health concern.

Notes:

208 - Identifying environmental reservoirs of *Salmonella* enterica on dairy farms in the northeastern United States

M.J. Craig[1], K.J. Cummings[1], L.B. Goodman[1], E. Frye[2], R.J. Franklin-Guild[3], J.D. Siler[1]

[1]Deparment of Public and Ecosystem Health, Cornell University, [2]Department of Population Medicine and Diagnostic Sciences, Cornell University, [3]Department of Population Medicine and Diagnostic Sciences, Animal Health Diagnostic Center. mjc437@cornell.edu

Session: *Salmonella*, 2025-01-21, 9:15 - 9:30

Objective: The presence of *Salmonella* enterica on dairy farms presents a threat to public health, animal health, and farm productivity. Particularly, *Salmonella* enterica serotype Dublin is associated with high risks of morbidity and mortality in calves. The objectives of this study were to 1) determine environmental reservoirs of *Salmonella* enterica on dairy farms in the northeastern United States and 2) identify environmental reservoirs of *Salmonella* Dublin specifically.

Methods: Environmental samples were collected from a variety of farm locations including calf housing, calf equipment room floors, farm equipment, and maternity pens. Samples were collected before a *Salmonella* Dublin outbreak (baseline time point) and 3-4 weeks after a confirmed clinical case of *Salmonella* Dublin (post-outbreak time point). At the Cornell University Animal Health Diagnostic Center, PCR was used to detect *Salmonella*. PCR-positive samples were then cultured and sent to NVSL for serotyping. Mixed-effects logistic regression modeling was used to evaluate significant predictors of *Salmonella* PCR positivity. *Salmonella* Dublin isolates were whole genome sequenced to characterize antimicrobial resistance, virulence, and stress response genotypes.

Results: In total, 575 environmental samples were collected from 23 dairy farms in the northeastern United States. There were 286 samples that were PCR-positive for *Salmonella*, 49 of which were culture-positive. Seven of 49 isolates (14%) were identified as *Salmonella* Dublin. The maternity pen was found to be associated with an increased odds of *Salmonella* PCR positivity (OR = 6.55; 95% CI = 3.91, 10.98), as well as calf equipment room floors (OR = 4.64; 95% CI = 1.95, 11.01). The post-outbreak time point of sampling was found to be associated with a decreased odds of *Salmonella* PCR positivity (OR = 0.37; 95% CI = 0.19, 0.74). *Salmonella* Dublin was isolated from maternity pens and calf housing areas from different farms. Sequencing of *Salmonella* Dublin isolates revealed a multidrug resistant genotype and a conserved profile of stress response and virulence genes.

Conclusions: These results suggest that hygiene and biosecurity are important for minimizing the spread of *Salmonella* on dairy farms. Farm areas such as maternity pens and calf equipment room floors should be targeted in efforts to mitigate the presence of *Salmonella*. For reducing environmental reservoirs of *Salmonella* Dublin, calf housing areas should be targeted in addition to the maternity pens and equipment room floors. This project is ongoing.

Financial Support: This project is funded by a USDA NIFA AFRI grant titled “Novel diagnostic approaches to comprehensively define *Salmonella* Dublin transmission dynamics and improve disease control in dairy cattle"

Notes:

209 - Prevalence and antimicrobial resistance in *Salmonella* Infantis isolates detected across the food chain in the United States 2013-2022.

Mohammad Nasim Sohail[1], Csaba Varga[1, 2]

[1]Department of Pathobiology, University of Illinois at Urbana-Champaign, [2]Carl R. Woese Institute for Genomic Biology, University of Illinois at Urbana-Champaign. mnsohail@illinois.edu

Session: *Salmonella*, 2025-01-21, 9:30 - 9:45

Objective: *Salmonella* Infantis (*S.* Infantis) is an emerging zoonotic foodborne pathogen. An increase in multidrug resistance among S. Infantis isolates has been observed in the US and globally. This study evaluates the prevalence and antimicrobial resistance (AMR) in S. Infantis across the United States of America between 2013 and 2022.

Methods: Publicly available AMR data of S. Infantis isolated from humans, retail meats, and food animals collected by the National Antimicrobial Resistance Monitoring System for Enteric Bacteria (NARMS) was analyzed. The broth microdilution method was used to assess the minimum inhibitory concentration of S. Infantis isolated from chickens (n=4225), turkeys (n=210), swine (n=673), cattle (n=186) and humans (n=920). Isolates were categorized as susceptible or resistant to 13 different antimicrobials based on breakpoints defined by the Clinical and Laboratory Standards Institute. Descriptive statistics were used to calculate proportions and 95% CI of AMR. The Mann-Kendall test assessed trends in AMR across the study period. Poisson regression models were built to compare the incidence rate ratios of S. Infantis in 2013 (reference) to all the other years.

Results: The prevalence of S. Infantis among all nontyphoidal *Salmonella* serotypes was highest in chickens (19.21 %), followed by swine (9.08%), cattle (4.09%), turkey (3.7%), and humans (1.66%). A significant increase in the prevalence of S. Infantis isolated from chickens and turkeys, a moderate increase in cattle and humans, and no increase in swine was observed. Multi-drug resistance (MDR; resistant to at least one agent in ≥3 antimicrobial classes) was detected in 91.43% of isolates from turkey, 84.47 % of chicken, 22.61% of humans, 18.82% of cattle, and 8.90% of swine, respectively. High resistance to tetracycline, nalidixic acid, and sulfisoxazole and low resistance to cefoxitin and amoxicillin-clavulanic acid were detected among livestock, poultry, and human isolates. All livestock and poultry isolates were susceptible to azithromycin and ciprofloxacin and Human isolates showed less than 0.5 % resistance. The most common coresistance patterns were observed between tetracycline, nalidixic acid, and sulfisoxazole.

Conclusions: An increase in the prevalence of chicken and turkey-origin S. Infantis was detected, while the prevalence of isolates originating from swine, cattle, and human populations remained low. Among chickens and turkeys, the proportion of AMR and MDR was the highest. On the other hand, MDR isolates among humans were low, suggesting that other exposure sources might play a role in human S. Infantis infections. This study provided data on the prevalence and AMR of S. Infantis across the food chain that can help health authorities develop stewardship programs.

Financial Support: The authors are grateful to the National Antimicrobial Resistance Monitoring System for Enteric Bacteria (NARMS) for its pivotal role in data collection and availability.

Notes:

210 - Risk factor analysis of *Salmonella* Dublin in dairy-beef farms: A PCR-based environmental sampling study

Alejandra Arevalo-Mayorga[1], Jarred Kopkey[1], Amgad Riad[1], Jessica Pempek[1], Samantha Locke[1], Greg Habing[1]

[1]Department of Veterinary Preventive Medicine, The Ohio State University. arevalo.27@buckeyemail.osu.edu

Session: *Salmonella*, 2025-01-21, 9:45 - 10:00

Objective: This study aimed to identify risk factors associated with the presence of *Salmonella* Dublin in dairy-beef farms using PCR detection on environmental samples. We hypothesized that specific farm management practices and environmental conditions would be significantly associated with the presence of *S.* Dublin.

Methods: We conducted a cross-sectional study of 54 dairy-beef farms across seven states (KS, OH, WI, MO, NY, MN, and TX) from June 2023 to September 2024. Environmental samples (n = 261) were collected from high-risk areas on each farm, including pre-weaned barns alleyways, post-weaned pens, and milk mixing rooms. Samples were analyzed using a multiplex endpoint PCR targeting *S.* Dublin-specific gene markers. A comprehensive questionnaire was administered to gather data on potential risk factors, including herd management, animal movements, housing systems, calf management practices, and biosecurity measures. Descriptive statistics were calculated for farm characteristics and *S.* Dublin prevalence. Univariable and multivariable logistic regression models were used to identify risk factors associated with *S.* Dublin detection. Variables with $p < 0.20$ in univariable analysis were included in the initial multivariable model, which was refined using backward elimination.

Results: *S.* Dublin was detected by PCR in 37% (20/54) of farms, with 16% (42/261) of environmental samples testing positive. Post-weaned pens had the highest prevalence at 8% (21/261), compared to pre-weaned alleyways (5%) and milk mixing rooms (3%). The final multivariable model identified the number of employees (OR: 1.84, 95% CI: 1.37-2.47, $p < 0.001$), and lack of site-specific PPE use (OR: 4.48, 95% CI: 2.01-9.97, $p < 0.001$) as risk factors significantly associated with *S.* Dublin detection. Non-significant variables included age at exit ($p = 0.999$) and breed ($p = 1.000$), suggesting these factors may not independently influence *S.* Dublin's presence.

Conclusions: This study reveals a considerable prevalence of *S.* Dublin with post-weaned pens at higher risk. The identified risk factors highlight the importance of employee management and biosecurity measures in *S.* Dublin control. Our findings emphasize the need for targeted interventions focusing on employee training, biosecurity protocols, and proper PPE use to mitigate *S.* Dublin prevalence in dairy-beef operations. Future research should explore the effectiveness of specific interventions based on these identified risk factors and evaluate their impact on reducing *S.* Dublin prevalence in dairy-beef operations. Additionally, longitudinal studies are warranted to assess long-term trends in *S.* Dublin 's presence and associated risk factors.

Notes:

211 - Investigation of *Burkholderia pseudomallei* seroprevalence in pigs slaughtered at selected pig abattoirs in Uganda

John E. Ekakoro[1], Arnold Lubega[2], Edrine B. Kayaga[2], Dickson Ndoboli[2], Andrew P. Bluhm[3], Eddie M. Wampande[2], Jason K. Blackburn[3], Karyn A. Havas[4], Michael H. Norris[5]

[1]Department of Diagnostic Medicine and Pathobiology, Rowan University, [2]School of Veterinary Medicine and Animal Resources, Makerere University, Uganda, [3]Department of Geography, University of Florida, [4]Department of Public and Ecosystem Health, Cornell University, [5]School of Life Sciences, University of Hawai'i at Manoa. ekakoro@rowan.edu

Session: Epidemiology 4, 2025-01-21, 8:30- 8:45 AM

Objective: *Burkholderia pseudomallei* is a Gram-negative bacterium that causes melioidosis, a disease of humans and animals. It is primarily transmitted through direct contact with contaminated soil and surface water. The epidemiology of this pathogen in Africa, including Uganda, is largely unknown. The objectives of this study were to estimate the seroprevalence of *B. pseudomallei* in pigs slaughtered in central Uganda and to identify potential hotspots for this pathogen in the country.

Methods: Pig serum samples were collected from pigs slaughtered at six abattoirs located in the Kampala metropolitan area of central Uganda. A total of 1035 pig sera were analyzed for serological responses to *B. pseudomallei* with type A and type B LPS using OPS type A and OPS type B ELISAs. The sera were analyzed using an in-house enzyme-linked immunosorbent assay (ELISA) developed at the Emerging Pathogens Institute at the University of Florida.

Results: Of the 1035 samples, 75 (7.25%, 95% CI: 5.8-9%) were seropositive to the OPS-A ELISA using a two standard deviations (SD) cutoff and 19 (1.84%, 95% CI: 1.2-2.9%) at 3 SD. For the OPS-B ELISA, 93/1035 (8.99%, 95% CI: 7.4-10.9%) were seropositive at the 2 SD cutoff, and 28/1035 (2.71%, 95% CI: 1.9-3.9%) at the 3 SD cutoff. Seropositivity was higher in the months of October, November, and December for both the OPS-A ELISA and OPS-B ELISA at the 2 SD and 3 SD cutoffs and particularly lower in the dry months of January, February, June, July, and August. Local Moran's I of Empirical Bayes Smoothing seroprevalence detected two high-high spatial clusters in Masaka and Kalungu, both on the northwestern shore of Lake Victoria and a high-low spatial outlier in Nakaseke in the central region.

Conclusions: Pigs slaughtered in central Uganda are exposed to *B. pseudomallei*, and there is a higher seroprevalence in the rainy months. Public health awareness campaigns about melioidosis may be needed.

Financial Support: Funding was provided by Cornell University's Atkinson Center for Sustainability. This project benefited from excess serum samples as well as equipment and training provided by a research project sponsored by the U.S. Defense Threat Reduction Agency, under grant number HDTRA1-20-1-0007.

Notes:

212 - Molecular epidemiology and population structure of *Brachyspira hyodysenteriae* and its impact on swine dysentery

Maria Hakimi[1], Anugrah Saxena[1], Huigang Shen[1], Orhan Sahin[1], Eric R Burrough[1], Ganwu Li[1]

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

Session: Epidemiology 4, 2025-01-21, 8:45- 9

Objective: Swine dysentery (SD), caused by *Brachyspira hyodysenteriae*, is a severe mucohemorrhagic diarrheal disease affecting grower-finisher pigs. The disease poses a significant economic burden to swine-rearing countries, mainly due to a decline in research during a period when the disease became less common. With its recent reemergence and rising antimicrobial resistance (AMR), important gaps remain in understanding this spirochete's genomic diversity and implication for virulence. The objective of this study is to conduct a comprehensive phylogenomic analysis of *B. hyodysenteriae* isolates from diverse geographical regions, focusing on population structure, molecular epidemiology, and the prevalence of AMR and virulence-associated genes.

Methods: Whole genome sequencing was performed by Illumina MiSeq platform on 117 *B. hyodysenteriae* U.S isolates obtained from Iowa State University Veterinary Diagnostic Laboratory. Additionally, 134 publicly available genome assemblies from GenBank were included, resulting in a total of 251 genomes. Phylogenomic analysis was conducted using core genome SNPs, and multilocus sequence typing (MLST) was used to determine sequence types (STs). Pan-genome analysis was performed using Panaroo, and functional characterization of gene families was conducted with eggnog-mapper. Additionally, AMR and virulence genes were screened using ABRicate against multiple databases, and a custom database for virulence genes.

Results: The phylogenomic analysis of *B. hyodysenteriae* isolates revealed nine distinct lineages across geographical regions, with lineages L9 and L7 predominating in the U.S. and L2 primarily found in Belgium. A total of 69 different STs were identified, including 20 novel STs. The pan-genome analysis indicated an open pangenome that expands as more genomes are analyzed. Across 251 genomes, the pan-genome contained 5,231 genes, including 1,648 core, 2,619 accessory, and 964 unique genes. COG category analysis linked core genes to energy production and conversion as well as translation. In contrast, accessory and unique genes were more abundant in replication, recombination, and repair.Through additional pan-genome and core genome analyses, we identified genetic traits linked to specific lineages, including known antimicrobial resistance (AMR) genes and key virulence factors. Notably, we uncovered four plasmid genes, previously associated with more virulent B. hyodysenteriae isolates, within specific lineages and geographical regions. The analysis of AMR and virulence genes also revealed notable associations with specific STs, with key virulence genes, particularly those related to hemolysin and iron uptake, present in the majority of isolates.

Conclusions: This study revealed substantial genetic diversity in *B. hyodysenteriae*, with distinct lineages and sequence types across geographical regions. Notably, it is the first report of an open pan-genome in this pathogen, providing valuable insights into its adaptability and evolutionary potential. The identification of virulence and AMR genes underscores the potential for increased pathogenicity and resistance. Together, these findings emphasize the need for further research to enhance disease control strategies for swine dysentery.

Notes:

213 - Characterizing hot spots for avian-equine influenza spillover

Andrew Park[1], Carlos Molinero[1], TJ Odom[1], Ayanna Johnson[2], Annakate Schatz[1], Mafalda Viana[3], Daniel Perez[1], Daniela Rajao[1], Batchuluun Damdinjav[4], Pablo Murcia[3]

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

Session: Epidemiology 4, 2025-01-21, 9 - 9:15 AM

Objective: (1) To integrate data on proximity to water sources, bird species diversity, climate data, and influenza subtype richness to evaluate drivers of influenza seropositivity in horses in Mongolia; (2) To build mathematical models that evaluate how exposure is likely to translate to seropositivity across time and space.

Methods: (1) Seroprevalence data from field surveys was combined with database-retrieved information to test for relationships between putative predictors of risk of influenza transmission from birds to horses using a combination of statistical and data visualization approaches. (2) SIR-type models were developed and parameterized to demonstrate the complex relationship between exposure risk and seropositivity rates in populations.

Results: (1) Predictors relate to each other whereby landscape features (water availability, climate) determine local bird communities. In turn these communities are known to carry specific influenza subtypes. The set of related predictors explains some of the variation in influenza positivity. (2) The models demonstrate that exposure rate, exposure seasonality, transmission rate, waning immunity rate, and sampling intensity interact to create both strong and weak correlations between exposure risk and seropositivity rate.

Conclusions: (1) A holistic approach to identifying hotspots for cross-species transmission is useful because predictors are inter-related and collectively shed light on identifying hotspots for cross-species transmission. (2) Models caution against a simplistic assumption that high exposure risk always translates to high seropositivity rates because interactions between dynamical processes may act to mask, and even reverse, the correlation. These findings are not unique to the avian-equine system in Mongolia and therefore have implications for other influenza spillover events.

Financial Support: This work was funded by EEID USDA National Institute of Food and Agriculture (Grant 2021-67015-33406) and the Biotechnology and Biological Sciences Research Council (Grant BB/V004697/1)

Notes:

214 - Epidemiological evaluation of equine metabolic syndrome in Arabian horses and their subgroups

Elaine Norton[1], Jennifer Hesser[2], Audrey Johnston[1], Brianna Young[1]

[1]Animal and Comparative Biomedical Sciences, University of Arizona, [2]College of Veterinary Medicine, University of Arizona. elainenorton@arizona.edu

Session: Epidemiology 4, 2025-01-21, 9:15- 9:30 AM

Objective: Estimate prevalence and individual and environmental risk factors influencing equine metabolic syndrome (EMS) in Arabian horses and their subgroups.

Methods: 591 Arabians (70 stallions, 398 mares, 123 geldings) from 43 farms were enrolled in the study. Phenotypic data included basal insulin, non-esterified fatty acids (NEFA), triglycerides (TG), insulin post oral sugar test at 60 and 90 minutes (INS-OST), body condition score (BCS), and cresty neck score (CNS). Horses were categorized based on management and owner-reported subgroups: English (n=91), Western (n=56), leisure (n=78), lesson (n=37), halter (n=81), broodmare (n=195), or breeding stallion (n=25). Binary outcome variables were defined as obesity (BCS≥8), hyperinsulinemia (insulin>20µIU/mL), post-OST hyperinsulinemia (max-INS-OST>45µIU/mL), and hypertriglyceridemia (TG>43mg/dL), with prevalence calculated across and within subgroups. Mixed model analyses were performed with EMS quantitative measurements as outcome variables, farm as a random effect and sex, age, CNS, BCS, sampling month, and subgroup as predictors. Marginal R2 and partial correlation coefficients estimated phenotypic variation explained by each explanatory variable. Pairwise comparisons of the estimated marginal means (emmeans) identified significant differences between categorical predictors (subgroup, sex, and sampling month) at Tukey-corrected p-values <0.05.

Results: Across our cohort, 25% were obese (BCS≥8), 58% had a CNS of ³3, 15% had fasting hyperinsulinemia, 25% had post-OST hyperinsulinemia, and 19% had hypertriglyceridemia. Obesity and post-OST hyperinsulinemia were most common in broodmares (32.7%, 23.1%) and horses in English disciplines (26.3%, 18.7%). Breeding stallions had the highest prevalence of a CNS 33 (67.1%) but hypertriglyceridemia, hyperinsulinemia, and post-OST hyperinsulinemia were absent in this group. Subgroup (R2=5-10%), month sampled (R2=0.01-5%) and CNS (R2=0.5-3%) were significantly associated with all quantitative outcome variables. Pairwise comparisons of the emmeans showed that stallions had an average of 6.2 µIU/mL lower max-INS-OST compared to mares (p=0.008).

Conclusions: These results confirm that Arabians are a high-risk breed for EMS with differences in prevalence and risk across subgroups and sex.

Financial Support: This research was supported by Animal and Comparative Biomedical Services at the University of Arizona and USDA-NIFA (award #2023-67016-40110).

Notes:

215 - Equine parvovirus-hepatitis infection in horse herds over four years

Joy E. Tomlinson[1], Gerlinde R. Van de Walle[2]

[1]University of Pennsylvania - New Bolton Center, [2]Cornell University College of Veterinary Medicine. jet371@gmail.com

Session: Epidemiology 4, 2025-01-21, 9:30- 9:45 AM

Objective: Equine parvovirus-hepatitis (EqPV-H) is a hepatotropic virus that causes acute hepatitis in horses. Severe disease, known as Theiler's disease, carries high case fatality rate and can occur in outbreaks over a period of months. Viral shedding and horizontal transmission from acutely infected horses is documented, however transmission from chronically infected horses is unknown. Our objective was to monitor infection dynamics over time in horse herds to determine transmission rate from chronically infected horses and to identify sources of EqPV-H introduction into herds.

Methods: Seven university and privately owned horse herds were enrolled. Serum was collected quarterly from all horses. Mare and foal pairs had serum and any administered plasma collected at 1 day, 1 and 2 months of age. EqPV-H infection status was determined by paired serology using luciferase immunoprecipitation system (LIPS) for anti-VP1 antibodies and qPCR for VP1.

Results: In total, 343 horses were tested at least once and 144 horses were tested over a year. The proportion of seropositive horses was 28 - 57% per year. One adult horse became PCR positive and seroconverted. No novel exposure (e.g. new viremic animal, equine biologic product treatment) was identified. Ten foals became PCR positive and seroconverted by 2-6 months of age, 9 of which had received seropositive plasma.

Conclusions: EqPV-H seronegative adult horses living with chronically infected adult horses rarely develop EqPV-H infection. Transmission of EqPV-H to foals is common. USDA licensed plasma products which have tested PCR negative but are seropositive might be able to transmit EqPV-H.

Financial Support: Funding provided by NIH, National Institute of Allergy and Infectious Diseases (NIAID) K08AI141767 to J.E. Tomlinson; the USDA NIFA Agriculture & Food Research Initiative Competitive Grant numbers 2020-67015-31297 to G.R. Van de Walle and 2022-67015-36343 to J.E. Tomlinson.

Notes:

216 - A multi-host SIR model for community transmission of SARS-CoV-2 among animal species

Mayank Gangwar[1], Andrew M. Kramer[1]

[1]University of South Florida. mgangwar@usf.edu

Session: Epidemiology 4, 2025-01-21, 9:45- 10

Objective: The COVID-19 pandemic has highlighted the critical need for comprehensive epidemiological models to understand multi-host disease transmission in diverse animal communities. This work aims to develop a flexible, extensible model of SARS-CoV-2 epidemiology across various animal species, emphasizing the understanding of cross-species transmission and potential spillback to humans. The study addresses the challenge of accounting for species-specific susceptibilities to different variants of SARS-CoV-2, along with the role of interspecies interactions-especially those concerning wildlife or domestic animals, in sustaining virus transmission.

Methods: We extended the classical Susceptible-Infectious-Recovered (SIR) model to include n species of potential hosts and directed transmission between them to account for possible trophic interactions. Accordingly, the model structure was kept flexible to simulate transmission dynamics for different variants of SARS-CoV-2 in various animal communities. We developed a software tool to set up and analyze the community transmission scenarios, exploring heterogeneity in community prevalence and outbreak persistence for the different model configurations. The model will be further parametrized using real data when active surveillance data on SARS-CoV-2 infection in wildlife species are available.

Results: Preliminary qualitative analyses show that community prevalence and persistence of SARS-CoV-2 outbreaks at the community level vary greatly with species-specific transmission rates and the structure of trophic interactions. The model amply demonstrates the possible outcomes for SARS-CoV-2 transmission in animal populations and the conditions under which cross-species spillover and continued outbreaks may occur.

Conclusions: The developed multi-host SIR model presents a robust analytical framework for understanding and evaluating the dynamics of the SARS-CoV-2 transmission in diverse animal communities. The model's adaptability makes it suitable for application to other multi-host pathogens, such as avian influenza, contributing to broader epidemiological research and preparedness efforts. This approach offers insights into predicting and managing outbreaks arising from zoonotic spillover events, enhancing surveillance strategies, and guiding public health interventions through a One Health approach that integrates public health, veterinary, and ecological research.

Financial Support: This work was funded by USDA-NIFA AFRI grant 2023-70432-40381 as part of the joint USDA-NSF-NIH-UKRI-BSF-NSFC Ecology and Evolution of Infectious Diseases program.

Notes:

217 - Coinfection of SPF turkeys with arthritic and enteric reovirus alters viral shedding and gut bacterial diversity

Saroj Khatiwada[1], Patricia A. Boley[1], Thamonpan Laocharoensuk[1], Kush K. Yadav[1], Carolyn M. Lee[1], Jelmer W. Poelstra[2], Gireesh Rajashekara[1], Scott P. Kenney[1]

[1]College of Food, Agriculture and Envoronmental Sciences, The Ohio State University, [2]Molecular and Cellular Imaging Center, The Ohio State University. khatiwada.20@osu.edu

Session: Virology 2, 2025-01-21, 8:30- 8:45 AM

Objective: Turkey arthritis reovirus (TARV) causes significant losses in the turkey industry due to arthritic lameness in 12 to 17-week-old turkeys. Arthritic lameness leads to increased culling and condemnation, decreased market body weight, and reduced carcass quality, necessitating more effective control measures. Turkey enteric reovirus (TERV) mainly causes transient enteritis and doesn't cause arthritis in turkeys. TARV pathogenesis is complicated due to a high prevalence of apparently nonpathogenic reoviruses in the intestines in commercial turkeys. Moreover, it is not known how subsequent infection of enteric reovirus and arthritic reovirus affect the progression and severity of reoviral arthritis. This study investigated the mechanism of coinfection (superinfection) of TARV with a non-arthritis causing TERV in reovirus free and immunologically naive specific pathogen free (SPF) turkeys.

Methods: SPF turkey poults were orally challenged with TERV MN1 strain at 1 day of age and TARV O'Neil strain at 8 days of age and were euthanized at 1- and 4- weeks post-infection (WPI) of TARV O'Neil. Intestinal contents, hock joints, tendons, and cloacal swabs were collected, and body weights were measured. DNA samples extracted from ileum and cecum contents were subjected to 16S rRNA V4-V5 sequencing. Statistical analysis among groups was performed by one-way ANOVA followed by post-hoc Tukey's test and Student's t test while differences in alpha and beta diversity were analyzed in R using Wilcoxon rank sum test and PERMANOVA respectively.

Results: A transient TARV-induced weight gain suppression was detected in the O'Neil only infected group but not in the coinfected group, and cloacal viral shedding also differed by infection status. There was a significant increase in diversity of bacterial communities in the coinfected group compared to mock at 4 WPI, while no such effect was observed at 1 WPI. Higher relative abundances of family Ruminococcaceae and genus Faecalibacterium were found in cecum and ileum at 4 WPI in the coinfected group compared to mock and O'Neil infected poults.

Conclusions: We observed a lack of TARV-induced weight gain suppression in coinfected poults along with a higher abundance of Faecalibacterium in cecum and ileum at 4 WPI compared to mock and O'Neil only infected poults. Future studies should focus on studying the pathogenesis of coinfection of reoviruses in commercial turkeys to translate the results observed in SPF turkeys. Additionally, the contribution of certain intrinsic microbial factors towards the onset, development, and severity of reovirus associated arthritis need to be addressed.

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:

218 - Activation of of bovine herpesvirus 1 (BoHV-1) infected cell protein 0 (ICP0) early promoter by stress

Clinton Jones[1]

[1]Oklahoma State University. clint.jones10@okstate.edu

Session: Virology 2, 2025-01-21, 8:45- 9

Objective: Acute bovine herpesvirus 1 (BoHV-1) infection induces apoptosis, inflammation and high levels of virus production. Viral spread to the peripheral nervous system leads to a life-long latent infection. Unlike other neurotropic herpesviruses, immediate early (IE) expression of BoHV-1 infected cell protein 0 (ICP0) and infected cell protein 4 (ICP4) are regulated by the immediate early transcription unit 1 (IEtu1) promoter. Alternative splicing of the IEtu1 mRNA leads to two distinct mRNAs that are translated into ICP0 and ICP4. A distinct early promoter also drives ICP0 expression to maintain high levels of this protein throughout productive infection. During stress-induced reactivation from latency, ICP0 protein expression is detected prior to the ICP4 protein. This finding suggests the ICP0 early promoter is active prior to the IEtu1 promoter during early stages of reactivation. Consequently, the objectives of this study were to better understand how stress-induced transcription factors regulate the ICP0 promoter. Hence, we tested whether the glucocorticoid receptor (GR) and specificity protein 1 (Sp1) or Sp3 cooperatively activate the BoHV-1 ICP0 early promoter and productive infection. Additional studies tested whether GR and Krüppel like factor 4 (KLF4) mediated transactivation is influenced by Sp1 or Sp3. The rational for testing these transcription factors is KLF4, Sp1 and Sp3 protein expression are induced by the synthetic corticosteroid dexamethasone in sensory neurons in trigeminal ganglia, an important site for BoHV-1 latency.

Methods: Transient transfection of ICP0 E promoter constructs was performed in neuronal cells. Additional studies tested whether transcriptional activity of the ICP0 E promoter is mediated by GR, KLF4, Sp1, and/or Sp3. The ability of GR and Sp1 or Sp3 to test whether these transcription factors stimulate BoHV-1 replication in permissive cells. The effect of an antibiotic, Mithramycin A, which preferentially binds GC rich DNA was also used to test whether it influenced ICP0 promoter activity.

Results: These results demonstrated GR and Sp1 or Sp3 cooperatively transactivated the ICP0 E promoter. Additional studies tested whether Sp1 or Sp3 enhanced the ability of 2 pioneer transcription factors (GR and KLF4) to transactivate ICP0 E promoter activity. Over-expression of KLF4 and Sp1 or Sp3 only had a modest effect on bICP0 E promoter activity. Mutation of consensus Sp1 binding sites proximal to the start site of ICP0 transcription reduced promoter activity more than mutating Sp1 binding sites further upstream from the start site of transcription. Interestingly, GR and Sp1 or Sp3 stimulate BoHV-1 productive infection. Mithramycin significantly reduced ICP0 E promoter activity in transient transfection studies.

Conclusions: These studies provide new evidence that helps to explain how stress and cellular stress-induced transcription factors regulate BoHV-1 gene expression and viral replication. We hope these studies will lead to new therapeutic strategies focused on reducing the incidence of BoHV-1 reactivation from latency.

Financial Support: This study was supported by grants the USDA-NIFA Competitive Grants Program (2021-67015 and 2023-07864),

Notes:

219 - Reduced susceptibility to heparan sulfate-adapted BVDV in primary cells from a CD46-edited bovine heifer

Alexandria C. Krueger[1], Brian L. Vander Ley[2], Michael P. Heaton[1], Aspen M. Workman[1]

[1]US Meat Animal Research Center, USDA-ARS, [2]Great Plains Veterinary Educational Center, University of Nebraska-Lincoln. alexandria.krueger@usda.gov

Session: Virology 2, 2025-01-21, 9 - 9:15 AM

Objective: Bovine viral diarrhea virus (BVDV) uses heparan sulfate (HS) and the protein receptor CD46 to bind and enter bovine cells. A CD46 gene-edited heifer was recently reported to have significantly reduced susceptibility to BVDV. A fundamental question is whether BVDV can adapt to circumvent the infection restriction imposed by the CD46 gene-edit. Here, our aims were two-fold: 1) determine whether BVDV can adapt in vitro to utilize the edited CD46 protein receptor to initiate infection, and 2) assess the ability of in vitro adapted BVDV isolates to infect primary cells ex vivo from the CD46 gene-edited heifer.

Methods: BVDV isolates were serially cultured 11 passages on CD46 gene-edited Madin-Darby bovine kidney (MDBK) cells, and changes in viral infectivity in edited cells was quantified by flow cytometry. Viral adaptation to HS was investigated by pre-incubating adapted BVDV isolates with heparin, a HS analog, or by treating cells with Heparinase I/III to remove cell surface HS. Flow cytometry was also used to compare infection efficiency between unadapted and in vitro adapted virus pairs in primary cells from the CD46 gene-edited heifer and an unedited control.

Results: Adapted BVDV isolates used a HS dependent entry mechanism to infect CD46 gene-edited MDBK cells with efficiency comparable to the unedited MDBK cells. Thus, BVDV did not adapt in vitro to use the edited CD46 protein for infection. Importantly, infection by HS adapted BVDV was reduced in primary skin fibroblasts from the CD46 gene-edited heifer and monocytes and lymphocytes remained highly resistant compared to the unedited control in ex vivo experiments.

Conclusions: Ex vivo studies indicate that primary cells expressing the edited CD46 protein receptor maintain varying levels of resistance to in vitro adapted BVDV in a manner that correlates with HS expression. Additional research is needed to determine whether HS adapted viruses would be naturally selected for in CD46 gene-edited cattle and, if so, whether they would have similar tropism, pathogenesis and disease outcomes in vivo.

Financial Support: Alexandria C. Krueger is supported by an appointment to the Agricultural Research Service (ARS) administered by the Oak Ridge Institute for Science and Education (ORISE). Funding for this research was provided by the USDA, ARS appropriated project 3040-32000-034-00D

Notes:

220 - Influenza D virus infection dynamics at the bovine respiratory epithelial barrier: physical and functional impacts

Ruth Nissly[1], Michael Ling[1], Suresh Kuchipudi[2]

[1]Pennsylvania State University, [2]University of Pittsburgh. rah38@psu.edu

Session: Virology 2, 2025-01-21, 9:15- 9:30 AM

Objective: Deltainfluenzavirus influenzae, or influenza D virus (IDV), is the most recently discovered species of orthomyxovirus. IDV infects the respiratory tract of several ruminant species, swine, and other organisms, including some evidence of human infection. However, the main reservoir species is cattle. Some studies have associated IDV infection with bovine respiratory disease complex (BRDC), the leading cause for morbidity and mortality in cattle. However, IDV is also frequently detected in healthy cattle. Our previous studies and those of others found no association between presence of IDV and BRDC. Therefore, a better understanding of IDV respiratory infection dynamics is needed to decipher mechanisms by which IDV infection may promote or prevent BRDC development.

Methods: The initial antimicrobial, inflammatory, and cell-recruitment signaling responses to infection of the respiratory epithelial barrier are critical for determining outcome of infection. We created in vitro models of the cattle respiratory epithelium by culturing primary bovine tracheal and bronchial airway epithelial cells at the air-liquid interface (AEC ALI) to produce pseudostratified, differentiated epithelial cells. We used this model to perform experimental infection studies with a field isolate of IDV. Viral RNA and viral RNA copies were quantified from apical and basolateral sides of the AEC ALI barrier. Barrier integrity was monitored daily using trans epithelial electrical resistance (TEER). Histological and immunofluorescent staining of AEC ALI was performed to visualize effects of IDV infection on epithelial barrier composition through the five-day infection period. Single cell RNAseq was performed on IDV-infected and mock-infected cells to identify cell types present in the AEC ALI and to assess viral and host gene expression profiles in each cell type.

Results: Our AEC ALI model closely mimics respiratory in vivo tissue including development of cell-cell adhesions, cilia and production of mucus. We used the bovine AEC ALI to characterize IDV infection dynamics including susceptible and resistant cells, viral replication kinetics, impact of infection on epithelial barrier integrity and composition, and host innate immune signaling. The cells initially targeted by IDV were exclusively ciliated cells. Ciliated cells that did not become infected had high expression of several interferon-induced genes including MXs, RSAD2, and ZBP1, suggesting these gene products are critical for limiting IDV spread in this cell type. Long-term (5+ days) infection with IDV was maintained, with live virus shed to both basolateral and apical sides of the epithelial barrier despite barrier integrity remaining intact. Nevertheless, epithelial remodeling occurred following infection with IDV, altering the proportion and physical location of cell types involved in production of mucosal antimicrobials including mucus and beta-defensins.

Conclusions: Our results indicate that IDV infection may fundamentally alter susceptibility to other BRDC pathogens through changes in epithelial barrier structure and function.

Financial Support: This research was supported through Pennsylvania Department of Agriculture award C940001067, Pennsylvania Department of Health agreement 4100088558, and Pennsylvania Agricultural Experiment Station, Hatch project 4882.

Notes:

221 - A novel multi-omics perspective on postnatal deficiencies in cattle following transient fetal infection with Bovine Viral Diarrhea Virus

Jessica N. Kincade[1], Jordan M. Eder[2], Erin M. McDonald[2], Darcy M. Deines[2], Brie M. Wright[2], Alexander P. R. Bally[2], Reyes A. Murrieta[2], Terry E. Engle[3], Hana Van Campen[1], Thomas R. Hansen[1]

[1]Dept. of Biomedical Sciences, Colorado State University, [2]Zoetis, Research Innovation Center, [3]Dept. of Animal Sciences, Colorado State University. jkincade@colostate.edu

Session: Virology 2, 2025-01-21, 9:30- 9:45 AM

Objective: Bovine Viral Diarrhea Virus (BVDV) is an economically important pathogen within the cattle industry. Capable of vertical transmission, the pathogenesis of fetal BVDV is directly related to the gestational age of the fetus. Infections occurring before ~125 days of gestation generate a persistently infected (PI) calf that develops immunotolerance to the virus, while fetal infection occurring after ~150 days of gestation generates a transiently infected (TI) calf capable of clearing the virus. These TI heifers have lower body weight at birth, 4 months of age, and 17 months of age when compared to controls. At birth, TI heifers display differential methylation of genes involved in growth, development, and the immune system. By 4 months of age, alterations were identified in genes associated with inflammation, ROS production, and insulin secretion. During a feedlot trial, the same TI heifers had elevated ceruloplasmin and oxidized glutathione, indicative of inflammation. We hypothesized that variations within the methylome of TI heifers persist to 17 months of age, influence the transcriptome, and result in aberrant regulation of the immune system, growth, and metabolism.

Methods: Pregnant heifers were inoculated with non-cytopathic BVDV type 2 or phosphate-buffered saline (PBS) on day 175 of gestation to generate TI (n=11) and control (n=12) calves. Blood samples collected from the calves at 17 months of age were subjected to complete blood count (CBC) analysis. White blood cells isolated from whole blood were analyzed using spectral cytometry, reduced representation bisulfite sequencing (RRBS), and single-cell transcriptomics.

Results: No differences were found in CBC values between treatment groups. Using flow cytometry, TI heifers were found to have decreased CD4+/CD8b+ T cells and natural killer T cells. Methylome analysis of 5 randomly selected TI and 5 control heifers revealed differential methylation at 5,508 CpG sites (DMS), 73 CpG islands, and 81 promoters. Using Ingenuity Pathway Analysis (IPA), variations of the methylome were found to be associated with the immune and cardiac systems, metabolism, and coagulation. Pathways identified include CXCR4 signaling, IL1 and IL8 signaling, leukocyte extravasation signaling, cardiac hypertrophy signaling, pancreatic secretion signaling, GNRH signaling, thrombin signaling, and GP6 signaling, among others. Comparison of the methylomes from the same 5 TI and control heifers at birth, 4 months of age, and 17 months of age revealed persistent differential methylation in 341 genes, 5 CpG islands, and a single promoter region. Genes identified here were associated with the immune system (promoter: MMP9, DMS: FcγRII, CD34, IL22, LIF, Notch1, VPREB1, IRF4, etc.) and metabolism (CpG Island: CELA3B, DMS: LPL, ADM2, POMC, etc.). Single cell transcriptomics revealed increased expression of GAB2 in a CD4+ T cell subset which may indicate increased generation of Th2 cells. Decreased expression of TEX9 in a B cell subset may suggest decreased memory B cell class switching.

Conclusions: Late gestation fetal infection with BVDV induces abnormalities of immune cell populations, the methylome, and transcriptome that persist to 17 months of age in TI heifers. These alterations can potentially influence growth and response to disease in TI cattle.

Financial Support: This research was funded by USDA NIFA grant 2019-67015-29866 and USDA NIFA AFRI pre-doctoral fellowship 2023-67011-40513. Flow cytometry and single-cell transcriptomic data were provided in kind by Zoetis Inc.

Notes:

222 - Biomarker discovery in pregnant cattle infected with bovine viral diarrhea virus

Heather Thomasovich[1], Jon Beever[2], Ky Pohler[3], Michael Rivera Orsini[1], Andi Lear[1]

[1]Large Animal Clinical Sciences, University of Tennessee, [2]Institute of Agriculture Genomics Center, University of Tennessee, [3]Department of Animal Science, Texas A&M University. hthomaso@vols.utk.edu

Session: Virology 2, 2025-01-21, 9:45- 10

Objective: This study aims to identify biomarkers in the blood of dams infected with bovine viral diarrhea virus (BVDV) that are associated with fetal BVDV infection by (1) evaluating pregnancy-associated glycoprotein (PAG) concentrations throughout gestation in PI and CTRL groups and by (2) isolating exosomes to evaluate the content in pregnant uninfected and BVDV infected cattle.

Methods: Nulliparous pregnant beef heifers were divided into 2 treatment groups, PI group (heifers carrying a persistently infected (PI) fetus, n=4), and a CTRL group (heifers carrying a non-infected fetus, n=4). Cattle were intranasally inoculated with BVDV-1b strain (BJ6) or sham media at 75 days of gestation. Whole blood was collected at 45, 95, 120, and 250 days of gestation, and serum and plasma were isolated. Infection status of calves was confirmed at birth via antigen capture ELISA. The exosome population was further isolated from plasma and flow cytometry was used to confirm the presence of CD63+ and PLAP+ placenta-derived exosomes, which were then evaluated by NTA, proteomics, and RNA extraction. Serum was used to determine PAG concentrations using a commercially available ELISA.

Results: No significant difference was observed in PAG concentrations between the PI group at 45 GD (5.19 +/- .58), 95 GD (5.40 +/- .67), and 120 GD (6.67 +/- .78) and CTRL group at 45 GD (6.60 +/- 1.07), 95 GD (6.98 +/- 1.43), and 120 GD (7.48 +/- 1.33). CD63+ placenta-derived exosome concentration increased significantly (p £ .0003) only in the CTRL group between time points 45 GD (729 +/- 5.29), 95 GD (837 +/- 196.65), 120 GD (1486.67 +/- 497.42) and 250 GD (1645 +/- 69.55), but no significant differences were found between time points in the PI group at 45 GD (961.17 +/- 265.48), 95 GD (967.8 +/- 366.35), 120 GD (1521.6 +/- 91.23), and 250 GD (1569.4 +/- 160.14). There were no significant differences in CD63+ or PLAP+ exosome concentrations between PI and CTRL treatment groups. 188 proteins, primarily involved in inflammatory and immune response, were differentially expressed between PI and CTRL groups.

Conclusions: Exosomes were successfully isolated from the plasma of dams carrying fetuses persistently infected with bovine viral diarrhea virus. We found differentially expressed proteins between CTRL and PI groups, representing differences in immune and inflammatory processes. A significant difference was found in CD63+ exosome concentrations between time points in the CTRL group, but not in the PI group. No significant differences between PI and CTRL treatment groups have been found in early and mid-gestation PAG concentrations, MFI, or exosome size and concentration. Limitations of this study include a small sample size and nonspecific PLAP antibody. Still, we hope to use this pilot study to elucidate trends that can guide future studies with a larger sample size.

Financial Support: Boehringer-Ingelheim and UTCVM COE Grant 2023. USDA NIFA Award No. 2022-67015-36502

Notes:

223 - Identifying immune correlates of protection against *Anaplasma marginale*

Olalekan Chris Akinsulie[1], Roberta Koku[1], Shelby Jarvis[1, 2], Sally Madsen-Bouterse[1], Reginaldo Bastos[2], Susan M. Noh[1, 2]

[1]Department of Veterinary Microbiology and Pathology, Washington State University, [2]Animal Disease Research Unit, USDA-ARS. olalekan.akinsulie@wsu.edu

Session: Vaccinology 2, 2025-01-21, 8:30- 8:45 AM

Objective: *Anaplasma marginale* is an intra-erythrocytic bacterium that causes bovine anaplasmosis, characterized by acute anemia and a notable reduction in cattle production worldwide. The current prevention methods are inadequate and rely primarily on the use of acaricides and antibiotics. Acaricides must be applied repeatedly to adequately control tick populations, which is untenable in many cattle production systems. Administration of long-acting tetracyclines, often used to prevent disease, is strongly discouraged due to global efforts to reduce antibiotic use. Thus, a vaccine is needed. Protective immunity can be achieved via vaccination with native outer membrane proteins. However, a major limitation in progress toward vaccine development is a lack of correlates of protective immunity. Because antibodies likely play a major role in immune protection, this work aimed to characterize the IgG Fc-mediated effector responses induced by antibodies specific for a subset of high-priority vaccine candidates in immune cattle. Through data analysis, we will then identify the combination of vaccine candidates and Fc-mediated effector functions that correlate with protective immunity.

Methods: The Fab domain of IgG provides specificity to the antibody response while the Fc- domain binds the Fc-receptors (FcRs) present on most immune cells. The interaction between the Fc-domain with different classes of FcRs triggers effector responses including monocyte and neutrophil phagocytosis, complement fixation, γδ+ T cell activation, and platelet activation. We used fluorescent bead-based assays and flow cytometry to measure the magnitudes of monocyte and neutrophil phagocytosis induced by the Fc region of antigen-specific antibodies using recombinant *A. marginale* outer membrane proteins, including Msp5, Msp1a, Msp1b, OmpA, and Omp7/8/9 conserved regions (CR), and Omp7/8/9 variable regions (VR) and immune serum from cattle at the time of control of *A. marginale* infection.

Results: The most significant levels of monocyte phagocytosis were induced by antibodies directed against OmpA, Msp1b, Msp1a, and Omp7/8/9 CR compared to a significantly lower response to Omp7/8/9 VR and Msp5. For neutrophil phagocytosis, antibodies directed against Msp1a and Msp1b had a significantly higher response than OmpA, Omp7/8/9 VR, Omp7/8/9 CR, and Msp5. Using the Pearson correlation coefficient, we statistically tested the linear relationship between each effector response directed against each antigen, and disease severity as measured by packed cell volume (PCV) and percent infected erythrocytes (PIE). There was a moderate negative correlation between monocyte phagocytosis induced by anti-OmpA, anti-Msp1b, and anti-Omp7/8/9 CR antibodies. Additionally, there was a moderate negative correlation between neutrophil phagocytosis mediated by anti-Msp1a antibody and disease severity.

Conclusions: We conclude that Fc-mediated monocyte phagocytosis directed against OmpA, Msp1b, and Omp7/8/9 CR and neutrophil phagocytosis directed against Msp1a are likely important anti-*A. marginale* effectors. Further investigation is required to quantify each antigen-specific antibody and the magnitude of other Fc-mediated responses, including complement fixation, γδ+ T cell activation, and platelet activation, and their correlation with PIE/PCV. Ultimately, this work will help identify correlates of immunity and aid in rational antigen selection for vaccine design downstream.

Financial Support: This project is supported by funding from USDA-ARS. CRIS number: 2090-3200-0043-000D.

Notes:

224 - Parenteral vaccination with *Moraxella bovis* cytotoxin and porin adjuvanted with Emulsigen®-D to prevent bovine pinkeye

John Angelos[1], Judy Edman[1], Scott Wetzlich[1]

[1]University of California, Davis. jaangelos@ucdavis.edu

Session: Vaccinology 2, 2025-01-21, 8:45- 9

Objective: Currently available parenteral *M. bovis* bacterins against infectious bovine keratoconjunctivitis (IBK; pinkeye) have variable efficacy. To determine if a novel parenteral *M. bovis* subunit vaccine composed of recombinant *M. bovis* cytotoxin plus recombinant *M. bovis* porin adjuvanted with Emulsigen®-D could prevent IBK and/or reduce severity of IBK, a randomized controlled field trial was conducted during April to August 2012 in 106 crossbred beef heifers in northern California.

Methods: Angus and Angus-Hereford crossbred heifers without evidence of active pinkeye were randomly assigned to received a subcutaneous dose of a vaccine comprised of recombinant *M. bovis* cytotoxin plus *M. bovis* porin adjuvanted with Emulsigen®-D (experimental vaccine group) or 0.9% saline (control group). Booster vaccinations were administered after 3 weeks. Ocular exams were performed once every 7 days for 16 weeks after primary vaccination to document development and severity of corneal ulceration associated with IBK. Blood samples were collected to assess immune responses to cytotoxin and porin on study days 0, 42, and 112. Florfenicol was administered if corneal ulcers >0.5 cm in widest diameter were identified. Nonparametric statistical methods were used to determine differences between the two study groups in the proportions of animals that developed IBK from day 21 through day 112, treatments, corneal ulcer severity, and immune responses to cytotoxin and porin. Animals that developed IBK as a result of a mechanical corneal trauma were excluded from the analysis.

Results: A numerically higher proportion of animals in the experimental vaccine group developed IBK, however, the cumulative corneal ulcer surface areas were lower in the experimental vaccine group compared to the control group. A numerically lower proportion of experimental group animals required florfenicol treatment for IBK. These differences were not statistically significant. Experimental vaccine group animals had significantly higher cytotoxin and porin specific serum IgG responses and serum cytotoxin neutralizing antibody titers compared to the control group animals.

Conclusions: Results from this study suggest that parenteral vaccination with *M. bovis* cytotoxin plus porin adjuvanted with Emulsigen®-D does not reduce the occurrence of IBK in cattle, however, it may help to reduce the severity of corneal ulceration associated with this disease.

Financial Support: This study was funded by USDA NIFA Hatch Formula Funds.

Notes:

225 - Ocular IgA and IgG responses in cattle vaccinated intranasally with native *M. bovis/M. bovoculi* cytotoxin adjuvanted with Carbigen®

John Angelos[1], Natalia Marin[1]

[1]Department of Medicine and Epidemiology, University of California Davis. jaangelos@ucdavis.edu

Session: Vaccinology 2, 2025-01-21, 9 - 9:15 AM

Objective: A lack of consistent effectiveness of parenterally administered vaccines against infectious bovine keratoconjunctivitis (IBK; 'pinkeye') has led to investigations of experimental intranasal vaccines to prevent this disease. As part of two randomized controlled field trials (RCTs) evaluating effectiveness of experimental intranasal *Moraxella bovis* + *Moraxella bovoculi* native cytotoxin vaccines, tear samples were collected from a subset of vaccinated cattle to quantitate *M. bovis* cytotoxin specific tear IgA and tear IgG responses in experimental vaccine group and control group steers.

Methods: During a 16 week period in 2022 (April 20 - August 9) and 2023 (April 5 - July 26), two RCTs were conducted in beef steers at a university field station in northern California. In both RCTs on study day 0, steers without active IBK were randomly assigned to receive either intranasal *M. bovis* + *M. bovoculi* native cytotoxin adjuvanted with Carbigen® (experimental vaccine group) or water plus Carbigen® (control group). Steers were boostered 21 days later. A total of 411 steers were enrolled between both RCTs. Tear samples were collected at 3 timepoints from 25 enrolled steers in the 2022 RCT and from 26 enrolled steers from the 2023 RCT: day 0 (prevaccination sample; timepoint 1), at either day 42 (3 week post booster sample for 2022 RCT) or day 49 (4 week post booster sample for 2023 RCT) (timepoint 2), and on day 111 (2022 RCT) or day 112 (2023 RCT) (timepoint 3). The population of sampled animals in each RCT represented approximately equal numbers of control and experimental vaccine group steers. Moraxella bovis cytotoxin-specific tear IgA and tear IgG concentrations were quantitated by ELISA, and fold changes in tear IgA and tear IgG concentrations from timepoints 1-2 and from timepoints 1-3 were calculated. Nonparametric statistical methods were used to evaluate for differences between experimental vaccine and control groups in tear IgA and tear IgG fold changes between timepoints; $P<0.05$ was set as the level of significance.

Results: Steers that received intranasal *M. bovis* + *M. bovoculi* native cytotoxin adjuvanted with Carbigen® had significantly higher median antigen-specific tear IgA fold changes versus control group steers from timepoints 1-2 and from timepoints 1-3. No significant differences in antigen-specific tear IgG fold changes were observed.

Conclusions: Intranasal administration in cattle of *M. bovis* + *M. bovoculi* native cytotoxin adjuvanted with Carbigen® stimulated *M. bovis* cytotoxin-specific IgA antibody responses in tear fluid. Further investigations are underway to determine if this experimental vaccine affects *M. bovis* cytotoxin neutralizing antibody responses in tear fluid, as well as whether or not it protects cattle against naturally occurring IBK.

Financial Support: This study was funded by the Russell L. Rustici Rangeland and Cattle Research Endowment and USDA NIFA Animal Health Formula Funds.

Notes:

226 - Evaluation of *Mycobacterium avium* subsp. *paratuberculosis* DIVA vaccine in Holstein calves

Maria A. Colombatti Olivieri[1], Mostafa Hanafy[2], Denise K. Zinniel[2], John P. Bannantine[1], Raul G. Barletta[2]

[1]USDA-ARS, National Animal Disease Center, [2]School of Veterinary Medicine and Biomedical Sciences, University of Nebraska-Lincoln. alejandra.colombatti@usda.gov

Session: Vaccinology 2, 2025-01-21, 9:15- 9:30 AM

Objetive: Johne's disease (JD) is caused by Mycobacterium avium subsp. paratuberculosis (Map) and primarily affects ruminants, with infection occurring in the first few months of life. We have developed live-attenuated strains capable of distinguishing vaccinated from infected animals (DIVA). DMAP52 marked and unmarked (unm) mutants exhibited lower macrophage survival compared to the WT strain. This study aimed to assess the immune response and pathogenicity in a calf challenge experiment.

Methods: Eleven male Holstein calves were used in two experimental groups of 5 animals infected at 2 to 3 weeks of age with three doses of 2 x 10^{11} CFU/mL of WT strain (K-10) and DMAP52-unm, while an uninfected animal was used as a control. The immune response was evaluated at pre-infection, two weeks, and at 1, 2, 3, 6, and 8.5 months post-infection (mpi) by antibody and IFN-gamma production, lymphocyte/monocyte populations, and a skin test. Map detection was performed using feces and tissues through culture and qPCR.

Results: The infectious dose administered to the WT group was significantly lower than that used in the DMAP52_unm group. Although the results between the two groups are not directly comparable, infection was confirmed in the WT group. Within 8.5 months of infection, no significant humoral or cellular immune response was detected in the DMAP52_unm group. One of the animals infected with DMAP52-unm tested positive on the skin test at 8.5 months post-infection, and no false positives for PPDb were detected. DMAP52_unm was detected in the spleen and liver from one animal by qPCR, but all feces and tissue cultures were negative. Unfortunately, due to a rotavirus outbreak and pneumonia, some calves died, resulting in 3-4 calves remaining in the mutant and WT strain groups.

Conlcusions: These findings suggest that the mutant strain is attenuated. However, further studies involving more animals, and a longer observation period are essential to determine the potential use of this strain as a vaccine.

Financial Support: This project was supported by the USDA-NIFA competitive program Agriculture and Food Research Initiative, grant No. 2020-67015-31416. Dr. Colombatti Olivieri is supported by the Oak Ridge Institute for Science and Education (ORISE).

Notes:

227 - Serological responses to S protein in vaccinated horses and horses with strangles

Noah Cohen[1], Emma Hughes[1], Charlie Bayne[2], Ellen Ruth Morris[1], Jocelyne Bray[1], Danielle Gonzales[1], Reagan Baker[1], Rafaela Klein[1], Wen Liu[1], Rebecca Legere[1], S. Garrett Wehmeyer[1], Angela Bordin[1], Igor Wierzbicki[2], David Gonzalez[2]

[1]Texas A&M University, [2]University of California, San Diego. ncohen@tamu.edu

Session: Vaccinology 2, 2025-01-21, 9:30- 9:45 AM

Objective: To evaluate the immunogenicity of a vaccine targeting the S protein (Ssee) of *Streptococcus equi* subspecies equi (SEE) and to determine antibody activity against Ssee protein in horses with strangles.

Methods: The first phase of the study was a prospective experiment using 20 University-owned Quarter Horses. The second phase of the study was a cross-sectional serosurvey of 78 privately owned horses with strangles. Horses were immunized intramuscularly with 0 (n=4), 200 (n=8), or 400 (n=8) µg of recombinant Ssee at weeks 0, 4, and 12. Serum and nasal secretions were collected from study horses at weeks 0, 4, 6, 12, 16, and 28 and tested by ELISA for IgG against Ssee; nasal secretions were also tested for IgA recognizing Ssee. Serum was tested for complement component 1 (C1) deposition onto Ssee by ELISA and opsonophagocytic killing (OPK) of SEE. Sera from horses with strangles were tested by ELISA for anti-Ssee IgG activity.

Results: Immunization with Ssee significantly ($P<0.05$) increased IgG activity against Ssee in serum and nasal secretions for up to 12 weeks after the 3rd immunization, and serum from vaccinated horses deposited significantly ($P<0.001$) more C1 deposition onto Ssee than serum from controls. Serum from horses with strangles had low levels of IgG activity against Ssee.

Conclusions: Immunizing horses with Ssee resulted in increased activity of functional IgG in serum and nasal secretions and horses with strangles had very low levels of serum IgG activity against Ssee, indicating that S protein immunization can be differentiated from natural infection.

Financial Support: Funding provided by the Morris Animal Foundation (Grant # D23EQ-014). Additional support provided by the Link Equine Research Endowment, Texas A&M University. Dr. Cohen is supported by the Glenn Blodgett Chair in Equine Studies at Texas A&M University.

Notes:

228 - Co-vaccination of cattle with RB51 and BCG enhances the BCG-specific CD4+ T cell response

Haley Sterle[1, 2], Ellie Putz[2], Steven Olsen[2], Paola Boggiatto[2]

[1]Iowa State University, [2]National Animal Disease Center, USDA-ARS. haley.greiman@usda.gov

Session: Vaccinology 2, 2025-01-21, 9:45- 10

Objective: *Brucella abortus*, the causative agent of bovine brucellosis, and *Mycobacterium bovis*, the causative agent of bovine tuberculosis (bTB), are two zoonotic pathogens that contribute to economic losses in the cattle industry and pose a public health risk worldwide. A T helper 1 (Th1) polarized immune response, characterized by CD4+ T cells that produce IFN-g, is known to contribute to protection against both intracellular pathogens. However, the commercially available brucellosis vaccine Brucella abortus strain RB51 (RB51) does not provide sterile immunity, and Bacillus Calmette-Guerin (BCG), a potential vaccine candidate for bTB, affords widely variable levels of protection against M. bovis challenge. Building on evidence that BCG vaccination may provide protection against unrelated pathogens, and previous results from our lab indicating that RB51 may prime bovine CD4+ T cells to recognize mycobacterial antigens, we sought to improve the Th1 polarized CD4+ T cell response to each vaccine by administering RB51 and BCG to cattle concurrently.

Methods: An in vitro recall response assay was conducted using peripheral blood mononuclear cells (PBMCs) isolated from BCG vaccinated, RB51 vaccinated, and BCG + RB51 co-vaccinated cattle. Briefly, PBMCs were incubated for seven days with complete RPMI media, g-irradiated RB51, 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 evaluate BCG- and RB51-specific T cell responses in each vaccination group. Data were analyzed using a simple auto regressive model (AR1), with weeks post vaccination set as a fixed effect. Pairwise comparisons of Least Squares Means were conducted to determine significant differences ($P \leq 0.05$) between contrasts of interest.

Results: Comparison of the RB51-specific CD4+ T cell response in RB51 vaccinated cattle and co-vaccinated cattle revealed that addition of BCG had no effect ($P \geq 0.092$) on the cell-mediated response to RB51. In contrast, co-vaccinated cattle had significantly higher numbers ($P < 0.05$) of proliferating CD4+ T cells in response to PPDb stimulation than BCG vaccinated cattle up to 24 weeks post vaccination. However, a validation experiment in a separate cohort of cattle yielded conflicting results; there was no difference ($P > 0.2$) in the number of proliferating CD4+ T cells in RB51 + BCG co-vaccinated cattle compared to BCG vaccinated cattle.

Conclusions: Co-vaccination of cattle with RB51 and BCG enhanced the CD4+ T cell response to PPDb in a primary study, but this phenomenon was not repeatable. This observation may have significant biological relevance to the development of an efficacious bTB vaccine, therefore future work will be focused on determining the factors that drove this unique response.

Financial Support: This research was funded by USDA CRIS 5030-32000-234. The authors did not receive funding from any outside grant or funding agencies.

Notes:

229 - Translating omics research into improved swine health

Laura Miller[1]

[1]Dept. Diagnostic Medicine/ Pathobiology, Kansas State University. lauracmiller@vet.k-state.edu

Session: Animal Vaccinology Research Network Symposium, 2025-01-21, 10:30 - 11:15

Swine health remains a significant challenge, with respiratory diseases causing substantial economic losses. Omics technologies offer a powerful approach to understanding the complex mechanisms underlying swine health and disease. By integrating genomics, transcriptomics, proteomics, and metabolomics, researchers can identify key genetic and molecular factors that influence disease susceptibility, immune response, and overall animal performance. This knowledge can be leveraged to develop innovative targeted interventions that improve swine health and reduce economic losses.One promising application of omics technologies in swine health is the development of novel vaccines against porcine reproductive and respiratory syndrome (PRRSV). By understanding the complex interplay between the virus and the host immune response, researchers can identify key targets for vaccine development. For example, our recent studies have shown that targeting the interferon (IFN) response can enhance vaccine efficacy and provide broader protection against diverse PRRSV strains.

Notes:

230 - Advancing systems vaccinology by leveraging AI

Yongqun "Oliver" He[1]

[1]Unit for Laboratory Animal Medicine, University of Michigan Medical School. yongqunh@med.umich.edu

Session: Animal Vaccinology Research Network Symposium, 2025-01-21, 11:15- 12:00

With the emergence of artificial intelligence (AI), leveraging systems vaccinology through AI has become a pivotal topic. AI encompasses three major subfields: Knowledge Representation and Reasoning (KRR), Natural Language Processing (NLP), and Machine Learning (ML). Ontologies, as the foundation of KRR, have played a critical role in standardizing and integrating vaccine knowledge and data, ensuring they are "AI-ready" and adhere to FAIR principles (Findable, Accessible, Interoperable, and Reusable). In NLP, Large Language Models (LLMs) like ChatGPT and Llama represent cutting-edge advancements, employing deep neural networks to generate human-like text. Meanwhile, ML technologies, including deep learning, have revolutionized vaccine design and mechanistic studies, paving the way for a transformative era for systems vaccinology.

Our research focuses on advancing systems vaccinology through AI. We have developed the community-driven Vaccine Ontology (VO), which provides a standardized, hierarchical structure for representing thousands of vaccines, their components, and induced responses in a machine-readable format. VO has been instrumental in integrating data into our VIOLIN vaccine knowledgebase and supporting external projects such as ImmPort, HIPC, and the Vaccine Adjuvant Compendium (VAC). VO has significantly enhanced vaccine data analysis and literature mining. Furthermore, we are developing vaccine-specific LLMs to improve literature mining and annotation, with ongoing evaluations of how VO can optimize their performance. Moreover, as part of the VIOLIN initiative, we have created advanced vaccine design tools, including Vaxign for filtering-based vaccine design, Vaxign-ML for antigen prediction using eXtreme Gradient Boosting, and Vaxign-DL, which leverages deep learning. These tools have been applied to identify vaccine candidates for diseases like Brucellosis and COVID-19. This talk will explore how these AI technologies synergize to advance systems vaccinology.

Notes:

231 - Fecal microbiota transplantation modulates jejunal host-microbiota interface in weanling piglets

Farnaz Yousefi[1], Alejandro Ramirez[2], Joy Scaria[3], Shankumar Mooyottu[1]

[1]Auburn University, [2]University of Arizona, [3]Oklahoma state university. fzy0027@auburn.edu

Session: Microbiome, 2025-01-21, 10:30- 10:45 AM

Objective: Weaning-associated enteric diseases are a major concern in the swine industry. This study investigates the effects of fecal microbiota transplantation (FMT) on the jejunum of weanling piglets, a segment of bowel less studied in terms of microbiomic changes despite its primary involvement in major post-weaning enteric diseases, including postweaning diarrhea (PWD).

Methods: Thirty-two 3-week-old piglets were divided equally into two groups: Control and FMT. The FMT group received fecal microbiota preparation from 3-month-old healthy pigs on the 1st and 3rd day after weaning. Half of each group was inoculated with an enterotoxigenic *E. coli* (ETEC) isolate ten days post-FMT. Piglets were euthanized in the third week (14^{th} and 18^{th} days post-FMT) after weaning to collect intestinal tissues and contents for microbiomic, metabolomic, and transcriptomic analyses.

Results: The jejunal microbiota showed a significant increase in alpha diversity in the third week post-FMT compared to the ileum and colon. FMT significantly enriched the jejunal microbiota composition, while multiple bacterial genera were specifically lacking in control weanling piglets. FMT was strongly associated with the enrichment of the genus Pseudoscardovia of the Bifidobacteriaceae family, which was found lacking in the jejunum of weanling control piglets and inversely associated with the abundance of the genus *Bifidobacterium* within the same family. Other genera associated with FMT included *Solobacterium*, *Shuttleworthia*, and *Pseudoraminibacter*, whereas bacteria such as *Erysipelotrichaceae* and *Acidaminococcus* were identified as most abundant in the control piglets. Metabolomic analysis revealed a significant modulatory effect of FMT on carbohydrate, amino acid, nucleotide, vitamin, and xenobiotic metabolisms, suggesting improved nutrient utilization. Transcriptomic analyses further confirmed the regulatory effects of FMT on gene expression associated with immune, metabolic, barrier, and neuroendocrine functions. Prior FMT treatment in the context of ETEC infection indicated a potential protective role, as evidenced by a significant shift in microbial diversity and metabolomic compositions and decreased diarrhea severity even though no effect on pathogen shedding was evident.

Conclusions: This study underscores the promise of FMT in enhancing jejunal health. In addition, the results suggest that FMT could be considered a potential strategy to address conditions associated with small intestinal dysbiosis in swine and other monogastric species with similar gut anatomy and physiology, such as humans.

Financial Support: This project was supported by Agriculture and Food Research Initiative Competitive Grant no. 2022-67015-40720 from the USDA National Institute of Food and Agriculture

Notes:

232 - Linking *Salmonella* populations in feedlot holding ponds to cattle feces and the overall microbial community

Valeria Lugo-Mesa[1], Enrique Doster[1], Brandy Burgess[2], Nikki Shariat[2], Benjamin J. Newcomer[1], Vinicius Machado[3], Juan Pineiro[4], Robert Valeris-Chacin[1], Matthew A. Scott[1], Paul S. Morley[1], Lee J. Pinnell[1]

[1]Veterinary Education, Research, and Outreach Program, Texas A&M University, [2]Department of Population Health, University of Georgia, [3]Department of Veterinary Sciences, Texas Tech University, [4]Department of Animal Science, Texas A&M AgriLife Research. valerialugo@tamu.edu

Session: Microbiome, 2025-01-21, 10:45- 11

Objective: The high incidence of *Salmonella* enterica-related foodborne illnesses in the U.S., along with increasing efforts from federal agencies and heightened public concern regarding salmonellosis, has made effective surveillance a priority. Carrier cattle can continuously shed *Salmonella* to the environment through fecal waste. In feedlots, liquid runoff carrying fecal waste is generated by rainfall and typically managed via holding ponds. The primary objectives of this study were to determine if cattle feedlot holding ponds contain *Salmonella*, characterize the diversity and composition of *Salmonella* populations in holding ponds and feces from nearby cattle pens, and identify correlations between *Salmonella* ecology and overall microbial populations. Further, this study compared the genetic potential of microbial populations with their metabolically active members.

Methods: Holding pond water samples (n = 13), were collected from 6-12 inches below the water surface using sterile 1L bottles and immediately placed on ice. From 4 pens closest to sampled ponds, 1 gram of feces was collected from 20 separate fecal pats using a sterile scoop, placed into sterile conical tubes, and immediately put on ice (n = 48). Fecal composites were homogenized thoroughly by vortexing. All samples were tested for *Salmonella* using selective enrichment and PCR, and CRISPR-SeroSeq was used to identify all serovars present in culture-positive samples. RNA and DNA were co-isolated using the RNeasy PowerSoil Total RNA Kit and DNA Elution Kit (Qiagen). RNA was reverse transcribed into cDNA using the qScript cDNA Synthesis Kit (Quantabio). Shotgun metagenomic (n = 61) and metatranscriptomic (n = 61) sequencing libraries were prepared using the Illumina DNA Prep Kit and sequencing was performed on an Illumina NovaSeq 6000. Using the AMR++ pipeline, sequencing reads were filtered for quality, taxonomically classified using Kraken2, and ARGs were identified using the MEGARes database. The R packages phyloseq, microbiome, btools, metagMisc, ANCOMBC, UpSetR, and vegan were used to analyze, visualize, and compare microbial diversity and composition.

Results: Nearly half of the sampled feedlot ponds and nearby fecal samples were culture and PCR-positive. Further, an average of 1.88 serovars across positive samples were identified by CRISPR SeroSeq. *Salmonella* was detected in low abundance across all ponds and fecal samples based on meta-omics sequencing, with overall higher abundance in culture-positive samples. No differentially abundant OTUs classified as *Salmonella* were identified between fecal and pond samples. While the taxonomic composition of fecal communities differed between the metagenome and metatranscriptome, there was no difference in fecal AMR gene pool composition. Similarly, the taxonomic composition of AMR gene pools did not differ between pond community metagenomes and metatranscriptomes.

Conclusions: *Salmonella* appears to be nearly ubiquitous, albeit at low abundance, and has similar population structure in holding pond water and cattle feces. However, its abundance did not significantly impact overall microbial community composition. Notably, the metabolically active members of fecal communities differed considerably from their genetic potential, whereas the resistome was similar. The similarities of *Salmonella* populations between holding ponds and feces lays the groundwork for exploring trends in community-wide *Salmonella* patterns in beef cattle through wastewater surveillance.

Financial Support: Research coordinated by the National Cattlemen's Beef Association, a contractor to the Beef Checkoff.

Notes:

233 - Defining microbiomes of ixodid ticks collected from the Chernobyl Exclusion Zone 30 years post nuclear catastrophe

Natanel Neumann[1], Ivan Ivanov[2], Aimee-Joy M. Hearn[3], John C. Blazier[4], Yuliya V. Rogovska Rogovska[1], Jiangli Wang[5], Sijia Li[6], Shuling Liu[7], Igor V. Nebogatkin[8], Artem S. Rogovsky[1]

[1]Department of Pathobiology & Diagnostic Investigation, Michigan State University, [2]Department of Veterinary Physiology and Pharmacology, Texas A&M University, [3]Department of Veterinary Pathobiology, Texas A&M University, [4]Institute for Genomics Sciences and Society, Texas A&M University, [5]School of Artificial Intelligence and Big Data, Hefei University, [6]Statistical Collaboration Center, Texas A&M University, [7]Department of Family Medicine, Oregon Health & Science University, [8]I.I. Schmalhausen Institute of Zoology of National Academy of Sciences of Ukraine. nneumann@msu.edu

Session: Microbiome, 2025-01-21, 11 - 11:15 AM

Objective: Advancements in DNA sequencing and bioinformatics have recently enabled scientists to perform in-depth analysis of tick microbiomes. The catastrophic 1986 accident at the Chernobyl nuclear power plant resulted in the largest release of radioactive material in human history, leading to long-term environmental consequences. Despite the significant ecological impact, few studies have investigated the long-term effects of Chernobyl's radiation on the microbial community. Of these few investigations, most did not utilize advanced statistical analysis, often relying on diversity metrics and having small sample sizes. This study aimed to characterize the microbial composition of Ixodes ricinus and Dermacentor reticulatus ticks collected within the Chernobyl Exclusion Zone (CEZ).

Methods: By sequencing the V6 region of the 16S rRNA gene, we analyzed a total of 160 individual microbiomes from 90 I. ricinus and 70 D. reticulatus ticks flagged from the highly restricted 10-km and 30-km zones surrounding the nuclear plant. Importantly, our metagenomic analysis also included individual microbiomes of 139 I. ricinus and 138 D. reticulatus ticks collected from four distinct control regions of Ukraine that have not been directly exposed to prolonged irradiation. The initial analysis consisted of diversity metrics followed up with the Linear Discriminant Analysis Effect Size (LEfSe) algorithm to quantify the most significant taxonomic features that would differentiate the microbial communities of ticks from the CEZ and those from the control regions.

Results: Our findings showed that the CEZ ticks consistently exhibited numerous statistically significant inter-sex and inter-regional variations in alpha and beta diversities as well as in bacterial relative and differential abundances. Several genera, namely, *Halomonas*, *Hymenobacter*, *Nocardioides*, *Rickettsia*, and *Williamsia* were significantly associated with the CEZ ticks, whereas A*grococcus*, *Bosea*, *Brevundimonas*, *Devosia*, *Geodermatophilius*, *Microbacterium*, and *Roseomonas* were significant features of the control ticks.

Conclusions: In addition to the geographical and climatic differences between the CEZ and control regions, we hypothesize that, based on our results, the microbiome of CEZ ticks has significantly adapted to decades-long irradiation. Given that numerous generations of ixodid ticks have been produced in the CEZ since 1986, the microbial composition of CEZ ticks represents the unique opportunity to study microbial adaptation to prolonged ionizing radiation exposure. Overall, our study contributed to understanding the microbial diversity and composition of *I. ricinus* and *D. reticulatus* ticks present in a highly radioactive contaminated environment.

Financial Support: The research was supported by the Texas A&M AgriLife Vector-Borne Disease Seed Grant and a start-up provided to A.S.R. by Department of Pathobiology and Diagnostic Investigation, College of Veterinary Medicine, Michigan State University

Notes:

234 - How relatedness between foals impacts the developing microbiome and resistome

Maggie Murphy[1, 2], Enrique Doster[1], Lee J. Pinnell[1], Cory Wolfe[1], Eryah Mora[3], Kelsey Shields[3], Jessica Looman[3], Hannah Looman[3], Gregg O. Veneklasen[3], John L. Pipkin[4], Paul S. Morley[1]

[1]Texas A&M University, VERO Program, [2]West Texas A&M University, [3]Timber Creek Veterinary Hospital, [4]Texas Tech University. mmurphy@wtamu.edu

Session: Microbiome, 2025-01-21, 11:15- 11:30 AM

Objective: The gut microbiome can vary between individuals with impacts ranging from the environment and group-living. Understanding of these impacts remains unclear, with other species, research has shown it difficult to parse out these effects as those related also are in a group-living situation. This study aims to fill research gaps on the developing equine microbiome and the impact relatedness may or may not have on this development.

Methods: Fecal samples were collected per rectum from 29 dam and foal pairs (n = 58) that were born and raised at Timber Creek Veterinary Hospital in Texas. Fecal samples were collected from foals at 7, 14, 28, and 60 days-of-age, while dams were collected at days 14, 28, and 60. Foals ranged in relatedness from unrelated to half and full siblings, as well as genetically identical clones. Following DNA isolation, the metagenomic library was prepared using the Illumina DNA Preparation kit and sequencing was carried out by the North Texas Genome Center on an Illumina NovaSeq 6000. Taxonomic classification was performed using Kraken 2, the standard database with analyses being completed using phyloseq in R.

Results: Sequencing deeply on animals that were in similar environments and were related at varying degrees allowed us to parse out potential small differences in the microbiome and resistomes of young foals and their dams. Ongoing analysis will explore the microbiome, including bacteria, viruses, and protozoa through shotgun metagenomic sequencing. Preliminary results suggest that environment has more of an impact on the microbiome development. As the microbiome reaches a stabilized state, there is a degree of influence by the environment. At a smaller scale there is the relatedness impact, but the degree to which we understand that is still on going.

Conclusions: Time is the most impactful influence on the development of the microbiome, with the relatedness of the animals providing a much lower percent of variance in the foals. This study will provide context for other studies regarding the knowledge of the developing microbiome and resistome in young foals. This study also provides insight into the viruses and protozoal communities in equine feces which has largely been regarded as needing more research.

Notes:

235 - Microbiota modulation of chickens impacts *Salmonella* colonization and host gene expression

Torey Looft[1], Melissa Monson[1], Carmen Wickware[1, 2]

[1]USDA-ARS, National Animal Disease Center, [2]Oak Ridge Institute for Science and Education. torey.looft@usda.gov

Session: Microbiome, 2025-01-21, 11:30- 11:45 AM

Objective: Exposing chicks to microbiota from feces or cecal contents of mature birds can reduce *Salmonella*, potentially through competitive exclusion. This study compared the anti-*Salmonella* potential of mature cecal contents (CC) to a defined community of 15 commensal bacteria (DC) in growing chickens. Additionally, changes in cecal microbiota composition and the effects of microbiota modulation on host cecal gene expression before and after inoculation with *Salmonella* were explored.

Methods: One-day-old White Leghorn chicks were divided into three groups and orally gavaged with DC, CC, or sterile PBS (control; CT). After one week, birds from each group were euthanized for cecal content and cecal tissue collection (pre-*Salmonella* sample), and remaining birds were orally gavaged with 1X108 colony forming units (CFU) of *Salmonella* enterica ser. Heidelberg (SH2813). Cecal contents were collected for evaluating *Salmonella* CFUs at 3-, 14-, and 28-days post-*Salmonella* inoculation (dpi). Additionally, at all timepoints (both pre- and post-*Salmonella* inoculation) cecal contents were used to extract DNA and sequence bacterial 16S rRNA for microbiota analysis and RNA was isolated from cecal tissues for transcriptomic analysis. *Salmonella* CFUs and community 16S alpha diversity were analyzed by Kruskal-Wallis. 16S Beta diversity was analyzed by perMANOVA while differential abundance was tested with ANCOM-BC. Transcriptomic data were analyzed with DESeq2 on normalized data.

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 microbiota from CC showed significant differences in alpha 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 on day 14 but diversity in DC was closer to CT on 28 dpi. Before *Salmonella* inoculation, 37 genes from CC birds and 676 genes from DC birds were differentially expressed compared to CT birds. At 3 dpi, significant expression changes were observed in 181 and 142 genes for the DC and CC groups, respectively, in comparisons to CT. When compared to CT at the later timepoints, the number of significantly differentially expressed genes increased in CC (588 and 838 genes at 14 and 28 dpi), with fewer differentially expressed genes in DC (290 and 59 genes at 14 and 28 dpi). Overrepresented gene ontology (GO) terms for T and B cell pathways and inflammatory responses were mostly identified in the CC group compared to CT at 14 and 28 dpi, while transporters were most enriched in the comparison of DC to CT pre-*Salmonella* inoculation. Overall, differentially expressed genes included transporters, tight junction, and immunological genes that play a role in essential intestinal functions and host interactions with the microbiota.

Conclusions: This study focused on leveraging the impact of commensal bacteria within the chicken intestine to reduce the duration and density of *Salmonella* colonization. The impact of host microbiota modulation on immunological and physiological gene expression suggests more work is needed to understand how microbial members contribute to poultry health and performance.

Financial Support: Research was supported through U.S. Department of Agriculture appropriated funds.

Notes:

236 - Microbiome and *Salmonella* dynamics in pork trim meat and contact surfaces in a commercial pork processing facility

Aaron Asmus[1], Tara Gaire[1], Kayla Heimer[2], Keith Belk[3], Randall Singer[1], Timothy Johnson[1], Noelle Noyes[1]

[1]University of Minnesota, [2]Hormel Foods Corporation, [3]Colorado State University. nnoyes@umn.edu

Session: Microbiome, 2025-01-21, 11:45- 12

Objective: The objective of this study was to describe the temporal dynamics of the microbiome and *Salmonella* in trim meat and contact surface samples collected over time across two fabrication lines in a single commercial pork processing facility.

Methods: Meat and associated contact surface samples were collected from the bootjack (BJ) and picnic trim (PT) fabrication lines of a pork processing facility over the course of 6 weeks in 2023. Samples were collected at the beginning and end of first shift. Samples were subjected to cultural isolation and qPCR-based detection and enumeration of *Salmonella*, as well as total DNA extraction. Three presumptive *Salmonella* colonies per positive culture plate were subjected to whole-genome sequencing and analysis. Total extracted DNA was subjected to 16S rRNA sequencing and microbiome analysis. Microbiome and *Salmonella* profiles were compared between meat and contact surface samples; between the two fabrication lines; between beginning- and end-of-shift; and between sampling dates.

Results: The microbiome profile differed significantly between the meat and contact surface samples, both across the two fabrication lines and within each individual fabrication line. Compositional differences in the microbiome were also strongly associated with both processing date and shift time. The abundance of key taxa associated with food safety and spoilage was also temporally dynamic across the production shift, and was different between the meat and contact surface samples. In particular, the abundance, prevalence and diversity of species with the genus Clostridium sensu stricto 1 increased significantly throughout the shift, and this increase was more pronounced in the PT line compared to the BJ line. Detection of *Salmonella* was temporally sporadic, with detections clustered around certain sampling dates. *Salmonella* load was generally very low. Multiple *Salmonella* serotypes were identified. Within each serotype, WGS analysis indicated very low levels of genetic diversity across sampling dates, fabrication line and sample type.

Conclusions: Our findings suggest that daily variations in the production process likely affect both the microbiome profile and *Salmonella* prevalence of trim meat and contact surfaces, across different fabrication lines. WGS results indicate that a common source(s) of individual *Salmonella* strains may exist, and this source may precede fabrication of the pork carcass. This study provides critical knowledge that can be used as a foundation for tailored processes to improve fresh pork safety and quality, potentially tailored to individual fabrication lines, time points within a shift, and/or production days. Additionally, this study provides a list of potential biological markers associated with food safety and quality that could be used by processors to develop and validate intervention strategies specific to different fabrication lines.

Financial Support: This work was funded by Hormel Foods Corporation.

Notes:

237 - Rapid chute-side antibiotic resistance detection tool to improve antimicrobial stewardship and optimize risk management while controlling bovine respiratory disease

Claudia Ossa-Trujillo[1], Hervé Volland[2], Anaïs Vogel[2], Thierry Naas[3], Christian Moguet[4], Milovan Stankov- Puges[4], Kenneth Aron[4], Hatem Kittana[5], Jessica Galloway-Peña[6], Sara D. Lawhon[6], Javier Vinasco[6], Keri N. Norman[1], H. Morgan Scott[6]

[1]Department of Veterinary Integrative Biosciences, Texas A&M University, [2]Département Médicaments et Technologies pour la Santé, Université Paris Saclay, France, [3]Assistance Publique-Hôpitaux de Paris, Hôpital Bicêtre, France, [4]Research and Development Department, NG Biotech, France, [5]Department of Diagnostic Medicine/Pathobiology, Kansas State University, [6]Department of Veterinary Pathobiology, Texas A&M University,. cossat@tamu.edu

Session: Diagnostic testing 2, 2025-01-21, 10:30- 10:45 AM

Objective: A chute-side lateral-flow immunoassay (LFIA) that can speed the detection of antimicrobial resistance (AMR) elements prevalent in bacterial pathogens would greatly benefit the management of bovine respiratory disease (BRD). Such a tool would be a useful adjunct both for controlling cattle disease and improving antimicrobial stewardship. With optimized risk management of both respiratory and enteric bacterial AMR, livestock can be kept healthy while the safety of the food supply is reassured.The primary aim of the study was to evaluate the sensitivity and specificity of two rapid diagnostic tools targeting (1) duplexed extended-spectrum beta-lactamase (CTX-M)/mobilized AmpC (CMY-2) proteins and (2) overall 3rd-generation cephalosporin (cefotaximase) activity in the feces of feeder cattle.

Methods: The primary aim was achieved using Bayesian latent-class models (BLCM) with the novel LFIA comparisons performed against standard microbiological endpoints. These analyses were conditioned on two populations of fecal bacteria of varying beta-lactamase prevalence driven by pre- and post-antibiotic therapy. A total of 400 beef cattle were included in a randomized field trial conducted on two large U.S. beef cattle feedyards. On Day 0, 200 individual fecal samples were collected per rectum on each operation, following which each animal received one of four antibiotic treatments (macrolide, fluoroquinolone, phenicol, or third-generation cephalosporin (or, no treatment in one feed yard)) following the single-dose labeled regimen used for BRD control (i.e., 50 cattle per group). Seven days later, 200 post-treatment fecal samples were collected and matched on animal ID. Fecal samples underwent screening for antimicrobial resistance microbiological analysis via spiral plating on selective media (MacConkey agar supplemented with 4 mg/L of ceftriaxone). These results were compared to those of the duplexed DetecTooLs (CTX-M/CMY-2) and cefotaximase activity DetecTooLs (both from NG Biotech, Guipry-Messac, France).

Results: Antibiotic metaphylaxis for BRD resulted in a transient increase in the prevalence of beta-lactam resistance elements and enzymatic activity on Day 7 compared to pre-treatment on Day 0. Based on this difference, BLCM analysis showed increasing sensitivity of the duplexed (CTX-M/CMY-2) assay, rising from 81% at 10^2 CFU/g feces to 99% at 10^5 CFU/g. Meanwhile, the test specificity diminished from 93% to 89% over the same range of bacterial counts. The cefotaximase activity assay exhibited a lower sensitivity over the same range of CFU (40-75%); however, specificity remained at 100% across the same range. Interestingly, macrolide use during metaphylaxis provided negligible selection pressure compared to non-treated controls. On the other hand, phenicols over-selected for beta-lactam resistance even when compared to the direct selection of a 3rd generation cephalosporin.

Conclusions: These findings suggest that rapid assays can guide antimicrobial stewardship decision-making for BRD metaphylaxis. Field application of such devices may require additional equipment and staff training. This initial study focused on enteric bacteria as a food safety aspect of stewardship in food animal production. Ongoing work in our group shifts the focus of these chute-side assays toward respiratory pathogens and macrolide resistance.

Financial Support: This research was funded by the International Consortium for Antimicrobial Stewardship in Agriculture (ICASA), a public-private partnership established by the Foundation for Food & Agriculture Research (FFAR). Grant ID: CASATWG-0000000011

Notes:

238 - A novel serological test for strangles in horses

Noah D. Cohen[1], Rie Nakajima[2], Ellen Ruth Morris[1], Rafael Ramiro de Assis[2], Emma V. Hughes[1], S. Garrett Wehmeyer[1], Jocelyne M. Bray[1], Reagan M. Baker[1], Danielle M. Gonzales[1], Angela I. Bordin[1], Philip Felgner[2]

[1]Texas A&M University, [2]University of California, Irvine. ncohen@tamu.edu

Session: Diagnostic testing 2, 2025-01-21, 10:45- 11

Objective: Serological testing for strangles can be helpful to diagnose complications and assess risk of complications from infection with *Streptococcus equi* subspecies *equi* (SEE) and to screen horses for exposure to SEE. Limitations in accuracy of the serological test available in the United States have been reported. Thus, we sought to identify novel protein targets for a serological test to improve detection of infection with or exposure to SEE.

Methods: In the first phase of the study, 100 genes that were either surface-associated or secreted by SEE were expressed in a cell-free expression system and resultant proteins were printed onto a microarray. The microarray was probed with serum from 30 horses with strangles, 30 strangles-free horses, and 18 horses infected with *Streptococcus equi* subsp, *zooepidemicus* (SEZ) to identify proteins for which sera from horses infected with SEE were more reactive. The protein(s) identified via the microarray were then tested using an enzyme-linked immunoassay (ELISA) format to estimate accuracy of the diagnostic test using receiver-operator characteristic (ROC) curve methods using sera from 100 horses with strangles and 100 serum samples from strangles-free horses, including horses infected with SEZ.

Results: A candidate protein was identified using the microarray method. Adapting this protein to an ELISA format, the area under the ROC curve was 97% and this value was significantly ($P < 0.001$) higher than the area under the ROC curve for an ELISA using a recombinant M protein of SEE.

Conclusions: A novel protein appears to be highly accurate for identifying horses infected with SEE. Testing for this protein should aid in improved control and prevention of strangles in horses.

Financial Support: Supported by grants from the Grayson-Jockey Club Research Foundation and The Foundation For The Horse. Additional support was provided by the Link Equine Research Endowment, Texas A&M University. Dr. Cohen is supported by the Glenn Blodgett Chair in Equine Studies.

Notes:

239 - Supervised machine learning to diagnose respiratory disease-affected bovine lung histopathology photomicrographs

Haleigh M Prosser[1], Abigail Finley[2], Brad J White[3], Eduarda M Bortoluzzi[3], Matthew A Scott[1]

[1]Veterinary Education, Research, and Outreach Program, Texas A&M University and West Texas A&M University, [2]Shreiber School of Veterinary Medicine, Rowan University, [3]Beef Cattle Institute, Kansas State University. hmprosser1@tamu.edu

Session: Diagnostic testing 2, 2025-01-21, 11 - 11:15 AM

Objective: Multiple syndromes constitute a respiratory disease diagnosis in feedlot cattle, including bronchopneumonia (BP), acute interstitial pneumonia (AIP), and bronchopneumonia with an interstitial pattern (BIP). Mortalities caused by these specific syndromes, especially late in the feeding period (>45 days on feed), cause economic stress and welfare concerns for producers. While respiratory syndromes present differently and may require different treatments, diagnosis must be made post-mortem by a trained veterinary anatomic pathologist. Because of time constraints, these diagnoses are not often made, leaving producers unaware of the specific syndrome affecting their cattle. Our research proposes using supervised machine learning (ML) algorithms to differentiate normal and respiratory disease-affected histopathology photomicrographs, then to diagnose the specific syndrome (BP, AIP, or BIP) affecting abnormal photomicrographs.

Methods: Four lung section (left caudal, right caudal, left cranial, and right cranial) samples were obtained in buffered formalin during on-site necropsy of 294 deceased feedlot steers and heifers, prepared and stained on slides, digitized, and diagnosed by a study-blinded, board-certified veterinary anatomic pathologist. Due to autolysis and inadequate labeling, 101 cases and 9 sections, respectively, were removed from further analysis. From these digitized histopathology slides, 4,168 x 4,168-pixel patches (~10X magnification objective) were hand-selected to compile balanced image numbers for each diagnosis, excluding a normal diagnosis, as only 220 healthy patches could be obtained. In an initial assessment, 220 normal and 220 BP patches were uploaded and labeled with histopathologic diagnosis using the Microsoft Azure Machine Learning Studio (Microsoft Corporation, Redmond, WA, USA). ML modeling was completed via the image classification feature of Azure's Automated ML studio with three selected model algorithms (ResNet50, residual networks; SE-ResNeXt, squeeze and excitation networks; and ViTb16r22, vision transformer networks). The algorithms utilized 20% of data for validation, and the best model was reported based on accuracy.

Results: A total of 8,345 patches (220 normal, 2,000 BP, 2,093 AIP, 2,016 BIP, and 2,016 other) at a 10X objective have been cropped. In the initial 440 image BP versus normal assessment, the ViTb16r22 visual transformer network was the most accurate model after random sampling trials within the automated ML platform. The best model accuracy within the image classification model was 98%, the highest weighted area under the curve (AUC) was 0.98, and the BP sensitivity estimate was 96%.

Conclusions: The initial assessment displays promising results for completion of automated ML for the full 4,168 x 4,168-pixel patch dataset. It is important to note that variability of diseased images in this initial assessment is likely less than in the eventual full assessment, as only one syndrome was included in the trial. Nonetheless, the estimated performance metrics indicate a potential for successful model utilization. We aim to identify a model to aid pathologists based on specific needs; the model may be used for slide screening, flagging abnormal images for manual review and discarding normal images, or a preliminary diagnostic, reporting an initial diagnosis back on-farm preceding manual pathologist review. Increased speed of histopathologic diagnoses would yield faster disease management and improved animal welfare in the feedlot setting.

Financial Support: This project is supported by Foundation for Food and Agriculture Resources (FFAR) International Consortium for Antimicrobial Stewardship in Agriculture (ICASA) Grant ID 22-000564, Texas A&M University, and Kansas State University.

Notes:

240 - A 4-Plex qPCR Assay for the quantification of pathogens in liver abscesses and ruminal tissues of feedlot cattle

Mina Abbasi[1], Reese Wilson[2], Xiaorong Shi[1], Leigh Ann George[1], Farinaz Baghaei Naeini[1], Jianfa Bai[1], Raghavendra G. Amachawadi[1], Dale Woerner[2], T. G. Nagaraja[1]

[1]Kansas State University, [2]Texas Tech University. minaabbasi@ksu.edu

Session: Diagnostic testing 2, 2025-01-21, 11:15- 11:30 AM

Objective: Liver abscesses (LA) are polymicrobial infections with *Fusobacterium necrophorum*, particularly subsp. necrophorum, as the primary etiologic agent and *Trueperella pyogenes* and *Salmonella* enterica as secondary agents. The objective of this study was to develop and validate a 4-plex quantitative PCR (qPCR) assay for the detection and quantification of four major bacterial pathogens, two subspecies of *F. necrophorum* (subsp. necrophorum and subsp. funduliforme), *T. pyogenes*, and S. *enterica*, in abscessed and healthy liver tissues, and matched ruminal tissues collected from beef-on-dairy cattle at slaughter.

Methods: The 4-Plex qPCR assay targeted the promoter region of the lktA gene (leukotoxin) for the two *F. necrophorum* subspecies, the plo gene (pyolysin) for *T. pyogenes*, and the invA gene (invasion protein A) for *S. enterica*. The assay was validated using several species and strains of targeted organisms and non-targeted organisms. A total of 424 tissue samples, which included 113 LA and 101 matched ruminal tissues, and 108 liver and 102 ruminal tissues from cattle with healthy livers were analyzed. Samples were homogenized and homogenates were subjected to DNA extraction and qPCR assay to detect and quantify the four pathogens. Homogenates were also subjected to culture methods, using selective, nonselective and enrichment media, to isolate and identify the four pathogens. Data were analyzed with liver status (LA or healthy) as the fixed effect and Fisher's exact test to assess prevalence of the pathogens and a t-test to assess log-transformed bacterial concentrations.

Results: Subsp. necrophorum was significantly more prevalent (61.1 vs. 3.7%) and present at higher concentrations (7.2 vs. 4.5 $\log_{10}$ CFU/g) in LA compared to healthy livers ($P < 0.01$). The prevalence of subsp. funduliforme was lower but the concentration was similar to subsp. necrophorum (33.6 vs. 7.4%; 6.9 vs. 4.5 $\log_{10}$ CFU/g, $P < 0.01$). The prevalence and concentrations of T. pyogenes (21.2 vs. 1.9%, 5.8 vs. 4.2 $\log_{10}$ CFU/g, $P < 0.05$) were different between LA and healthy livers, but not for S. enterica (0 vs. 0.9%, $P = 0.48$). In ruminal tissues, prevalence of subsp. funduliforme was higher than that of subsp. necrophorum. However, no significant differences were detected in prevalence and concentrations of subsp. necrophorum (47.5 vs. 41.2%, 5.3 vs. 5.1 l $\log_{10}$ CFU/g, $P = 0.4$ and $P = 0.09$), subsp. funduliforme (88.1 vs. 87.3%, 5.5 vs. 5.3 $\log_{10}$ CFU/g, $P = 1.0$ and $P = 0.08$), or *T. pyogenes* (3 vs. 2%, 4.73 vs. 0.05 log10 CFU/g, $P = 0.69$ and $P = 0.48$). The qPCR assay results on prevalence aligned with the culture-based isolation data.

Conclusions: The 4-plex qPCR assay is useful in detecting and quantifying the four major pathogens implicated in LA. A small proportion of healthy livers carried the four pathogens, but the concentrations were lower than in LA. The ruminal tissue prevalence and concentrations of the pathogens did not appear to be related to the occurrence of LA. A likely application of the qPCR assay is in differentiating bacterial species that contribute to (high concentration) and those that are prevalent, but not contributing to infection (low concentrations).

Financial Support: Foundation for food and agriculture research

Notes:

241 - Random forest models for antimicrobial minimum inhibitory concentration imputation

Gayatri Anil[1, 2], Joshua Glass[1], Abdolreza Mosaddegh[1, 3], Casey L. Cazer[1, 2]

[1]Department of Clinical Sciences, Cornell University, [2]Department of Public and Ecosystem Health, Cornell University, [3]Multidisciplinary Graduate Engineering Programs, Northeastern University. ga325@cornell.edu

Session: Diagnostic testing 2, 2025-01-21, 11:30- 11:45 AM

Objective: Antimicrobial resistance (AMR) is a public health threat because AMR genes and pathogens can pass between humans, animals, and the environment; thus, antimicrobial use in one sector affects the others. Idiosyncrasies in AMR data, including missing data and changes in testing protocols, such as antimicrobials and concentrations tested between labs and over time, make characterizing AMR trends challenging. Therefore, this study sought to use machine learning methods to develop a model to impute missing minimum inhibitory concentrations (MIC).

Methods: Cattle-associated *Escherichia coli* was selected for modeling because it is tested against a range of antimicrobials by the National Antimicrobial Resistance Monitoring System (NARMS). Phenotypic AMR data (MIC) from two publicly available datasets (NARMS-Cattle Carcass-Cecal and NARMS-Ground Beef) corresponding to 11,713 isolates collected between 2002-2019 were used. Random forest models were designed to predict the MIC of a given *E. coli* isolate for 10 antimicrobial agents across 8 drug classes. Predictors included isolate metadata and MICs of other antimicrobials. The dataset was divided into 70% training and 30% testing data, with models developed on training data and classification accuracy evaluated on the testing data. A specific random forest model was trained for each antimicrobial. Model performance was then evaluated on test data isolates by comparing predicted MICs to the NARMS reported lab-derived MIC values. Models were also assessed for their ability to accurately classify susceptible and resistant isolates (sensitivity) with a susceptible or resistant imputed MIC value respectively.

Results: The exact accuracy (EA) of each model as well as its accuracy in predicting the MIC to within one MIC category (+/-1) are as follows: amoxicillin-clavulanic acid (EA: 51%, +/-1: 97%), amikacin (EA: 35%, +/-1: 86%), ampicillin (EA: 49%, +/-1: 88%), chloramphenicol (EA: 52%, +/-1: 91%), ciprofloxacin (EA: 84%, +/-1: 99%), trimethoprim-sulfamethoxazole (EA: 91%, +/-1: 98%), sulfisoxazole (EA: 57%, +/-1: 85%), cefoxitin (EA: 47%, +/-1: 91%), tetracycline (EA: 64%, +/-1: 80%), and ceftiofur (EA: 59%, +/-1: 97%). Overall, resistant and susceptible sensitivities were over 80% for most antimicrobial models. In general, the strongest predictor variable in a given model was another drug in the same drug class, if such a drug was available as a predictor. Among the metadata predictors, year of isolate collection was consistently an important predictor across antimicrobial models.

Conclusions: The high overall +/-1 accuracy of the models indicate that the predicted MIC values are well correlated with lab-derived MIC values. FDA approved automated antimicrobial susceptibility testing devices have a margin of error of +/-1 2-fold MIC dilution, and since accuracy was assessed by comparing the model's predictions to NARMS laboratory-reported MIC values, +/-1 MIC category accuracy may better account for this variation than exact accuracy. In addition, the models are able to differentiate between susceptible and resistant isolates. These results indicate that the models can predict MIC values at a level of accuracy that would be helpful for imputation in resistance datasets. Imputation of missing AMR data would allow for better evaluation of AMR trends over time, helping inform stewardship policies.

Financial Support: This work is supported by the USDA National Institute of Food and Agriculture, AFRI project 2023-68015-4092. Student support was provided by the National Institute of General Medical Sciences of the National Institutes of Health under Award Number T32GM150453.

Notes:

242 - Development of digital PCR assay for detection of *Taylorella equigenitalis* causing Contagious Equine Metritis in horses

Vishakha Kulkarni[1], Smriti Shringi[1], Devendra H. Shah[1]

[1]Texas Tech University School of Veterinary Medicine. vishkulk@ttu.edu

Session: Diagnostic testing 2, 2025-01-21, 11:45- 12

Objective: Contagious Equine Metritis (CEM), caused by *Taylorella equigenitalis* (TE), is a highly contagious, sexually transmitted foreign animal disease for U.S. equine population. Past multistate CEM outbreaks in U.S. have resulted in significant economic losses and import restrictions. The current outbreak in Florida since May 2024 highlights the threat it poses to the U.S. equine industry. The transmission of TE among horses and CEM outbreaks can be mitigated by early detection and diagnosis. However, the current federally approved diagnostic procedures (live animal breeding, culture, followed by confirmatory 16S ribosomal-DNA PCR) are laborious, time-consuming, and show variable success. To address the need for rapid and reliable CEM diagnostics, we identified novel TE-specific targets using comparative genomic analysis on several TE genomes. The objective of this study is to develop and evaluate a digital PCR (dPCR) assay for the species-specific detection of TE.

Methods: Three TE-specific targets were chosen to design target-specific oligonucleotide sets (primers and probes)using Primer Express software. These sets were tested in singleplex, followed by multiplex dPCR assays. Both singleplex and multiplex dPCR assays were evaluated using target genes cloned pUC57 plasmids (positive amplification controls/PACs)The analytical specificity and limit of detection of the dPCR assays were evaluated using serially diluted PACs, a reference ATCC TE, and a closely related *T. asinigenitalis* (TA) strain. The specificity was further analyzed using additional TE strains from ATCC, 10 epidemiologically distinct TE strains from the National Veterinary Services Laboratory (NVSL), and 11 non-TE bacteria. Further validation of the multiplex assay will be performed using additional non-TE bacteria and spiked genital swab samples from horses (negative genital swabs spiked with known concentration of reference TE strain).

Results: The singleplex and multiplex dPCR assays demonstrated species-specific detection of only TE targets or strains and differentiated TE from the closely related *T. asinigenitalis* (TA) demonstrating high specificity. Two out of three target-specific oligonucleotide sets showed high efficiency in detecting TE strains. The limit of detection of the singleplex and multiplex dPCR assays was determined to be ≥ 10 genome copy equivalents per microliter using serial dilution of the PACs and reference TE DNA.

Conclusions: The results demonstrate that the two newly designed oligonucleotide sets (primers and probe) allowed species-specific and sensitive detection of *Taylorella equigenitalis*. Two oligonucleotide sets will be used in duplex dPCR assay. We are currently performing validation of duplex dPCR assay using TE strain, non-TE bacterial strains (including TA strains) and genital swab samples from horses. This digital PCR assay will improve surveillance, detection and diagnostic strategies for Contagious Equine Metritis caused by *Taylorella equigenitalis* thus providing enhanced biosafety and biosecurity tools for safeguarding national and global equine industry.

Financial Support: The financial support for this project is provided by TTU-SVM Start-up funds and the TTU-SVM Equine Health Research Award 2024.

Notes:

243 - Detections of human seasonal H3N2 influenza A virus reverse-zoonoses in swine during the 2022-2024 influenza season

Michael A. Zeller[1], Daniel Carnevale de Almeida Moraes[2], Giovana Ciacci Zanella[3, 4], Carine K. Souza[2, 4], Tavis K. Anderson[4], Amy L. Baker[4], Phillip C. Gauger[2]

[1]Veterinary Diagnostic Laboratory, Iowa State University, [2]Department of Veterinary Diagnostic & Production Animal Medicine, Iowa State University, [3]Department of Veterinary Microbiology & Preventive Medicine, Iowa State University, [4]National Animal Disease Center, USDA-ARS. mazeller@iastate.edu

Session: Swine influenza, 2025-01-21, 10:30- 10:45 AM

Objective: This study's objective was to investigate the frequency, phylogenetic relationship, and genetic characteristics of human spillover H3N2 influenza A virus detected in swine by the Iowa State University Veterinary Diagnostic Laboratory. Additional investigation was conducted to understand the internal gene constellations and the antigenic phenotype of the virus.

Methods: Samples were submitted from Colorado, Illinois, Indiana, Ohio, Oklahoma, Missouri, Nebraska, North Carolina, and Pennsylvania as well as Chile and Mexico. Samples included 23 oral fluids, 6 nasal swabs, 1 lung, and 1 virus isolate. The spillover H3N2 viruses originated from cocirculating human seasonal H3 lineages. The hemagglutinin gene was sequenced using the Sanger method and classified through Nextclade. Sixteen samples were submitted for additional whole-genome sequencing. Maximum-likelihood phylogenetic inference was employed to understand the relationship of the viruses on a genetic and geographic level.

Results: A total of 31 human-to-swine H3N2 transmissions from November 7, 2022 to May 27, 2024. Sixteen H3N2 detections occurred during the 2022-2023 human influenza season and 15 detections during the 2023-2024 season. During 2022-2023, lineages 3C.2a1b.2a.2a1 and 3C.2a1b.2a.2b were detected. In the 2023-2024 season, 3C.2a1b.2a.2a1 and 3C.2a1b.2a.2b lineages continued to be detected in swine by genetic sequencing, in addition to the 3C.2a1b.2a.2a.3a.1 lineage. Eighteen neuraminidase (NA) genes were sequenced, 11 were derived from the human seasonal H3N2 lineage, while the remaining sequences were mixed contemporary swine NA lineages. Whole-genome sequencing performed on 16 samples yielded 6 human-seasonal genomes and 7 cases had mixed infections with gene segments reflecting the evolutionary origins of endemic swine IAV (TRIG, classical, and/or pandemic lineages). Maximum-likelihood phylogenetic inference indicated multiple independent human-to-swine transmissions occurred ($n \geq 8$). Following spillover, phylogenetic evidence supported the transmission of human seasonal IAV in pig populations in North Carolina and Illinois for at least 12 months. Representative human seasonal spillover virus was antigenically distinct when measured using hemagglutination inhibition assays that included a panel of endemic swine lineages.

Conclusions: Human seasonal H3 continued to spill over into swine at regular intervals with onward transmission occurring between swine. Whole genome sequencing indicates co-circulation and a high likelihood of reassortment between human spillover IAV and endemic swine virus. Further interventions such as appropriate biosecurity practices and swine vaccinations with homologous strains are necessary to limit human IAV spillovers and prevent the endemic establishment of a new swine H3 lineage.

Notes:

244 - Impact of maternal antibodies and weaning on the replication and transmission of human H3N2 Influenza A in piglets

Giovana Ciacci Zanella[1, 2], Matias I. Cardenas[3], Celeste Snyder[1], Carl Hutter[1], Bailey Arruda[1], Meghan Wymore Brand[1], Daniel Perez[3], Tavis K. Anderson[1], Daniela Rajao[3], Amy L. Baker[1]

[1]USDA-ARS, [2]Dept. of Veterinary Microbiology and Preventive Medicine, Iowa State University, [3]Dept. of Population Health, University of Georgia. giovana.zanella@usda.gov

Session: Swine influenza, 2025-01-21, 10:45- 11

Objective: Modern swine production requires human-animal engagement, particularly from farrowing to weaning, and subsequently creates critical points for interspecies transmission of influenza A virus (IAV). Swine vaccination is routinely practiced in the U.S.A., with vaccines applied to sows to transfer maternal derived antibodies (MDA) to their piglets. Weaning is a highly stressful period for piglets, along with increased human interaction. Weaned piglets have a range of immunity depending on the immunity in their dam and efficiency of passive MDA transfer, so piglets may have matched, mismatched, or naïve immune statuses. Our work assessed the effect of matched and mismatched antibodies acquired from vaccinated sows and the stress of weaning on the susceptibility of piglets to a human-origin H3N2 IAV.

Methods: A virus (hu-like H3N2rg) was generated by reverse genetics to contain the HA and NA gene segments from a human seasonal H3N2, and the internal gene segments representative of endemic U.S. swine IAV. The hu-like H3N2rg was previously shown to replicate in the upper respiratory tract of weaned pigs but was mild in clinical disease and relatively lower in transmission efficiency. Sixty-six intranasally challenged seeder piglets were divided into maternal immunity and weaning groups: matched MDA (N=24), mismatched MDA (N=18), or no maternal antibodies (N=24); then further sub-divided into weaned or not weaned. At 2 days post inoculation, sixty-six naïve direct contact pigs were placed with seeders. IAV qRT-PCR and virus titration were performed on nasal swabs and bronchoalveolar lavage to evaluate shedding and transmission kinetics. Hemagglutination inhibition (HI) assays against the hu-like H3N2rg challenge virus were performed to determine antibody titers. Macroscopic and microscopic pneumonia scores, HI titer log2 geometric means, log10 transformed nasal swab, and BALF viral titers were analyzed using analysis of variance (t-tests and ordinary one-way ANOVA with Tukey's post-hoc multiple comparisons test of parameters with statistical differences.

Results: We found that weaned piglets had significantly higher levels of serum cortisol five hours post-weaning than the piglets that remained with the sows ($p=0.0341$). Matched MDA were effective in reducing shedding in challenged pigs on all five days post inoculation ($p < 0.0001$) and minimized the transmission of the human-like H3N2 to contacts. Also, there was an increase in shedding detected in the contact piglets that were placed with weaned pigs. This trend was significant on day 3 post-contact ($p= 0.0231$) and demonstrates that the stress of weaning is an impactful factor on IAV transmission rates between direct contacts. HI data showed that overall weaning did not impact the magnitude of MDA antibody titers at 5 days post inoculation $p > 0.05$.

Conclusions: Increased susceptibility and transmission rate increase the likelihood of a human-seasonal virus establishing in weaned pigs. Therefore, these results identify critical control points in production where changing practices can mitigate human-to-swine and swine-swine human-origin IAV transmission. Practices such as matching sow vaccine to circulating strains, increasing herd immunity prior to farrowing, improving worker policies and biosecurity during the pre- and post-weaning time-periods can prevent the establishment of novel viral lineages and onward transmission.

Financial Support: Funding was provided in part by NIH NIAID CEIRR (#75N93021C00015), National Pork Board and USDA-ARS.

Notes:

245 - Adaptation of a human-origin H3N2 Influenza A virus to pigs restricts receptor specificity for extended glycans and enhances sialic acid binding

Matias Cardenas[1], Pradeep Chopra[2], Brittany Seibert[1], Brianna Cowan[1], L. Claire Gay[1], Flavio Cargnin Faccin[1], C. Joaquin Caceres[1], Geert-Jan Boons[2], Daniel R. Perez[1], Amy L. Baker[3], Tavis K. Anderson[3], Daniel S. Rajao[1]

[1]Department of Population Health, University of Georgia, [2]Complex Carbohydrate Research Center, University of Georgia, [3]National Animal Disease Center, ARS-USDA. m.cardenas@uga.edu

Session: Swine influenza, 2025-01-21, 11 - 11:15

Objective: Influenza A virus (FLUAV) human-to-swine spillovers are common and have favored the emergence of novel FLUAV in pigs. Inactivated vaccines protect against antigenically similar infections by preventing FLUAV replication in the host. Nonetheless, available vaccines normally offer limited protection against antigenically distintic infections, allowing the virus to continue circulating in pigs. This replication favors the emergence of escape variants with reduced antibody recognition, but it remains unknown if it affects virus adaptation to a new host.

Methods: To understand the impact of immunization in the adaptation of a human-origin H3N2 virus to swine, pigs were vaccinated with a commercial inactivated vaccine and then infected intratracheally and intranasally with an antigenically distinct reassortant virus with human H3N2 surface gene segments and swine internal gene segments (hVIC/11). After 3 days, inoculated pigs were removed and new contacts were introduced. This cycle was repeated for a total of 4 contacts. Pigs were euthanized at 6 dpi/days post-contact, and nasal swabs and bronchoalveolar lavage fluid (BALF) were collected.

Results: BALF titers showed an increase of the virus in the lungs by contacts 2, and sequencing of nasal swabs showed the emergence of two major variants in the hemagglutinin (HA) receptor-binding site (RBS) accompanying this increase: the V186G and the F193Y and a single neuraminidase (NA) variant (D113A) while in non-vaccinated pigs only the NA change was observed. The HA variants not only exhibited a statistically significant reduction on antibody recognition compared to the challenge virus but also a reduced pool of glycans supporing FLUAV binding being limited to extended receptors with at leats 3 N-acetyllactosamine (LacNAc) repeats. Further analysis demonstrated that the HA RBS amino acid changes significantly enhanced 2,6-sialic acid (SA) binding affinity. As a result, the D113A NA change restored the HA/NA balance previously disrupted, although this effect was dependent on the HA present.

Conclusions: Together, our results demonstrate that immunization against mismatched strain(s) selected for mutations with reduced antibody recognition and restricted receptor recognition, being limited to extended glycans although a higher affinity for 2,6-SA was observed. The increased receptor affinity was further compensated by the D113A NA change. This data suggests that adaptation of human viruses to pigs could be mediated by 2,6-SA affinity rather than a switch in the receptor specificity of the virus, highlighting a sequential model in which changes in the HA trigger compensatory mutations in the NA.

Financial Support: This research was supported by the Agriculture and Food Research Initiative grant from the USDA National Institute of Food and Agriculture. Funding was also provided, in part, by The National Pork Board.

Notes:

246 - Size distribution and viral load of influenza A virus-laden particles emitted from pigs over the course of infection

Lan Wang[1], José Morán[2], My Yang[1], Bernard Olson[2], Chris Hogan[2], Montserrat Torremorell[1]

[1]Department of Veterinary Population Medicine, University of Minnesota, [2]Department of Mechanical Engineering, University of Minnesota. wang9036@umn.edu

Session: Swine influenza, 2025-01-21, 11:15- 11:30

Objective: Swine influenza is a highly contagious disease of pigs caused by influenza A virus (IAV). IAV transmits rapidly, mostly through nose-to-nose contact, contaminated fomites and air. Despite plenty of evidence that IAV can be transmitted through air, there is limited information on the dynamics of airborne IAV emission over the course of infection and the particle size distribution that IAV associates with. Particle size determines how far airborne particles travel, the deposition site in the respiratory tract and infectivity of virus-laden particles. Therefore, understanding the dynamics of size distribution of virus-laden particles is essential to better understand viral airborne transmission and to establish more effective control measures. In this study, we quantified nasal shedding from pigs experimentally infected with a swine-origin H1N1 virus, and characterized the size distribution of virus-laden particles emitted from pigs into the air over the course of infection.

Methods: Four H1N1 IAV inoculated pigs were placed in two connected isolators (2 pigs per isolator) upstream of a four feet long air-permeable tunnel. Two uninoculated sentinel pigs were placed into another isolator downstream of the tunnel at 1 day post inoculation (DPI). The airflow moved at 51 m^3/h unidirectionally from inoculated to sentinel pigs. Sentinel and inoculated pigs were sampled daily by collecting nasal swabs from 0 to 6 DPI. Air samples were collected daily from upstream and downstream isolators in duplicate using two Andersen cascade impactors (ACI). The two ACIs operated simultaneously at 90 L/m for 30 min collecting particles ranging from 0.22 to > 8 μm. Samples were tested by RT-qPCR to quantify viral RNA.

Results: Inoculated pigs shed IAV consistently from 1 DPI until the end of study at 6 DPI. Sentinel pigs shed IAV at 2 DPI after being exposed to aerosols from inoculated pigs, indicating IAV was transmitted through air shortly after infection of the inoculated pigs. Viral RNA was detected in airborne particles emitted from inoculated pigs as early as 1 DPI, ranging from 0.22 to > 8 μm. The viral load from 1 DPI to 6 DPI was ranging from $1.9*10^4$ RNA copies/m3 (6 DPI) to $3.3*10^5$ RNA copies/m3 (2 DPI), with the highest viral load found in the larger particles (> 6.5 μm) and the lowest viral load in particles between 0.22 μm and 1 μm. Over the course of infection, the amount of airborne IAV emitted by infected pigs in particles > 8 μm was significantly higher than those in particles < 1.7 μm.

Conclusions: Under the conditions of this study, a swine-origin H1N1 IAV could be readily transmitted in pigs through the air, with higher viral load found in larger particles over the course of infection.

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:

247 - Emerging swine influenza virus circulation in Korea challenges the protective efficiency of current SIV vaccines

Yeojin Shin[1], Do Hai Quynh[1], Minjoo Yeom[1], Hyoun-il Kim[2], Seongho Shin[3], Eunseo Bae[4], Woonsung Na[5], Daesub Song[1]

[1]Department of Virology, Seoul National University, [2]Optipharm Inc., South Korea, [3]Optipharm Animal Disease Diagnostic Center, South Korea, [4]Dental Research Institute, Seoul National University, [5]Department of Oral Microbiology and Immunology and Dental Research Institute, Seoul National University. yjshin530@snu.ac.kr

Session: Swine influenza, 2025-01-21, 11:30- 11:45

Objective: The aim of this study is to evaluate the protective efficiency of preexisting swine influenza vaccine against the recent circulating strains in Korea.

Methods: A total of 45 positive samples from domestic swine were collected from different regions in South Korea in 2023 and viruses were propagated in the allantoic cavity of 10-day-old embryonated chicken eggs. Eight viral fragments were amplified by segment specific primers followed by sequencing using an Illumina MiSeq platform. The obtained sequences from novel strains and those of vaccine strains were used to generate the segment phylogenetic trees using Mega-X(v.10.2.6). Novel isolated viruses were tested with HI test for cross reactivity with mouse antisera against vaccine strains in Korea. Three-week-old female SPF swine challenged intranasally with a 105.5 TCID50/ml of #14 strain A/swine/South Korea/14/2023(H1N2) and followed-up for 10 days. Contact group are cohoused day 1 post infection of challenge group.

Results: Overall, 3/45(6.6%) of the isolates were obtained, different two subtypes: 1 H1N1, 2 H1N2 subtypes in 2023. We additionally selected A/swine/South Korea/Hoam2/2018(H3N2) as a candidate for a trivalent vaccine. Phylogenetic trees showed that the HA genes of three distinct isolates and Korean H1 strains after 2020 clustered within 1C Eurasian avian-like lineage. Additionally, H3 Korean strains after 2020, including A/swine/South Korea/Hoam2/2018(H3N2), were grouped within cluster IV.Low amino acid homology(66%-73%) was present in the epitope region of the HA gene between the novel SIV isolates and vaccine strains. Mouse antibodies induced by infection with vaccine strains did not cross-react with three subtypes. Challenge and contact groups of #14 strain exhibited efficient nasal viral shedding, contact transmission, and multifocal lesions were detected in the lungs, with coalescing purple-red consolidation indicative of severe pneumonia.

Conclusions: The genetics and antigenicity of the current circulating SIVs strains in Korea are distantly related to the vaccine strains. Therefore, is it necessary to develop new vaccine candidate to better respond to the emerging SIV.

Financial Support: This paper was partially supported by the South Korea National Institute of Wildlife Disease Control and Prevention as part of “Specialized Graduate School Support Project for Wildlife Disease Specialists” Program. Additional funding was provided by the Natural Products Research Institute.

Notes:

248 - Detection of distinct hemagglutinin and neuraminidase Influenza lineages in single pigs across production stages

Joaquin Alvarez-Norambuena[1], Chong Li[1], My Yang[1], Marie Culhane[1], Montserrat Torremorell[1]

[1]Univesity of Minnesota. jalvare@umn.edu

Session: Swine influenza, 2025-01-21, 11:45- 12:00

Objective: Influenza A virus (IAV) is a common respiratory pathogen of swine. IAV infections with expansion of genetic diversity represent a threat to animal and public health due to mutation and reassort. Reassortment occurs when IAV with distinct genes infect the same cell and exchange gene segments evolving to new viral genotypes with distinct gene constellations. Little is known about farm management factors impacting reassortment. In this study, we aim to describe the level of IAV genetic diversity focusing on documenting the number and lineage of distinct hemagglutinin and neuraminidase gene segments found in individual pigs and production flows as a first step to evaluate factors involved in the emergence of IAV reassortment under field conditions.

Methods: We identified three production flows 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 weaned groups having pigs commingled from all three sow herds, and one group having pigs from two sow herds. Nasal swabs were collected from sixty pigs at approximately 3, 5, 8 and 16 weeks of age, with 240 pigs per cohort totaling 720 pigs total. Samples were confirmed by IAV matrix gene RT-qPCR. A subset of 94 RT-qPCR positive samples were selected for viral plaque purification assays to isolate individual virions. After isolation, plaques were propagated, amplified using universal IAV primers and submitted to the University of Minnesota Genomic Center for whole-genome sequencing. The sequence data was trimmed, and de novo assembled and aligned using sequences from OctoFLU. After alignment, phylogenetic trees were conducted for HA and NA segments using maximum likelihood methods in IQtree, and the HA and NA gene segments were subclassified into lineages based on different clades and clusters.

Results: A total of 56 samples were plaque purified yielding 409 plaques. Out of these, we obtained 395 HA sequences and 397 NA sequences. Five distinct HA lineages were documented in the breeding herd and the nursery site while one lineage was documented in the finishing pigs. Lineage H1A.1.1.3 was the most common lineage in the breeding herd and nursery while H1 lineage 1A.3.3.3-3c was the only lineage identified in the finishing pigs. There was three distinct NA lineages documented in the breeding herd and the nursery pigs, and only one lineage documented in the finishing pigs. N2 lineage 1998B was the most detected lineage in the sow farm, and N1 lineage C.3.2 was the only lineage noted in the finishing pigs. There were 23 samples (41.07%) with two or more HA lineages, and 19 samples (33.93%) with two or more NA lineages.

Conclusions: Our study provides evidence of multiple HA and NA gene segments at the individual pig level and across multiple production stages. Our results indicate the dynamic nature of IAV lineages in a population, with some being able to transmit and persist throughout all production stages, while others remain at low prevalence. Our results further contribute to the understanding of IAV diversity in pigs.

Financial Support: This project was supported by the USDA National Institute of Food and Agriculture Grant no. 2022-67015-36660.

Notes:

249 - Zinc finger antiviral protein affects Zika, Japanese encephalitis, and West Nile virus infection phenotypes

N.P. Khanh Le[1], P. P. Singh[1, 2], Uladzimir Karniychuk[1, 2]

[1]Department of Veterinary Biosciences, The Ohio State University, [2]School of Public Health, University of Saskatchewan, Canada. zdk427@mail.usask.ca

Session: Virology 3, 2025-01-21, 10:30- 10:45

Objective: The zinc finger CCCH-type antiviral protein 1 (ZAP), also known as ZC3HAV1, or PARP13, is a cellular protein. ZAP is expressed in at least 28 tested human tissues as well as mouse, pig, bird, and other vertebrate cells. ZAP exhibits broad antiviral activity, but its interactions with flaviviruses remain unclear. Flaviviruses pose significant global threats to humans and livestock. For example, in addition to the 2016 epidemic in humans, Zika virus (ZIKV) persists in swine herds in Mexico. Pigs are the primary amplifying zoonotic host for the Japanese encephalitis virus (JEV). JEV can also cause abortions in pregnant pigs and disease in piglets. West Nile virus (WNV) has resulted in substantial morbidity and mortality in birds, horses, and humans worldwide. Thus, we studied the role of endogenous ZAP in ZIKV, JEV, and WNV infections.

Methods: We performed immunohistochemistry, western blot, RT-qPCR, and TCID50 assays to compare the infection phenotypes of ZIKV, JEV, and WNV in wildtype (ZAP-WT) and ZAP knockout (ZAP-KO) monkey Vero cells. To explore ZAP-mediated indirect antiviral effects against ZIKV, we analyzed global cellular gene expression using RNA-seq in mock and infected ZAP-WT and ZAP-KO cells at 24 and 72 hours post-infection. Additionally, we employed the Comprehensive Identification of RNA-binding Proteins by Mass Spectrometry (ChIRP-MS) to identify "ZAP-independent" and "ZAP-dependent" interactomes associated with ZIKV RNA. We used GraphPad PRISM 9 software with unpaired t-test or one-way ANOVA multiple comparisons for statistical analysis.

Results: Quantitative immunohistochemistry revealed a significant reduction in ZIKV, JEV, and WNV envelope (E) protein expression in ZAP-WT compared to ZAP-KO cells ($n=9$, $P<0.05$). Western blot analysis showed that ZAP significantly decreased the expression of the NS5 protein for all three viruses ($n=2$). RT-qPCR and TCID50 assays indicated significantly higher ZIKV RNA loads and infectious titers in supernatants from ZAP-KO cells compared to ZAP-WT cells at 24, 48, and 72 hours post-inoculation ($n=4$, $P<0.05$). At 72 hours post-inoculation, ZAP-KO cells exhibited significantly higher intracellular ZIKV RNA loads than ZAP-WT cells ($n=6$, $P<0.01$). Comparative RNA-seq analysis demonstrated that ZAP influences global gene expression in ZIKV-infected Vero cells, enhancing antiviral transcriptional responses. The ChIRP-MS analysis identified 209 unique proteins interacting with ZIKV RNA exclusively in ZAP-positive cells; these interactions were absent in ZAP-KO cells, indicating a "ZAP-dependent" ZIKV RNA interactome.

Conclusions: Endogenous ZAP is associated with altered infection phenotypes of ZIKV, JEV, and WNV. ZAP also enhances transcriptional responses against ZIKV and modifies the ZIKV RNA interactome with cellular proteins. Further comprehensive studies are necessary to fully elucidate the complexities of ZAP-mediated antiviral activity and its potential implications for combating flavivirus infections.

Notes:

250 - Susceptibility assessment of intestinal organoids from North American big brown bat to swine enteric coronavirus

N.C. Twu[1], R.K. Nelli[1], J.C. Mora-Diaz[1], L. Yen[1], A. Saxena[1], R.M. Ruden[1], P. Sitthicharoenchai[2], L. Gimenez-Lirola[1]

[1]Department of Veterinary Diagnostic and Production Animal Medicine, Iowa State University, [2]Department of Population Health and Pathobiology, North Carolina State University. nctwu@iastate.edu

Session: Virology 3, 2025-01-21, 10:45 – 11:00

Objective: In North America, the big brown bat (Eptesicus fuscus) is one of the abundant and widely distributed bat species with frequent human and animal encounters. Bats are considered reservoirs for several zoonotic coronaviruses (CoVs), including SARS-like-CoVs and MERS-CoV, making their susceptibility to various CoVs critical to understand. Some swine coronaviruses, for example, porcine epidemic diarrhea virus (PEDV) and swine acute diarrhea syndrome coronavirus (SADS-CoV) are believed to have bat origins. However, research on bat susceptibility to swine CoVs is limited due to their asymptomatic nature, ethical and ecological concerns, maintenance challenges, and the lack of commercial reagents for bats. This study developed an in vitro model using intestinal organoids (enteroids) from Eptesicus fuscus to evaluate susceptibility to PEDV, transmissible gastroenteritis virus (TGEV), and porcine deltacoronavirus (PDCoV).

Methods: Small intestine tissues were collected from 3 big brown bats, and crypt cells were isolated and cultured in Matrigel to develop differentiated enteroids, termed BEMCs. The BEMCs were dissociated and seeded in transwell inserts (BETCs) to form polarized epithelium. The cell phenotypes of bat small intestine tissue, BEMCs, and BETCs were characterized via immunohistochemistry (IHC) and gene expression analysis, including CoV receptors. BETCs were inoculated with PEDV (USA/Colorado/2013), TGEV (Purdue and Miller strains), and PDCoV (Michigan/8977/2014) with/without trypsin and observed for 48 h post-inoculation (hpi). Susceptibility to these CoVs was assessed by observing cytopathic effects (CPE), detecting viral nucleocapsid protein (NP) in infected cells via immunofluorescence (IFA), and quantifying viral NP RNA in supernatants, cell pellets, and subnatants using RT-qPCR.

Results: Isolated crypt cells were cultured into enteroids and able to subculture for over 10 passages. BEMCs displayed crypt and villus domains with a central lumen. Dissociated BEMCs cells were seeded in transwell inserts, attached within a day, grew into a monolayer, and became confluent between 5-7 days. The morphology of BEMCs and BETCs mirrored that of the small intestine epithelium. Goblet cells secreting mucus were identified by periodic-acid-Schiff Alcian blue (PAS-AB) staining. IHC and gene expression analysis confirmed that BEMCs and BETCs share identical cell phenotypes with bat small intestine tissue and express CoV receptors. BETCs inoculated with PEDV, TGEV Purdue/Miller, and PDCoV supplemented with trypsin exhibited moderate to severe degrees of classic CPE, such as cell rounding, cytoplasmic stranding, and detachment, from 24 to 48 hpi. In contrast, in the non-trypsin-supplemented inoculation groups, mild CPE was observed in PEDV and TGEV Purdue/Miller inoculated BETCs only at 48 hpi, and no CPE was observed in PDCoV inoculated BETCs. Viral NP of PEDV, TGEV, and PDCoV were detected in infected BETCs by IFA. Virus-related fluorescence intensity was higher in trypsin-supplemented inoculation groups for all viruses. RT-qPCR confirmed viral RNA in supernatants, cell pellets, and supernatants of BETCs.

Conclusions: This study developed enteroid cultures from big brown bats and demonstrated their trypsin-independent susceptibility to swine enteric CoVs (PEDV, TGEV, and PDCoV). This in vitro model is valuable for investigating viral pathogenesis and potential cross-species transmission between bats and other animals.

Notes:

251 - Equine arteritis virus hijacks the CXCL16/CXCR6 axis to infect Equine CD3+ T lymphocytes

Come J. M. Thieulent[1], Mariano Carossino[1], Udeni B. R. Balasuriya[1]

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

Session: Virology 3, 2025-01-21, 11:00 - 11:15

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 (CXCL16S) was previously identified as a cell entry receptor for EAV. Stallions with a CXCL16S allele (homozygote for CXCL16S [CXCL16S/S], and heterozygote for CXCL16 [CXCL16S/r]) are more likely to become LTPI and have a subpopulation of CD3+ T lymphocytes susceptible to EAV infection in vitro, while stallions with only the CXCL16R allele (homozygote for CXCL16R [CXCL16r/r]) are not. CXCR6 is the receptor for the chemokine CXCL16, which exists as a transmembrane (tmCXCL16) or soluble form (sCXCL16). The role of the CXCL16/CXCR6 axis in EAV infection remains unclear. The objective of this work was to further elucidate the role of the CXCL16/CXCR6 axis in the infection of CD3+ T lymphocytes by EAV.

Methods: Peripheral blood mononuclear cells (PBMCs) collected from healthy horses (CXCL16S/S [n=6]; CXCL16S/r [n=6]; CXCL16r/r [n=6]) and HEK-293T cells expressing equine CXCL16S and CXCL16R were used in this study. Virological and classic cell culture (e.g., cell transfection, immunofluorescence, plaque assays), fluorescence-activated cell sorting (FACS), and single-cell RNA sequencing (scRNA-seq) were used to evaluate the role of CXCL16 and CXCR6 in CD3+ T lymphocyte susceptibility to EAV infection.

Results: Purified equine CD3+ T lymphocytes are resistant to EAV infection, while equine CD3+ T lymphocytes in the presence of other mononuclear cells are susceptible to EAV infection in vitro, suggesting that infection of CD3+ T lymphocytes is partly mediated by other cellular factors. The CXCL16-CXCR6 protein-protein interaction has been modeled in silico, and the amino acids involved in this interaction have been identified. Antibodies targeting these amino acids specifically block EAV infection of equine CD3+ T lymphocytes in a dose-dependent manner. A CXCR6 antagonist (ML339) blocks the infection of equine CD3+ T lymphocytes to EAV infection, confirming the role of CXCR6 in EAV infection. scRNA-seq of infected PBMCs with EAV has been performed, and the results are being analyzed. Purified CD3+ T lymphocytes will be co-cultured with HEK-293T cells expressing CXCL16S/CXCL16R or recombinant sCXCL16S/sCXCL16R, and infected with EAV to evaluate if the sCXCL16 may contribute to infection of CD3+ T lymphocytes.

Conclusions: Collectively, our data provide further evidence that both CXCR6 and CXCL16 play a major role in EAV infection of CD3+ T lymphocytes. This data supports our hypothesis that EAV infection of CD3+ T lymphocytes is associated with EAV binding to sCXCL16S and that the subsequent interaction between EAV-bound sCXCL16S and CXCR6 expressed on the surface of equine CD3+ T lymphocytes facilitates viral entry. Experiments are still in progress to confirm this hypothesis.

Financial Support: 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 National Institute of Food and Agriculture to UBRB.

Notes:

252 - Investigating the relationship between the cellular protein ZFP36L1 and autophagy in suppressing Norovirus replication

Malabika Bhowmik[1], Tooba Momin[1], Abiageal Newell[1], Mrigendra Rajput[1]

[1]University of Dayton. bhowmikm1@udayton.edu

Session: Virology 3, 2025-01-21, 11:15 - 11:30

Objective: ZFP36L1 is a cellular RNA-binding protein that belongs to the CCCH-type zinc finger protein family and plays a significant role in host immune responses. The zinc ions in the protein coordinate its structure, enabling ZFP36L1 to interact with mRNA and regulate its turnover within cells. The ability of ZFP36L1 to bind RNA provides antiviral activity against a broad range of viruses, including Japanese encephalitis virus (JEV), dengue virus (DENV), influenza A virus (IAV), human coronavirus (HCoV), and murine norovirus-1 (MNoV-1). Autophagy is a powerful innate response tool that host cells use to defend against viral infection. However, viruses too have acquired the potent ability to hijack and subvert autophagy for their benefit. Our preliminary study suggested a relationship between ZFP36L1 and autophagy. The current study is carried out to determine the interplay between ZFP36L1 and autophagy in suppressing MNoV-1 replication.

Methods: To measure the effect of autophagy on MNoV-1, RAW 264.7 cells were treated with either an autophagy-suppressing drug, 3-methyladenine, or an autophagy-enhancing drug, rapamycin while mock-treated cells were used as control. After infection with MNoV-1, virus titer in those cells was measured using TCID50. Similarly, to assess the effect of ZFP36L1 on MNoV-1 replication, wild-type, stably overexpressed or ZFP36L1 knockdown RAW 264.7 cells were infected with MNoV-1, virus titer in those cells was measured using TCID50. To determine the relationship between ZFP36L1 and autophagy, mock-treated HEK cells or cells treated with either an autophagy-suppressing drug, 3-methyladenine, or an autophagy-enhancing drug, rapamycin was analysed for ZFP36L1 expression using qPCR and western blot. Similarly, wild-type, stably overexpressed or ZFP36L1 knockdown HEK cells were analysed for autophagy induction by measuring the expression of autophagy marker, light chain 3B (LC3B) using qPCR and western blot.

Results: Our results showed that ZFP36L1 overexpression or autophagy suppression significantly reduced MNoV-1 titer. While knockdown of ZFP36L1 or increased autophagy significantly enhanced MNoV-1 titer. The qPCR and western blot results reveal an inverse relationship between ZFP36L1 and autophagy.

Conclusions: The current study shows an inverse relationship between cellular ZFP36L1 expression and the induction of autophagy. Elevated levels of ZFP36L1 were found to suppress both autophagy and MNoV-1 replication, suggesting that ZFP36L1 plays a significant role in modulating cellular autophagy to inhibit MNoV-1 replication. Further research is needed to better understand the mechanisms by which ZFP36L1 regulates autophagy.

Financial Support: Research is supported by University of Dayton, Department of Biology fund provided to Dr Mrigendra Rajput

Notes:

253 - ZFP36L1 suppresses virus replication beyond poly(a) tail deadenylation

Tooba Momin[1], Malabika Bhowmik[1], Abiageal Newell[1], Mrigendra Rajput[1]

[1]University of Dayton. momint1@udayton.edu

Session: Virology 3, 2025-01-21, 11:30- 11:45

Objective: Among microbes, RNA viruses have a very high mutation rate. This, combined with a unique genetic composition, enhances their ability to survive and increases their potential for cross-species transmission. These characteristics make RNA viruses strong candidates as etiological agents for future pandemics. Therefore, a strategy that targets the conserved replication cycle of RNA viruses could be a promising approach to suppress viral titers and reduce viral shedding from infected individuals, including both animals and humans.ZFP36L1, a CCCH-type zinc finger protein that controls mRNA turnover in cells, could offer such a strategy. ZFP36L1 has shown its antiviral activity against various RNA viruses, including flaviviruses, retroviruses, and alphaviruses. Our preliminary investigations have shown that ZFP36L1 reduces replication of the human coronavirus OC43 (HCoV-OC43). This study aims to further explore the mechanisms by which ZFP36L1 suppresses viral replication.

Methods: Wild-type, stably overexpressed or ZFP36L1 knockdown HCT-8 cells were infected with HCoV-OC43 and analyzed for virus titer, virus-induced cytopathic effect, and individual viral transcripts by qPCR. To investigate whether ZFP36L1 suppressed viral replication through a poly(A) tail deadenylation mechanism, CNOT1, a key regulator of mRNA decay, was knocked down in ZFP36L1-overexpressing cells, and the impact of CNOT1 knockdown on viral titer was measured. Each testing program was repeated three times with three replicates, and results were analyzed by paired T-test and one-way ANOVA at a 95% level of significance. A p-value less than 0.05 was considered statistically significant.Computational analysis, including RNA-Protein Interaction Prediction (RPISeq) software and homology modeling, were performed to predict potential interaction between ZFP36L1 and viral genome. Results of the computational predictions were validated through RNA immunoprecipitation assay while functional analysis of ZFP36L1 and viral genome interaction was confirmed by luciferase reporter assays.

Results: ZFP36L1 overexpression significantly reduced the titer of HCoV-OC43, while its knockdown resulted in increased viral titers compared to virus-infected wild-type cells ($p<0.05$). Knockdown of CNOT1 in ZFP36L1-overexpressing cells did not reverse the ZFP36L1-mediated suppression of viral titers, indicating that the reduction of HCoV-OC43 titers by ZFP36L1 was independent of poly(A) tail deadenylation. Computational analyses predicted a strong interaction between ZFP36L1 and the HCoV-OC43 nucleocapsid protein, which was confirmed by RNA immunoprecipitation assays. Additionally, luciferase assays demonstrated that the interaction between ZFP36L1 and the nucleocapsid protein suppressed viral genome replication.

Conclusions: Our findings showed that ZFP36L1 plays a critical role in suppressing HCoV-OC43 replication, primarily through its strong interaction with the viral nucleocapsid protein, which inhibits viral replication. This study uncovers an additional mechanism of ZFP36L1-mediated viral suppression. It was previously known that ZFP36L1 inhibits viral replication either through poly(A) tail/3' UTR deadenylation or by reducing viral protein translation and viral protein trafficking.While our current study focused on HCoV-OC43, ZFP36L1's role in viral suppression may extend to other coronaviruses, such as Transmissible Gastroenteritis Virus (TGEV), Porcine Epidemic Diarrhea Virus (PEDV), and human viruses like Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) and SARS-CoV-2, which will be explored in future studies.

Financial Support: Research is supported by University of Dayton, Department of Biology fund provided to Dr. Mrigendra Rajput.

Notes:

254 - Chronic hepatitis in horses with equine hepacivirus infection

Mason C. Jager[1], Daniela Luethy[2], Thomas J. Divers[1], Gerlinde R. Van de Walle[1], Joy E. Tomlinson[2]

[1]Cornell University College of Veterinary Medicine, [2]University of Pennsylvania - New Bolton Center. jet371@gmail.com

Session: Virology 3, 2025-01-21, 11:45– 12:00

Objective: Equine hepacivirus (EqHV) is closely related to hepatitis C virus (HCV), which causes persistent infection and chronic hepatitis in people. EqHV causes subclinical hepatitis during acute resolving infection, however, there is limited information on hepatitis associated with chronic infection. We report 28 clinical cases of chronic hepatitis in horses infected with EqHV.

Methods: Mixed retrospective and prospective case series. Horses presented with the following inclusion criteria: 1) chronic hepatitis, defined as at least one month duration of elevated serum liver biomarkers and/or elevated serum liver biomarkers with findings of chronicity on liver histopathology, such as fibrosis; 2) serum or liver EqHV RT-qPCR positive; and 3) liver histopathology performed. Liver biopsies were independently reviewed scored by 16 individual features.

Results: Twenty-eight horses met inclusion criteria. Two horses had acute resolving infections and bacterial cholangiohepatitis. Eight horses died within 5 months and persistent infection could not be verified. Eighteen horses had persistent hepaciviral infection of 35 months follow-up. These 18 horses were median 16 (range, 5-24) years old, 6 mares and 12 geldings, and all light breeds. Median duration of documented hepatitis was 18 (5-120) months with median duration of documented EqHV viremia of 14 (5-42) months. Predominant histopathologic findings were lymphocytic inflammation and nodules, bridging and dissecting fibrosis, and individual hepatocyte necrosis.

Conclusions: The definitive cause of hepatitis in these horses cannot be determined. The similarities between these cases and HCV suggest it is likely that EqHV causes chronic hepatitis and liver failure in horses.

Financial Support: National Institutes of Health, National Institute of Allergy and Infectious Diseases K08AI141767; U.S. Department of Agriculture, National Institute of Food and Agriculture 2022-67015-36343

Notes:

255 - Heterologous prime-boost immunization of two-component PWDVax protected weaned pigs against F18 enterotoxigenic *Escherichia coli* (ETEC) post-weaning diarrhea (PWD)

Chongyang Zhang[1], Siqi Li[1], Ipshita Upadhyay[1], Sai Simha Reddy Vakamalla[1], Kathryn L. Lauder[1], Weiping Zhang[1], Chance Hansen[1], Courtney Hayes[2], Kristen Ann Massey[2], Nicole L. Herndon[3]

[1]Department of Pathobiology, University of Illinois at Urbana Champaign, [2]Department of Clinical Veterinary Medicine, University of Illinois at Urbana Champaign, [3]Division of Animal Resource, University of Illinois at Urbana Champaign. zhang405@illinois.edu

Session: Vaccinology 3, 2025-01-21, 10:30- 10:45

Objective: Post-weaning diarrhea (PWD) caused predominantly by enterotoxigenic *Escherichia coli* (ETEC) continuously causes significant economic losses to swine producers worldwide. Currently, there are no effective countermeasures against this major swine disease. The vaccine is considered practical and cost-effective, but developing effective PWD vaccines has been difficult historically. Since ETEC bacteria causing PWD produce F4 (K88) or F18 fimbria and various combinations of enterotoxins heat-labile toxin (LT), heat-stable toxin type Ib (STa), heat-stable toxin II (STb), and Shiga toxin type 2e (Stx2e; Stx2e also causes edema disease), an effective PWD vaccine would need to induce protective immunity ideally against two fimbriae and four toxins. Based on novel epitope- and structure-based multiepitope-fusion-antigen (MEFA) vaccinology platform, we created an epitope-based polyvalent fimbria-toxin protein (fimbria-toxin MEFA) to precisely target all PWD virulence determinants (fimbriae F4 and F18 and toxins LT, STa, STb, and Stx2e), developed a two-component multivalent PWD vaccine candidate, PWDVax, applied a heterologous prime-boost immunization schedule, and evaluated the efficacy of the vaccine against F18 ETEC-associated PWD.

Methods: In this study, we created a new fimbria-toxin MEFA monomer protein as the acellular component and additionally, live *E. coli* bacteria to express a GM1-binding AB5 holotoxin-structured fimbria-toxin MEFA protein as the cellular component. 28 piglets born to two sows were randomly assigned to the control group and the vaccination group. The vaccination group was first immunized with the fimbria-toxin MEFA monomer protein intramuscularly on day 4 and followed with live *E. coli* bacteria producing GM1-binding holotoxin-structured fimbria-toxin MEFA orally on day 19. The control group was sham-vaccinated with the same amount of phosphate-buffered saline. All pigs were orally inoculated with 2.5 x 109 CFU doses of an ETEC F18 field strain on day 32. Efficacy was assessed by evaluating diarrhea, clinical observations, dry fecal matter percentage and colonization of the challenge bacteria in small intestines. IgG in blood and SIgA in feces and intestines responses to the target fimbriae and toxins were measured.

Results: The vaccination resulted in significant reductions in clinical PWD. When challenged with an F18 ETEC strain, the immunized piglets were protected from 87.5% watery diarrhea and 66.7% any diarrhea. Quantitative colonization based on pig ileum tissue showed that the pig vaccinated with PWDVax had over two logs reduction of F18 ETEC bacterial colonization in small intestines. The levels of antibody results showed a significant elevation of IgG and SIgA antibody responses to target antigens (F4 and F18 fimbriae and LT, STb, STa, and Stx2e toxins). The fecal dry matter from the feces collected from the control pigs was significantly less than that of the vaccinated pigs ($p<0.05$).

Conclusions: Our results suggested PWDVax protects against F18 ETEC-associated PWD and potentially becomes an effective PWD vaccine. The two-component vaccine and heterologous prime-boost immunization strategies can be informative for neonatal vaccine development.

Financial Support: This project was supported by Agriculture and Food Research Initiative Competitive Grant no. 2017-67015-26632 and 2017-67015-31471 from the USDA National Institute of Food and Agriculture.

Notes:

256 - An in vivo-simulated vaccine to prevent bovine respiratory and other diseases due to *Histophilus somni*

Thomas Inzana[1], Dianjun Cao[1], Amelia Woolums[2], Theresa Merrilee[2], Bindu Subhadra[1], Emily Gareri[1], Kelsey Harvey[2]

[1]Long Island University, [2]Mississippi State University. thomas.inzana@liu.edu

Session: Vaccinology, 2025-01-20, 6:00 - 8:00

Objectives: We propose 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. We have shown that the natural growth state of *H. somni* is a biofilm, and that during chronic infection (e.g. BRD, myocarditis, etc.), a biofilm in the host is prevalent. Furthermore, half of the bacterial genome is differentially expressed when the bacteria form a biofilm, compared to bacteria grown planktonically. In addition, iron binding proteins are expressed by bacteria in the host that are not expressed in rich culture medium, and antibodies to such proteins may reduce bacterial growth in vivo. Our current objectives are to develop a vaccine that will mimic the antigenic profile of the bacterium when in the host, and combine the vaccine with a novel adjuvant designed to induce a more protective host immune response with less inflammation. To accomplish our goal our aims are: 1) Produce in vivo-like outer membrane vesicles (ivOMV) and biofilm matrix (BM) material containing antigens expressed by the bacteria in the host; 2. Immunize calves with the ivOMV-BM vaccine to assess the vaccine's capability to induce humoral and cellular immunity.

Methods: ivOMVs were prepared by growing the bacteria in broth medium, then adding EDDHA (ethylenediamine-N,N'-bis((2-hydroxyphenyl)acetic acid) to sequester free iron. After 5 hours incubation the cells were removed by low speed centrifugation, the supernatant filtered, and the OMVs recovered by ultracentrifugation. The BM was obtained from biofilm cultures that were grown for 5 days in a 1 L flask. The bottom 70 mls of biofilm was recovered, vortexed, and the bacterial cells removed by low speed centrifugation. The supernatant was pelleted at 30,000 x *g* for 15 min, and resuspended in phosphate buffered saline. The adjuvant BECC438, which has been previously shown to induce a strong immune response to bacterial antigens, was added. To assess immunogenicity of the vaccine, two groups of calves were immunized with two different doses of ivOMV-BM intramuscularly, and sera and plasma were collected prior to immunization and weekly thereafter for antibody and cytokine analyses, respectively.

Results: Two groups of calves were immunized twice three weeks apart with a low dose and high dose of ivOMV-BM vaccine combined with adjuvant. Sera and plasma were collected weekly for 8 weeks to determine IgM, IgG_1, and IgG_2 titers, and concentrations of IFN-gamma, IL-1-alpha, IL-1-beta, IL-4, IL-6, IL-10, IL-17A, and TNF-alpha, respectively. An early IgM response was noted, which fell off and was followed by an increasing IgG response. The antibody response to OMV was greater than to BM, but both were greater than to a commercial bacterin. Specific antibody classes, subclasses and cytokine responses will be described.

Conclusions: Preliminary results indicated that the ivOMV-BM vaccine is immunogenic, though a stronger response to OMV than BM was noted. Additional immunogenicity tests will be used to confirm the optimal vaccine dose prior to challenge and efficacy studies.

Financial Support: USDA-NIFA Award 2023-67015-39655

Notes:

257 - Intrauterine vaccines using nanoparticles encapsulating antigens and adjuvants to make semen-friendly vaccine formulations

Pooja Choudhary[1], Ramin Mohammadi[2], Donaldson Magloire[1], Siew Hon Ng[1], Kezia R. Fourie[1], Zahed Khatooni[1], Haoming Liu[1], Azita Haddadi[1], Heather L. Wilson[1, 2]

[1]Vaccine and Infectious Disease Organization, University of Saskatchewan, [2]Department of Veterinary Microbiology, University of Saskatchewan. heather.wilson@usask.ca

Session: Vaccinology 3, 2025-01-21, 11 - 11:15

Objective: Our lab has shown that intrauterine (i.u.) immunization administered at breeding can trigger humoral and cell-mediated immunity without impacting sperm function or fertility. To augment the immune response, we encapsulated the antigen and adjuvants (confidential) with polymer-based nanoparticles (NPs). We used a combination of intramuscular (i.m.) and i.u. routes as primary and booster immunizations to assess which response triggers the most robust immune response in gilts.

Methods: NP were used to encapsulate recombinant porcine epidemic diarrhea virus spike protein (PEDVS) as antigen and an proprietary adjuvant (Adj). Gilts (n=3, each group with a repeat cohort trial underway to increase the animal numbers per group) were immunized at 1st estrus and again at 2nd estrus with PEDVS+Adj-NPs as follows: Group 1 =i.m. then i.m. route, Group 2 = i.m. then i.u. routes, Group 3 = i.u. then i.u. routes, and Group 4 received empty NPs at i.m. then i.u. routes. Gilts were euthanized 53 days after the initial vaccine administered at 1st estrus and the crown/rump sizes were measured as were the number of viable and non-viable fetuses. Blood samples were collected at 0, 20 and 53 to quantify PEDVS-specific immunoglobulin G (IgG), and neutralizing IgG titers, and IFN-γ secretion from peripheral blood mononuclear cells (PBMC) T-cells. Intestinal and uterine tissues were harvested on day 53 post primary vaccination, and mucosal PEDVS-specific IgG and IgA were quantified.

Results: Gilts immunized by the i.m./i.m. routes and i.m./i.u. routes with PEDVS+Adj-NP showed comparable PEDVS-specific IgG antibodies in serum that were significantly higher than titres from the control gilts from day 53 serum. Gilts immunized by the i.m./i.u. routes showed significantly higher PEDVS-specific IgG antibodies in the jejunum and the uterus and significantly higher serum neutralizing antibodies relative to the titres from control gilts. Gilts immunized by the i.m./i.u. routes showed significantly higher antigen-specific IFN-γ production from the PBMCs relative to the IFN-γ titres from the control gilts. The gilts immunized via the i.u./i.u. routes failed to show induction of PEDVS-specific humoral or cell-mediated immunity and all groups responded with low mucosal IgA titres. The NPs did not appear to impact fertility as the number of live/still born fetuses and the crown/rump ratios were comparable across groups.

Conclusions: The NPs successfully encapsulated the antigen and the Adj such that the latter did not impact sperm function or fertility. The i.u. vaccine administered as booster to the priming i.m. vaccine succeeded in inducing significantly elevated antigen-specific antibodies in serum and mucosal tissues, neutralizing antibodies in the serum and T cell immunity. The primary i.u. vaccine and booster i.u. vaccine failed to trigger immune responses suggesting that the priming i.m. vaccine was needed. We envision gilts reviewing an i.m. vaccine during sorting at 1st estrus followed by i.u. vaccination combined with the semen at breeding will be an attractive route of immunization amenable to current husbandry practices.

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

Notes:

258 - Vaccination with a *Lawsonia intracellularis* vaccine mitigated some disease parameters but failed to affect shedding

Alison Jeffery[1], Kezia R. Fourie[1, 2,] Dylan Chand[1], Pooja Choudhary[1], Siew Hon Ng[1], Haoming Liu[1, 2], Donaldson Magloire[1, 2], Zahed Khatooni[1], Emil Berberov[1], Heather L. Wilson[1, 2]

[1]Vaccine and Infectious Disease Organization, University of Saskatchewan, [2]Department of Veterinary Microbiology, University of Saskatchewan. heather.wilson@usask.ca

Session: Vaccinology 3, 2025-01-21, 11:15- 11:30

Objective: *Lawsonia intracellularis* (LI) is an economically important bacterium that is the causative agent of ileitis in pigs. In developing a subunit vaccine, we have focused on different immunization routes and adjuvants. In this study we performed a challenge study to measure the efficacy of two immunogenic formulations that may aid in protecting animals against disease.

Methods: A challenge trial was undergone to measure immunogenicity and protection against disease. Group 1 and Group 2 piglets (n=20 each) were vaccinated one of two vaccines, where each contained three recombinant L. intracellularis proteins; F, G, and Y and one of two proprietary adjuvants; A1 or A2. Group 3 piglets (n=20) were mock vaccinated with saline. Vaccination or mock vaccination of all groups occurred on days 0 and 21. On day 42, all animals were challenged with L. intracellularis, and on day 63 all animals were necropsied. To measure immunogenicity, serum and mucosal antibody responses were assessed. To determine protection, average daily weight gain (ADWG), bacterial shedding in feces and gross lesions were evaluated.

Results: Both vaccines induced significant systemic humoral immune responses after vaccination but weak mucosal responses. Group 3 animals had lower ADG than Group 2 animals. Group 1 animals had significantly lower quantities of the pathogen in feces than any other group. Group 1 animals had significantly lower gross lesion scores and gross lesion lengths than other groups.

Conclusions: Currently, there is no subunit vaccine for L. intracellularis. Here, vaccination with a subunit L. intracellularis vaccine comprised of three recombinant antigens determined that the vaccine was immunogenic. More importantly, formulation with adjuvant A2 provided some protection as evidenced by some mitigation of disease. This formula is promising and should be studied further.

Financial Support: The Governments of Saskatchewan and Canada contributed funding under the Canadian Agricultural Partnership. VIDO receives funding from the Government of Saskatchewan through Innovation Saskatchewan, the Ministry of Agriculture & the Canada Foundation for Innovation through the Major Science Initiatives Fund.

Notes:

259 - Engineering a recombination-resistant live attenuated vaccine candidate for Porcine Epidemic Diarrhea Virus

Mingde Liu[1, 2], Bikash Aryal[1], Xiaoyu Niu[1, 2], Qiuhong Wang[1, 2]

[1]Department of Animal Sciences, The Ohio State University, [2]Department of Veterinary Preventive Medicine, The Ohio State University. liu.6202@buckeyemail.osu.edu

Session: Vaccinology 3, 2025-01-21, 11:30- 11:45

Objective: Porcine epidemic diarrhea virus (PEDV) is a lethal coronavirus for neonatal piglets. Because newborn piglets lack sufficient time to generate protective immunity against PEDV, they rely on passive immunity via the colostrum and milk from immunized sows/gilts. Currently, feedback method for priming pregnant sows/gilts followed by boosting with the commercially available killed or subunit vaccines is used on farms for PEDV control and prevention. However, feedback approach poses a risk of transmitting other pathogens. There is an urgent need for effective and safe live attenuated vaccines (LAVs) to replace feedback material, but none is available. However, safety is the biggest concern for LAVs because vaccine strains can contribute to the emergence of new variants via recombination and mutation, leading to vaccination failure and complicating PEDV surveillance. We aimed to develop recombination-resistant, efficacious PEDV LAV candidates carrying attenuation mutations in multiple genes.

Methods: We generated RMTv1 by optimizing our previously reported recombination-resistant PEDV mutant RMT that carries re-modeled transcriptional regulatory sequence-core sequences (TRS-CSs). Three additional PEDV mutants (RMTv1-nsp1+nsp15, RMTv1-nsp1+nsp16, and RMTv1-nsp15+nsp16) were engineered by introducing combinations of mutations in nsp1, nsp15, and/or nsp16 into the RMTv1 backbone. The replication efficiency of these mutants was evaluated in Vero and IFN-sufficient LLC-PK1 cells. Viral genetic stability and sensitivity to IFNs were assessed in Vero cells. Type I and type III IFN induction was measured in LLC-PK1 cells. Neonatal gnotobiotic piglets were orally inoculated with RMTv1 and RMTv1-nsp1+nsp15 mutant and challenged with virulent PEDV at 21 days post-inoculation to examine their virulence and immunogenicity.

Results: The RMTv1 backbone and three mutants were successfully rescued. All three mutants had an unexpected single nucleotide mutation/deletion in the 5'-UTR TRS region. One similar mutation was also detected in the passage 10 of RMTv1. In Vero cells, RMTv1 exhibited reduced replication efficiency and formed smaller plaques than wild-type (WT) PEDV. RMTv1-nsp1+nsp15 and RMTv1-nsp15+nsp16 replicated similarly to RMTv1, whereas RMTv1-nsp1+nsp16 showed enhanced replication. In LLC-PK1 cells, RMTv1 exhibited reduced replication than WT PEDV and all three mutants displayed even lower replication efficiency. Because RMTv1-nsp1+ nsp 15 demonstrated the highest genetic stability after passaging 10 times in Vero cells among the three mutants, we further evaluated it. RMTv1 and RMTv1-nsp1+15 induced similar but significantly higher levels of IFNs than WT PEDV. Also, the RMTv1-nsp1+nsp15 was most sensitive to IFN-β treatment, followed by RMT-V1, then by WT PEDV. In gnotobiotic piglets, RMTv1 and RMTv1-nsp1+15 showed attenuation compared with the WT PEDV. RMTv1-nsp1+15 exhibited a 0% mortality rate. Post-challenge, none of the RMTv1- or RMTv1-nsp1+15-inoculated piglets developed severe diarrhea, while 50% of the mock-challenge group pigs died. Additionally, similar serum virus-neutralizing antibodies were elicited in the pigs of RMTv1-nsp1+15 and RMTv1 groups, and the titers were significantly boosted post-challenge.

Conclusions: Generation of a recombination-resistant RMTv1 backbone marks significant progress in PEDV LAV development. Further attenuation was achieved by inactivating interferon antagonists. In this study, RMTv1-nsp1+15 mutant caused no mortality and induced strong protective immunity in neonatal pigs, suggesting it a promising LAV candidate warranting further evaluation in sows/gilts.

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:

260 - Immunological lessons on how to best protect pigs against porcine reproductive and respiratory syndrome virus.

Andrew R. Kick[1], Jessica Proctor[2], Amanda F. Amaral[3], Lizette M. Cortes[4], Juliana Bonin-Ferreira[4], Phillip C. Gauger[5], James M. Hammer[6], Glen W. Almond[4], Jeremy Pittman[7], Elisa Crisci[4], Tobias Kaeser[8]

[1]Department of Chemistry & Life Science, United States Military Academy, [2]BioAgilytix, [3]Department of Population Health and Pathobiology, North Carolina State University, [4]College of Veterinary Medicine, North Carolina State University, [5]Veterinary Diagnostic and Production Animal Medicine, Iowa State University, [6]Elanco Animal Health, [7]Smithfield Foods, [8]Department of Biological Sciences and Pathobiology, University of Veterinary Medicine Vienna, Austria. tobias.kaeser@vetmeduni.ac.at

Session: Vaccinology 3, 2025-01-21, 11:45- 12

Objective: This presentation looks back at six years of our research into how vaccines can contribute to protect pigs against the porcine reproductive and respiratory syndrome virus - PRRSV.

Methods: During this time, we performed PRRSV infection trials with a detailed analysis of the host cellular and humoral immune response. We also performed various vaccination and challenge trials to study maternal immunity and immunity induced in weaners against homo- and heterologous PRRSV strains. We also performed a field study to evaluate the role of PRRSV in the porcine respiratory disease complex.

Results: Our results demonstrate that on the one side, maternal immunity is mainly driven by antibodies, especially the transfer of neutralizing antibodies. Furthermore, autogenous inactivated vaccines can be highly beneficial to induce these neutralizing antibodies against farm-prevalent strains. To protect growing pigs with vaccination at weaning, the systemic CD4 T-cell response is a useful strain-specific correlate of protection. However, in mucosal tissue, cytotoxic T cells seems to strongly contribute to protection and TCR-gamma/delta T cells seem to be active during post-viremic phases.

Conlusions: In conclusion, our data show that every part of adaptive immunity plays a specific role in protecting against PRRSV; and they indicate that timing and the use of appropriate vaccination strategies is crucial to limit the impact of PRRSV on pig production.

Financial Support: This research was sponsored by the North Carolina State University, the North Carolina and Virginia Pork Councils, and Elanco Animal Health Inc.

Notes:

261 - Stochastic modelling of economic risk and net return distributions for feedlot steers marketed at alternative endpoints

Lucas M. Horton[1], Ted C. Schroeder[2], Marshall N. Streeter[3], John P. Hutcheson[3], David G. Renter[1]

[1]Department of Diagnostic Medicine and Pathobiology, Kansas State University, [2]Department of Agricultural Economics, Kansas State University, [3]Merck Animal Health. lhorton@vet.k-state.edu

Session: AVEPM - Schwabe Symposium, 2025-01-20, 5:45 – 6:00

Objective: To assess economic risks associated with extending days-on-feed (DOF) in U.S. feedlot steers by evaluating the distributions of net returns when feeding cattle to heavier, more extreme endpoints (EPs). Later-fed EPs were compared against a contemporary industry standard, focusing on how market conditions and animal performance factors influence net return variability.

Methods: A stochastic simulation model was developed to represent pen-level outcomes for feedlot steers marketed at four EPs, each separated by 14 additional DOF. Endpoint 1 (EP1) aligned with current industry norms, while later EPs (EP2, EP3, EP4) represented progressively extended feeding durations. The model used parameter estimates derived from a recent commercial feedlot trial and industry data (2021 to mid-2024) on fed cattle prices, feed costs, and carcass-based premiums/discounts. By simulating cattle performance, health, carcass characteristics, and market conditions, the model generated net return differences for later EPs compared to EP1. Conditional random forest models were applied to identify variables appearing to be the most influential on net returns.

Results: Feeding steers to later EPs expanded net return distributions, reflecting greater financial uncertainty. Although potential gains existed at longer DOF, the probability of economic losses also increased. Compared to marketing on a live basis, selling on a carcass-based grid accounting for premiums and discounts commonly shifted net returns downward when feeding to later EPs – particularly for EP3 and EP4 – due to discounts from increased heavyweight carcasses, as well as greater proportions of Yield Grade 4 and 5 carcasses. As EPs extended, negative net returns became more frequent for both live and grid marketing scenarios. The single most influential variable on net return differences was the change in fed cattle prices when delaying marketing from EP1 to subsequent EPs, effectively reflecting different prices received when marketing on different weeks. Other key economic drivers included the base fed cattle price, corn price, and – when grid marketing – the Quality Grade grid. The most important non-economic variable was mortality, while all other animal health, performance, and carcass variables had marginal to minimal impact on net return differences from EP1, and were ranked lower than primary economic factors

Conclusions: Decisions to feed cattle beyond traditional industry EPs may be largely governed by market volatility rather than changes in animal performance or carcass attributes. In other words, what may be perceived as the optimal biological EP, may not align with the optimal economic EP. While extending DOF can offer opportunities for improved returns under favorable market conditions, it simultaneously heightens exposure to negative outcomes when conditions deteriorate. Producers should prioritize market signals – particularly fed cattle price trends – over incremental performance changes when considering later marketing. Effective risk management and flexible marketing strategies are essential to navigate the heightened uncertainty associated with longer feeding periods.

Notes:

P001 - Performance of an ASF real-time PCR assay on the Magnetic Induction Cycler (MIC) and QuantStudio 5 thermal cyclers

Kate Schumann[1], Danielle Marcone[1, 2], Bailey Harach[1, 2], Leslie Blakemore[1]

[1]United States Department of Agriculture, [2]Oak Ridge Institute for Science and Education. Danielle.Marcone@usda.gov

Session: African Swine Fever, **2025-01-20, 6:00 - 8:00**

Objective: The Bio Molecular Systems (BMS) Magnetic Induction Cycling system (MIC) is a 48-well, quantitative real-time PCR platform. Compared to traditional 96-well Peltier-block systems, such as the Applied Biosystem QuantStudio™ 5 (QS5) real-time PCR thermal cycler, the MIC's small size, portability, and relatively low cost are attractive attributes. The comparison of the ASF real-time PCR assay on the MIC and the QS5 real-time PCR thermal cyclers was to provide additional platform options to both the National Veterinary Services Laboratory (NVSL) and external laboratories.

Methods: This methods comparison study evaluated analytical sensitivity, precision, diagnostic sensitivity, and diagnostic specificity of the ASF real-time PCR assay across both platforms. Materials were sourced from international field samples and domestic casework.

Results: Analytical sensitivity was 10^{-6} for ASFV Georgia and ASFV Davis, and 10^{-5} for ASFV Malawi across both platforms. Inter- and Intra-assay standard deviations were 0.07, 0.30 for the MIC and 0.05, 0.29 for the QS5. Diagnostic sensitivity was 97.22% for the MIC and the QS5. Diagnostic specificity was 100% for both.

Conclusions: Overall, for criteria evaluated, the MIC PCR platform performed at a comparable or better level to the QS5 platform. This indicates that the MIC may be a suitable, alternative platform for the current ASF real-time PCR assay. Based on the results of this work, future studies to transfer additional assays to this system are anticipated.

Notes:

P002 - Performance characteristics of real-time PCR master mixes for the detection of African swine fever virus

Bailey Harach[1], Danielle Marcone[1], Leslie Blakemore[1], Kate Schumann[1]

[1]APHIS, USDA. bailey.harach@usda.gov

Session: African Swine Fever, **2025-01-20, 6:00 - 8:00**

Objective: Under the method of disease control and outbreak response for African swine fever (ASF), one-step real-time PCR assays, using commercial master mixes, are often performed. Limiting factors like cost and reagent availability, can hinder the work done in diagnostic laboratories. It is important to have alternative master mixes available as to not impede any outbreak control or surveillance work necessary to combat the spread of disease.

Methods: The current master mix used for the National Veterinary Services Laboratory (NVSL) ASFV real-time PCR assay is the TaqMan™ FAST Virus 1-Step Master Mix [ThermoFisher Scientific]. Here, we compared the performance characteristics of three commercial master mixes using the current ASFV real-time PCR assay. Two candidate master mixes, the VetMAX™ FAST Multiplex Master Mix (with ROX) [ThermoFisher Scientific] and the UltraPlex® 1-step ToughMix® Low ROX™ (4X) [Quantabio] were evaluated. To execute this methods comparison study analytical sensitivity, diagnostic sensitivity, diagnostic specificity, as well as repeatability were evaluated. Three strains of ASFV were used: ASFV Georgia, ASFV Davis, and ASFV Malawi.

Results: Between the master mixes the analytical sensitivity was 10^{-6} for ASFV Georgia for all, 10^{-6} for ASFV Malawi for all, and 10^{-6} for ASFV Davis with VetMAX and UltraPlex but was 10^{-5} for TaqMan. Inter- and Intra-assay standard deviations were 0.06 and 0.32 for TaqMan, 0.29 and 0.38 for VetMAX, as well as 0.69 and 0.3 for UltraPlex. Diagnostic sensitivity was 97.14% for TaqMan and 100% for both VetMAX and UltraPlex, and diagnostic specificity was 100% for all three systems.

Conclusions: Overall, results were comparable for all three master mixes. We concluded that the two candidate master mixes are appropriate alternatives when using the NVSL ASFV real-time PCR assay. The results of this study suggest the feasibility of implementing these master mixes in additional assays in the future.

Notes:

P004 - Comparison of library preparation methods for the sequencing of African swine fever virus using illumina platform

Amy Berninger[1], Jim L. Pierce[1], Lizhe Xu[2], Roger W. Barrette[2], Patrick T. Ababio[3], Steven M. Lakin[4], Theophilus Odoom[3], Bonto Faburay[4], Vivian O'Donnell[2]

[1]Oak Ridge Institute for Science and Education, [2]Foreign Animal Disease Diagnostic Laboratory, USDA, [3]Accra Veterinary Laboratory of Veterinary Sercives Directorate, [4]National Bio- and Agro-defense Facility, USDA. alb396@cornell.edu

Session: African Swine Fever, **2025-01-20, 6:00 - 8:00**

Objective: Evaluate the performance of three different DNA library preparation kits (Illumina DNA Prep, Nextera XT DNA Library Prep Kit, and the ExpressPlex Library Prep Kit) to sequence the whole genome of African Swine Fever Virus (ASFV) across different sample types, nature of infection, and DNA template used as input.

Methods: To evaluate the capability of the three library preparation kits to characterize the ASFV genome, the following samples were used: 4 blood samples from swine experimentally infected with ASFV, provided by the Foreign Animal Disease Diagnosticians School and 17 swine tissue samples collected in outbreak regions of Ghana. Samples were stored at -70°C until further processing and then extracted using the MagMAX™ CORE Kit (Thermofisher) on the KingFisher™ Flex magnetic particle processor (Thermofisher). Two sequencing approaches were tested for the blood samples: whole genome shotgun sequencing (using extracted blood as input) and amplicon sequencing (using tiled amplicon pools as input). Amplicon generation of ASFV was achieved by following the ASFV Tiled PCR amplification protocol, using LongAmp Taq DNA polymerase (NEB) as a modification. Whole genome shotgun sequencing was not performed for the field samples from Ghana due to lower genome resolution, and only amplicon sequencing was performed. Libraries were prepared using Illumina DNA Prep Kit (Illumina), Nextera XT (Illumina), and ExpressPlex™ Library Prep Kit (SeqWell). Samples were multiplexed and loaded using a 500-cycle v2 sequencing kit (Illumina) on the MiSeq System. The 4 extracted blood samples and 4 tiled amplicons pools (blood experimental samples) and the 17 tiled amplicon pools (Ghana field samples) were sequenced three times, corresponding with the three library preparation kits. Bioinformatic analysis was performed using our own ASFV fast pipeline.

Results: When sequencing the whole ASFV genome, our lab considers a “resolved” genome generating a minimum of 8x depth of coverage to adequately characterize the virus. When only extracted DNA was used as input, using blood from experimentally infected pigs, even while the Illumina DNA Prep Kit resulted in the highest depth and coverage for the samples, none of the kits evaluated reached enough depth and coverage to fully resolve the genomes. However, when ASF tiled amplicons were used as input, using the same samples, the Illumina DNA Prep kit successfully resolved genomes for all four samples, while Nextera XT and the ExpressPlex kit did not resolve any genome. Furthermore, ASF tiled amplicons generated from field samples resulted in a resolution of 65% of samples (11/17) with Illumina DNA prep, 18% (3/17) with Nextera XT, and the ExpressPlex kit did not resolve any of the samples.

Conclusions: Results indicated that the Illumina DNA Prep kit achieved the highest success rate in ASF genome resolution when sequenced, with the greatest number of samples generating a minimum of 8x depth of coverage. In this work, we evaluated three different DNA library preparation kits as a proof of concept to sequence the ASF genome. However, further evaluation is also recommended to optimize the sequencing output of each library preparation kit.

Notes:

P005 - Alternative searching in fighting against African swine fever disease in Benin: A pilot study

Okri F. H. Ohouko[1], Koffi Koudouvo[2], Victorien T. Dougnon[1], Jean R. Klotoe[1], Jacques Adouko[1], Arnaud Soha[1], Farougou Souaïbou[3], Issaka Youssao[4]

[1]Research Unit in Applied Microbiology and Pharmacology of natural substances, University of Abomey-Calavi (Benin), [2]Laboratory of Physiology and Pharmacology of Natural Substances, University of Lome (Togo), [3]Research Unit on Communicable Diseases (URMAT), University of Abomey-Calavi (Benin), [4]Laboratory of Animal Biotechnology and Meat Technology, University of Abomey-Calavi (Benin). ohoukofrjus@yahoo.com

Session: African Swine Fever, **2025-01-20, 6:00 - 8:00**

Objective: The present study aims to explore the curative properties of two antiviral medicinal plants, Acacia nilotica (L.) Willd. ex Delile (AN) and Aloe vera (L.) (AV) in pigs infected with African swine fever virus (ASFV).

Methods: The fruits of AN and the leaves of AV, collected and identified at the national herbarium respectively under the number YH 422/HNB and YH 423/HNB have been used for aqueous extract and gel preparation. The experimental design consisted of a positive control (piglets infected with ASFV and not treated), a negative control (piglets not infected with ASFV and not treated) and two treatments groups for each plant considered. Each group were made of 5 piglets of 8 weeks old. Piglets of positive control group and treatment group were infected by intra-muscular injection with 3 mL of viral broth constituted of spleens, kidneys, hearts, gastric lymph nodes from PCR-confirmed ASF pigs. Treatments groups received orally the aqueous extract of AN/gel of AV after the confirmation of viral infection. The first treatment group received 1mL and the second 2mL of aqueous extract of AN/gel of AV per kilogram (Kg) of body weight (BW). Data such as rectal temperature, mortalities, clinical signs, macroscopics lesions have been collected and compared. Hematological and biochemistry parameters have been dosed. The one-way ANOVA analysis of variance have been used for blood parameters comparison. The study received the authorization of the ethical committee review board of the University of Abomey-Calavi under the number 11248411.

Results: Piglets infected showed clinal signs of ASF disease such as vomiting associated with respiratory distress, simple diarrhea leading to bloody diarrhea, inappetence and hyperthermia from the 3rd day post-inoculation. These manifestations progressed to redness of the scrotum, ears and legs, especially in infected and untreated piglets. No difference has been registered in white blood cells from the 1st day of inoculation to the 3rd post-inoculation in all batches. At the 5th day post-infection, piglets of the positive control group were all dead. Treatments groups which received aqueous extract of AN/gel of AV shows temperature normalization at seventh day post-infection (4 days post-treatment). However, piglets treated with 1mL survived till the 7th and 9th days post-inoculation respectively for AV and AN, while 2mL treatments of both groups' dead at 10th days post-inoculation.

Conclusions: This experiment shows the effectiveness of ASF disease induction in piglets. However, the evaluation of the therapeutic effect of the aqueous extract of the fruits of Acacia nilotica and the gel of the leaves of Aloe vera did not reveal an efficacy in the management of pigs infected with ASF virus. More exploration needs to be done for better management of the disease in infected piglets.

Financial Support: Our acknowledgment to the Benin government throughtout its support under the fund "Programme d'Appui aux Doctorants" of the Ministry of Higher Education.

Notes:

P006 - First report of genotype II for the African swine fever virus outbreak in Benin Republic in 2023

Okri F. H. Ohouko[1], Vivian O'Donnell[2], Edward Spinard[3, 4], Mark Dinhobl[3, 4], Amy Berningere[5], Lizhe Xub[2], Jacob Fenster[3,4], Manuel V. Borca[3, 4], Bonto Faburay[2, 5], Victorien T. Dougnon[1], Douglas P. Gladue[6]

[1]Research Unit in Applied Microbiology and Pharmacology of Natural Substances, University of Abomey-Calavi (Benin), [2]U.S. Department of Agriculture, Animal and Plant Inspection Service, Plum Island Animal Disease Center, [3]U.S. Department of Agriculture, Agricultural Research Service, Foreign Animal Disease Research Unit, Plum Island Animal Disease Center, [4]U.S. Department of Agriculture, Agricultural Research Service, Foreign Animal Disease Research Unit, National Bio and Agro-Defense Facility, [5]U.S. Department of Agriculture, Animal and Plant Inspection Service, National Bio and Agro-Defense Facility, [6]Seek Labs, Salt Lake City. ohoukofrjus@yahoo.com

Session: African Swine Fever, **2025-01-20, 6:00 - 8:00**

Objective: The aim of this study was to characterize the isolates from the African swine fever virus outbreak in Benin

Methods: Two isolates (BEN-AACB2 and BEN-OPNB1) were collected from the 2023 outbreak in swine in southern Benin (Department of Atlantique), which were thought to have perished from African Swine Fever. DNA was extracted and DNA Libraries were constructed for illuminia using the Nextera XT kit and Nanopore, with the Rapid BC Kit v 14 (ONT), and R10.4 flow cells. De novo assembly was performed using SPAdes and Genomes were annotated using the default settings of TheTransporter.

Results: Each sample's minion reads and two sets of illumina paired-reads resulting in a 180,642 (BEN-OPNB1) and a 184,758 (BEN-AACB2) nucleotide length contig each with a GC content of 38.6%. Both genomes were Genotype 2 (historic genotype II) and Biotype 2. Both genomes exhibited the 14 gene deletion that has been observed in the Georgia variants causing outbreaks in western Africa and analysis of the 3' end of the genome revealed both genomes were more similar to the Ghana 2022 isolates than Nigeria-RV502 as they did not contain the reverse complement of the 5' region.

Conclusions: This study present insight on African swine fever dynamic in Benin. It constitutes a guide for the country to establish action plan for better managing the disease for the pork industry protection. To the scientific community, research should focus on developing local vaccine to rapidly tackle the emergence of the strain circulating.

Financial Support: This work is supported by a grant from the U.S. Civilian Research & Development Foundation (CRDF Global) that was funded from the National Bio and Agro-Defense Facility

Notes:

P007 - An analytical framework for prioritizing African swine fever disease surveillance in domestic and wild pigs

Mary J. Woodruff[1], Greg Franckwiak[2], Vienna R Brown[2], Dana Cole[2], Ryan S. Miller[1]

[1]Center for Epidemiology and Animal Health, USDA-APHIS, [2]National Feral Swine Damage Management Program, USDA-APHIS. mary.woodruff@usda.gov

Session: African Swine Fever, **2025-01-20, 6:00 - 8:00**

Objective: Preserving the health of U.S. animal agriculture and wildlife relies on effective foreign animal disease (FAD) surveillance. Surveillance supports rapid pathogen detection, minimizes outbreak severity, and mitigates economic impact. Spread of FADs among wild and domestic animals is increasingly a concern and must be addressed by surveillance systems. However, designing such systems is challenging due to limited ecological and epidemiological data in wildlife and technical challenges associated with non-invasive methods. The question remains: where to allocate limited surveillance resources while maximizing early detection of African swine fever (ASF) in wild and domestic pigs?

Methods: To support optimal surveillance allocation, we developed an adaptive risk-based analytical approach that identifies locations at risk of disease introduction. We extended a previously established disease spillover framework at the wildlife-livestock-human interface, incorporating four key risk factors: (1) disease introduction, (2) wild host abundance, (3) domestic host abundance, and (4) host connectivity. For each risk factor, we normalized and standardized data by the county area (sq km), then weighted these risk factors based on data quality, disease risk likelihood, or expert recommendations. Our introduction risk data, sourced from four federal agencies, included ports of entry, individuals, aircraft, and vessels arriving from disease-positive countries, agricultural product seizures, and census demographics. Wild host abundance was estimated from a catch-effort model informed by USDA Wildlife Services data. Domestic host abundance was obtained from USDA National Agriculture Statistics Services. Host connectivity included the distribution of landfills and niche production farms, presumed interfaces between wild and domestic pigs. We calculated county-level relative ranks using these risk factors by multiplying the weighted risk factors. Next, we generated one overall relative risk rank per county by multiplying these risk ranks together and binned overall risk-rank into low, medium, high, and very high (95-percentile) categories. County relative risk ranks are provided to decision makers annually at national and state scales to support targeted surveillance for ASF.

Results: We identified a total of 167 counties at high risk (95-percentile) of ASF introduction and where we recommend allocating targeted surveillance of wild and domestic pigs. High-risk counties were distributed throughout the Southern U.S. and California. Notable high-risk clusters included eastern Texas, central California, and eastern Florida. Counties with ports of entry or wild pig populations tended to be higher risk when prioritizing both wild and domestic pig surveillance.

Conclusions: Our data-driven insights directly inform USDA national surveillance strategies, enabling the allocation of limited surveillance resources to areas of greatest risk. This approach highlights the critical role of interagency collaboration and large-scale data integration in supporting national biosecurity. We present one application of our adaptive, risk-based targeted surveillance approach. Applying this framework across species and pathogens can support maximizing surveillance resources and mitigate disease introduction risks. By providing actionable insights to decision makers, we help safeguard the health of U.S. animal agriculture and wildlife.

Financial Support: Postdoctoral fellowship funding for Mary J Woodruff provided through ORISE

Notes:

P008 - Development of vectored subunit vaccine candidates for ASFV

Pablo S. Britto de Oliveira[1], Yonghai Li[2], Jessica C. G. Noll[1], Natasha N. Gaudreault[2], Leonardo C. Ribeiro[1], Diego G. Diel[1], Juergen A. Richt[2]

[1]Population Medicine and Diagnostic Sciences, Cornell University, [2]Diagnostic Medicine/Pathobiology, Kansas State University. psb86@cornell.edu

Session: African Swine Fever, **2025-01-20, 6:00 - 8:00**

Objective: African Swine Fever Virus (ASFV) causes an often-lethal disease of pigs, both wild and domestic that is characterized by a wide variety of clinical signs in affected animals. ASFV is a large DNA virus and attempts to develop vaccines capable of protecting against the infection and the disease have been largely unsuccessful. In this project, we aim to rationally design, engineer and test vectored subunit vaccines with respect to protection against ASFV infection and disease. The objectives of this project are to: 1) define ASF vaccine-induced immune responses, and 2) evaluate ASF subunit vaccine safety and efficacy in pigs. Here we tested vectored vaccines containing ASFV proteins that are delivered by two different virus vectors, vesicular stomatitis virus (VSV) and a parapoxvirus called Orf virus (ORFV). Previously we have shown that heterologous immunization with rVSV and rORFV virus vectors expressing ten different ASFV antigens provide partial protection (50%) against virulent ASFV challenge. Next, we performed an immunization/challenge study in pigs with these virus-vectored ASFV antigens with the addition of an adjuvant.

Methods: The immunogenicity and protective efficacy of the rVSV- and rORFV-ASFV vector libraries administered with adjuvant were evaluated in 3- to 4-week-old ASFV negative pigs (n=6 pigs per group). Animals were immunized with the respective candidate vector vaccines via intramuscular (IM) immunization. Each vaccine preparation included the 2'3'-cGAMP VacciGradeTM adjuvant. Animals were immunized on day 0 and boosted on day 21 and 35 post-primary immunization. Following immunization, animals were monitored daily and body temperatures were taken. Additionally, serum and PBMCs were collected. On day 48, all animals were challenged IM with 25 HAD_{50} of a virulent genotype II ASFV virus strain. Animals were monitored daily and clinical signs and body temperature recorded until the end of the study at 15 days post challenge (dpc). Blood and oropharyngeal swabs were collected up to 15 dpc. Full necropsy was conducted on all animals. Tissues including spleen, tonsil and various lymph nodes (e.g., mediastinal, mesenteric, renal, etc.) were collected.

Results: Average rectal temperatures post-vaccination were similar to the non-vaccinated pigs. Post-challenge, the vaccinated pigs developed fevers starting at 8 dpc, and all animals succumbed to the ASF disease by 14 dpc (n=6). In contrast, the mock-vaccinated animals (n=5) survived until the end of the observation period of 15 dpc, but were all ASFV PCR positive.

Conclusions: The addition of adjuvant to our subunit vaccine regime did provide protection against ASF but instead resulted in enhanced disease as compared to non-vaccinated pigs or pigs vaccinated without adjuvant.

Financial Support: USDA NIFA

Notes:

P009 - Comparative study in domestic pigs between the recently emerged ASFV hybrid Vietnamese field strain rASFV I//II and the ASFV field strain Georgia 2010

Elizabeth Ramirez-Medina[1], Alyssa Valladares[1], Leeanna Burton[1], Leandro P. Sastre[1], Mark Dinhobl[1], Vivian K. O'Donnell[2], Manuel V. Borca[1]

[1]U.S. Department of Agriculture, Agricultural Research Service, Foreign Animal Disease Research Unit, Plum Island Animal Disease Center (PIADC), [2]Foreign Animal Disease Diagnostic Laboratory, USDA Plum Island Animal Disease Center (PIADC). elizabeth.ramirez@usda.gov

Session: African Swine Fever, **2025-01-20, 6:00 - 8:00**

Objective: African swine fever (ASF), is a disease of domestic and wild swine that has spread throughout Africa, Central Europe, East and Southeast Asia. The clinical presentation of the disease heavily depends on the virulence of the ASFV strain. In 2023, newly emerging highly virulent ASF viruses (rASFV I/II) containing genetic elements from both p72 genotype I and II ASF viruses were reported in Northern Vietnam. In this report, we evaluate the clinical presentation of the disease in domestic pigs inoculated with either the ASFV recombinant field isolated rASFV I//II or the ASFV field isolate Georgia 2010.

Methods: Two groups of pigs were inoculated intramuscularly (IM); one with rASFV I/II (10^2 HAD_{50}) and the other with ASFV- Georgia 2010 (10^2 HAD_{50}). Clinical signs of the disease were monitoring daily and sample collection of blood and nasal swabs was performed.

Results: Animals inoculated IM with either virus rASFV I/II and ASFV-Georgia 2010 showed an acute disease being all animals in both groups euthanized between the 5- and 6-days post infection. Viremia titers in all the groups closely followed the clinical presentation of the disease, both in length and extent.

Conclusions: Results suggest that rASFV I/II, produces an acute homogeneous disease as ASFV-Georgia 2010. This is the first comparative report on the virulent phenotype of rASFV I/II field strain isolated in Vietnam and the ASFV-Georgia 2010, that provides information that may be used in developing epidemiological management measures to control ASF.

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

Notes:

P010 - Analyzing multidrug resistance patterns across the food supply chain using association rule mining

Joshua Glass[1], Gayatri Anil[1, 2], Kristina Ceres[2], Laura Goodman[2], Casey Cazer[1, 2]

[1]Department of Clinical Sciences, Cornell University, [2]Department of Public and Ecosystem Health, Cornell University. jg2527@cornell.edu

Session: Antimicrobial use, **2025-01-20, 6:00 - 8:00**

Objective: Agricultural antimicrobial use may lead to the emergence of multidrug resistant microbes in food animal populations and at subsequent levels of the food chain. Multidrug resistance (MDR) poses a unique threat by limiting treatment options for animal and human illness. Analyzing MDR poses challenges to researchers due to the complexity and number of possible MDR patterns. The objective of this study was to apply association rule mining to the analysis of MDR patterns present in cattle-associated *Escherichia coli*, incorporating both phenotypic and genotypic indicators of resistance.

Methods: Data were retrieved from the National Antimicrobial Resistance Monitoring System (NARMS) to include 12,932 *E. coli* isolates tested against 12 to 14 antimicrobials from 2002 to 2021. Data were divided into separate datasets based on year and source (retail meat vs cecal samples). Minimum inhibitory concentrations were used to determine each isolate's phenotypic resistance profile based on NARMS interpretive criteria. Associations among these resistance profiles were analyzed using association rule mining. Associations based on the presence of resistance genes were also mined if the genotypic data was available. To compare rules across datasets, the percentage of rules from one dataset that were captured in another dataset were calculated. Phenotypic and genotypic rules were compared by aggregating resistances and genes to the antimicrobial class level.

Results: On average, almost all (~98%) phenotypic rules were captured by the genotypic rules; however, a lower percentage (~45%) of the genotypic rules were captured by the phenotypic associations. MDR patterns from both phenotypic and genotypic data were more consistent across years among cecal isolates (~50% the same year to year) than retail meat isolates (~25% the same year to year). The average percentage of phenotypic cecal rules captured within phenotypic retail meat rules decreases across years (going from ~95% in 2013 to ~25% in 2019). Whereas the average percentage of phenotypic retail meat rules captured within phenotypic cecal rules increases across years (going from ~45% in 2013 to ~85% in 2019).

Conclusions: Association rule mining was able to effectively provide insights into complex associations among MDR patterns present in cattle associated *E. coli*. The high percentage of phenotypic associations represented in the genotypic associations suggests that genotype is a good predictor of phenotypic resistance; while the comparatively lower percentage of genotype class-associations represented within the phenotype class-associations is largely driven by drug-specific aminoglycoside resistance genes. This discrepancy may suggest that more drugs need to be phenotypically tested to have a fuller understanding of the variation in MDR patterns. It also may be important to consider how phenotypic and genotypic data could result in different classifications of MDR to promote consistency in MDR categorization. The greater consistency of the phenotypic rules generated from the cecal samples (along with the finding that these rules better capture the retail meat rules than the converse), may suggest that the cecal dataset provides a fuller representation of the MDR profiles present in the food system.

Financial Support: This work is supported by the USDA National Institute of Food and Agriculture, AFRI project 2023-68015-4092

Notes:

P011 - Overview of an ongoing Zoetis antimicrobial susceptibility surveillance program for major veterinary pathogens in the United States and Canada

Bryce L. Lunt[1], Lacie Gunnett[1], David J. Asper[1], Michael T. Sweeney[1], Véronique Moulin[1], Dipu Mohan Kumar[1], Abhijit Gurjar[1]

[1]Zoetis. bryce.lunt@zoetis.com

Session: Antimicrobial use, **2025-01-20, 6:00 - 8:00**

Objectives: To continuously monitor for changes in susceptibility in antimicrobials used in veterinary medicine against target pathogens from across the United States and Canada.

Methods: Over 85,000 bacterial strains isolated as pathogens from beef and dairy cattle, pigs, horses, cats, and dogs have been collected by Zoetis since 1998 from diagnostic laboratories across the United States and Canada as part of a surveillance program designed to monitor the ongoing level of susceptibility towards the major antimicrobials used for specific disease indications in each of these animal species. Typically, thirty-three animal species/pathogen combinations are tested against sixty-two animal species/drug combinations submitted from up to thirty-six laboratories each year. In order to limit over-representation of a geographical area, isolate submission from laboratories is limited to a maximum number of strains of each pathogen per year. Additionally, the number of isolates from a particular herd, ranch or household is limited to reduce the risk of overrepresentation of clones or epidemiologically related strains and subsequent data bias. Each isolate is considered by the submitting lab as the etiological agent responsible for disease. Isolates are randomly chosen by the laboratory without regard to susceptibility or previous antibiotic use. Laboratories are requested to include information regarding previous antibiotic use, when available. Minimal inhibitory concentration values for all isolates are determined using a broth microdilution system, which conforms strictly to the standards of the Clinical and Laboratory Standards Institute (CLSI) for testing veterinary pathogens and that is held to rigorous quality control standards beyond those recommended by CLSI.

Results: This long-term Zoetis surveillance program provides valuable information about numerical changes in in vitro activity of the most commonly used antimicrobials in veterinary medicine and provides a valuable and robust collection of historical strains of clinical pathogens. These data are extensive in geography and time; and for many animal species/drug combinations, more than twenty years of data have been collected across the United States and Canada using a consistent methodology.

Conclusions: Responsible stewardship of veterinary anti-infectives involves activities that aim to understand the development of resistance, minimize any effects of antimicrobial use, and sustain the efficacy of current veterinary drugs. Zoetis is committed to monitoring antimicrobial susceptibility through this surveillance program and surveillance for antimicrobial resistance among veterinary pathogens is an important component of stewardship advocated by Zoetis.

Notes:

P012 - Targeting calcium homeostasis is the leading pathway to control *Clostridioides difficile* infection

Ahmed Abouelkhair[1], Nader Abutaleb[1], Mohamed Seleem[1]

[1]Department of Biomedical Sciences and Pathobiology, Virginia Polytechnic Institute and State University.
aabouelkhair@vt.edu

Session: Antimicrobial use, **2025-01-20, 6:00 - 8:00**

Objective: *Clostridioides difficile* is the leading cause of deadly antibiotic-associated diarrhea in hospitalized patients, causing approximately 500.000 cases and 30,000 fatalities per year. This led the United States Centers for Disease Control and Prevention (CDC) to announce *C. difficile* as the "most urgent public health threat." The current generation of anti-*C. difficile* drugs show suboptimal results, with high rates of treatment failure and recurrence because of insufficient sporicidal efficacy and gut dysbiosis (a disruption of the gut microbiota). Therefore, to tackle *C. difficile*, innovative, selective therapeutic agents are urgently needed. In this study, we report the discovery of ionomycin, a calcium ionophore metabolite produced by *Streptomyces conglobatus*, as a potent anti-*C. difficile* agent that can selectively suppress *C. difficile* in the presence of high concentrations of calcium, in an attempt to develop an alternative strategy to conventional antibiotics.

Methods: In this work, we tested a microbial metabolite library (~527 compounds) against *C. difficile* using high-throughput cell screening, and we found that ionomycin had strong efficacy. We were able to verify ionomycin's anti-*C. difficile* activity by using the broth microdilution technique in compliance with clinical and Laboratory Standards Institute (CLSI) standards. After that, we utilized a range of in vitro experiments, one of which was a time-kill assay, to confirm ionomycin's anti-*C. difficile* activity and determine the speed at which ionomycin eliminates a high inoculum of *C. difficile*. Furthermore, the mechanistic studies were assessed using membrane depolarization experiments. Additionally, we evaluated the anti-*C. difficile* efficacy against the gut microbiota, spore germination by the spore outgrowth assay, and *C. difficile* toxin inhibition through the use of ELISA. To validate the previously outlined in vitro results, the in vivo efficacy of ionomycin in the acute and recurrent *C. difficile* mouse model has been investigated using C57Bl/6 mice. All statistical analyses and visualizations of this study were performed using GraphPad Prism version 10 for Windows. $P < 0.05$ was considered statistically significant.

Results: Our findings reveal that ionomycin had potent anticlostridial activity against 30 *C. difficile* clinical isolates, inhibiting the growth of 50% and 90% of the isolates tested (MIC_{50} and MIC_{90}) at 1 μg/mL and 2 μg/mL, respectively. In the time-kill assay, ionomycin reduced the high bacterial inoculum by 3 $\log_{10}$ in 8 hours, outperforming vancomycin and fidaxomicin, the two anti-*C. difficile*-approved drugs. Curiously, ionomycin also exhibited sporicidal activity; it eradicated the *C. difficile* spore load in 4 days and prevented spore growth and toxin production from the remaining germinating cells. Importantly, ionomycin had a very limited efficacy against human gut microbiota. Mechanistic studies showed that the addition of a calcium chelator reduced the bactericidal activity of ionomycin and boosted it when calcium was supplemented. Ionomycin also altered the membrane potential of *C. difficile*, and calcium supplementation strengthened this effect. Moreover, ionomycin performed better in the *C. difficile* mouse model than the control drug, vancomycin.

Conclusions: These results, collectively, indicate that ionomycin represents a promising anticlostridial activity that merits further investigation for developing novel anti-*C. difficile* drugs that target calcium homeostasis

Financial Support: NIH grant R01AI130186

P013 - Antimicrobial use in Canadian feedlots

Sheryl Gow[1], Tara Funk[2], Kayla Strong[3], Alyssa Butters[4], Dana Ramsay[2], Booker Calvin[5], Craig Dorin[6], Steve Hendrick[7], Sherry Hannon[5]

[1]PHAC, [2]University of Saskatchewan, [3]Independent Contractor, [4]University of Calgary, [5]Telus Agriculture, [6]Veterinary Agri-health Services, [7]Cattle Health Management. sheryl.gow@phac-aspc.gc.ca

Session: Antimicrobial use, **2025-01-20, 6:00 - 8:00**

Objective: The Canadian feedlot antimicrobial use (AMU) and antimicrobial resistance (AMR) surveillance network (CFAASP) was established in partnership with the Canadian government, feedlot stakeholders, veterinary practitioners, and feedlot producers. This surveillance network aims to (1) provide representative estimates of AMU in Canadian feedlots and to (2) monitor AMU trends over time.

Methods: Participating feedlots retrospectively provided key information specific to a random selection of production lots from the previous year, including the amount, concentration, route of administration, and indication. Additional demographics such as age, weight, inventory, and BRD risk categorization data were provided as part of the minimum requirements for sentinel feedlot surveillance.

Results: Measured in mg/kg biomass, there was a net decrease (-10.6%) in total AMU from 2019 to 2022. Over 95% of total AMU across all years was administered in feed. The prevention and control of liver abscesses, histophilosis, and bovine respiratory disease were the primary drivers of AMU in feedlot cattle. There was a net increase (+19.5%) in Category II AMU from 2019 to 2022, driven by the increasing proportion of in-feed streptogramins from 2019 (0%) to 2022 (11%). Conversely, Category I AMU remained consistently low across all years. Specifically, cephalosporin and fluoroquinolone use comprised 4% and 3% of the total injectable AMU in 2022. In 2022, the most frequently used classes of antimicrobials were tetracyclines (72%), macrolides (16%), and streptogramins (10%), accounting for 98% of the total AMU.

Conclusions: Allowing veterinarians and producers to review current practices data from this project helps support the preservation of antimicrobial effectiveness, thus improving cattle health and welfare and protecting public health. The CFAASP provides robust data to support AMU transparency and antimicrobial stewardship discussions.

Financial Support: Alberta Beef Producers (ANH.11.19), Alberta Cattle Feeders Association, Beef Cattle Research Council (ANH.11.19), Beef Farmers of Ontario, Canadian Agricultural Partnership Alberta (SUR-049245) and Ontario (CAP-AHAR-OR-A-000166), Public Health Agency of Canada, Saskatchewan Ministry of Agriculture MISC.01.18 CIPARS AMR, Saskatchewan Cattlemen's Association.

Notes:

P014 - Biochar broiler house amendment reduces contamination with extended spectrum beta-lactamase producing *E. coli*

Getahun Agga[1], Nanh C. Lovanh[1], Karamat R. Sistani[1]

[1]USDA-ARS, Food Animal Environmental Systems Research Unit, Bowling Green, Kentucky. getahun.agga@usda.gov

Session: Antimicrobial use, **2025-01-20, 6:00 - 8:00**

Objective: Biochar has been shown to reduce ammonia emission, which is particularly important health problem in broiler chicken houses. However, the potential benefit of biochar as an intervention to reduce antimicrobial resistance in food animal production is not studied. The objective of the project was to evaluate the impact of biochar poultry litter amendment as a mitigation measure to reduce the prevalence of extended spectrum beta-lactamase (ESBL) producing *E. coli* in broiler production system.

Methods: Biochar was surface applied to the back half of house 1 before day old chicken were placed. After the brooding period, the back of the house was opened, and the chicken were allowed to freely move. House 2 was not amended with biochar and left as control. Poultry litter samples were collected as grab samples on days 0 (baseline), 9, 23, 40 and 56 (at harvest) from the floors of the two broiler chicken houses over one full grow cycle. Samples were pre-enriched in peptone water and streaked on commercially available CHROMagar ESBL. A single isolated colony typical of ESBL-producing *E. coli* was re-streaked onto a second plate to obtain a pure colony and confirmed by PCR. Data were analyzed using logistic regression.

Results: Biochar poultry house amendment significantly reduced theprevalence of ESBL-producing *E. coli* compared to no amendment (OR= 0.3; 95% CI=0.17-0.49). The prevalence of ESBL-producing *E. coli* sequentially increased from the front to the back of the broiler houses showing distinct spatial variation, regardless of biochar amendment. Similarly, the prevalence increased with the age of the chicken, reaching the maximum at 23 days of age.

Conclusions: In conclusion, biochar can be utilized to mitigate the burden of antibiotic resistant bacteria in broiler production. Furthermore, significant spatial and temporal variations occur with respect to the dynamics of ESBLs-producing *E. coli* within broiler chicken productionsystem.

Notes:

P015 - In vitro colistin pressure revealing antimicrobial adaptation strategies of animal derived *Klebsiella pneumoniae*

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

[1]Seoul National University. stevekyung25@snu.ac.kr

Session: Antimicrobial use, **2025-01-20, 6:00 - 8:00**

Objectives: Colistin is considered the last-resort antibiotic for the clinical treatment of multidrug-resistant Gram-negative bacterial infections, including those caused by *Klebsiella pneumoniae*, an opportunistic pathogen of serious worldwide concern. However, the process of colistin resistance development in *K. pneumoniae* remains largely unknown. Therefore, adaptive laboratory evolution (ALE) was utilized with *K. pneumoniae* isolates to gain further understanding of colistin adaptation.

Methods: In this study, two isolates of *K. pneumoniae* were subjected to ALE under colistin pressure to identify differentially expressed RNA at each stage of resistance development. Each isolate served as the ancestor of three mutant strains resulting from independently conducted ALE experiments. Consequently, all strains surpassed the resistance determinant values suggested by CLSI standards by the second day of antibiotic pressure. Strains isolated from each stage underwent transcriptomic RNA-seq analysis. Isolates from the final stage of ALE were subjected to whole-genome resequencing to identify genomic variations.

Results: As a result of ALE, six colistin resistant isolates were acquired. The resistant ratio of the bacterial population exceeded 60%, at least by the 7th day of colistin pressure. The whole-genome resequencing revealed multiple variant genes potentially associated with colistin adaptation, requiring further assessment. The RNA-seq results revealed that in the early stage, pathways related to quorum-sensing, such as the autoinducer-2 pathway, are involved, while in the later stage, pathways related to colistin target modification mechanisms are involved. This indicates that different reactions and pathways contribute to colistin survival in the early and later stages.

Conclusions: In this study, it was identified that bacterial cells utilize various strategies to respond to colistin pressure at different stages. The findings presented in this study will contribute to a deeper understanding of colistin resistance development in *K. pneumoniae* and provide useful insights for further studies aimed at establishing new strategies to prevent the emergence of colistin-resistant *K. pneumoniae*.

Financial Support: This study was supported by National Research Foundation (NRF-RS-2024-00392205), BK21 FOUR Future Veterinary Medicine Leading Education and Research Center and Research Institute for Veterinary Science, Seoul National University, Seoul, Republic of Korea.

Notes:

P016 - Use of systemic ceftiofur and ampicillin in dairy cows: A randomized clinical trial

Juliano L. Goncalves[1], Amanda T.F. Silva[2], Karla Vasco[1], Shannon Manning[1], Lixin Zhang[1], Bo Norby[1], Rinosh Mani[1], Pamela L. Ruegg[1]

[1]Michigan State University, [2]Federal Rural University of Pernambuco. goncal25@msu.edu

Session: Antimicrobial use, **2025-01-20, 6:00 - 8:00**

Objective: This study aimed to evaluate differences in the abundance and duration of shedding of resistant Gram-negative (GN) bacteria in the feces of healthy cows treated with ceftiofur (CEF) or ampicillin (AMP), as compared to a control group that did not receive antibiotic treatment.

Methods: Healthy lactating Holstein cows were randomly assigned to receive once daily treatments for 5 days with CEF (2.2 mg/kg subcutaneously, n = 8), AMP (11 mg/kg intramuscular, n = 8) or sterile saline (25 cc, subcutaneously, n = 8). Fecal grab samples were collected before treatment (day 0) and weekly, starting at day 7 after treatment for 11 weeks. Briefly, fecal samples were diluted and inoculated using a spiral plater in duplicate on MacConkey agar, non-supplemented or supplemented with AMP or CEF. The inoculated plates were incubated at 37°C for 24 h, after which bacterial counts were determined. The abundance of GN bacteria resistant to AMP or CEF was enumerated using a logarithmic scale and as a proportion of the total number of GN bacteria. Generalized linear mixed models for repeated measurements were used to compare the abundance of resistant bacteria among treatments over 12-weeks.

Results: The abundance of GN bacteria did not vary within or among treatments over the weeks of clinical trial. The abundance of GN bacteria resistant to AMP and CEF did not differ among treatment groups within a given week. After treatment, systemic treatment with AMP or CEF did not increase the proportion of resistant GN bacteria in feces of treated cows as compared to a control group that did not receive antibiotic treatment. A slight reduction in the abundance of GN bacteria resistant to AMP was observed over the course of AMP treatment, with a reduction of 1.4 log cfu by week 4 and 2.1 log cfu by week 11, reaching levels lower than those measured in the first week post-treatment. Resistance to CEF fluctuated over the 11-week period, but no differences were observed within or among the treatment groups. Regardless of the treatment group, variation in the proportion of GN bacteria resistant to AMP (but not CEF) was observed among individual cows. Interestingly, the proportion of GN bacteria resistant to AMP varied among individual cows within treatment. Both before and after administration of antimicrobials, one cow in each treatment group contributed > 50% of bacteria that were resistant to AMP. Moreover, these 3 cows continued to shed bacteria resistant to AMP in the 11 following weeks.

Conclusions: Our findings indicated that systemic treatments of AMP and CEF in healthy cows had only minor transient effects on abundance and duration of resistant bacteria shedding in feces.

Financial Support: Supported by the Michigan Alliance for Animal Agriculture Grant #AA-22-0020. We thank all the employees from the Dairy Cattle Teaching and Research Center at Michigan State University, as well as the collaborators from the top milk quality.

Notes:

P017 - A probiotic engineered for controlled release of antimicrobials targeting swine pathogens

In Young Choi[1], Jee-Hwan Oh[1], Jan Peter van Pijkeren[1]

[1]Department of Food Science, University of Wisconsin-Madison. ichoi37@wisc.edu

Session: Antimicrobial use, **2025-01-20, 6:00 - 8:00**

Objective: *Streptococcus suis* is as an important swine and zoonotic pathogen that causes severe disease in humans and significant financial strain on the pig industry. While antibiotics are commonly used to treat *S. suis*, broad application of antibiotics raises concerns about antibiotic resistance. Our long-term goal is to develop probiotic bacteria as microbial therapeutics. The objective of this project is to engineer *Limosilactobacillus reuteri* to deliver antimicrobials, including phage-derived lysins, to target *S. suis* in the gastrointestinal tract. *L. reuteri* was engineered to accumulate therapeutic proteins inside the cell, which are released following natural lysis in the gastrointestinal tract. We hypothesized that controlled lysis will yield more robust lysis and antimicrobial release thereby increasing therapeutic efficacy

Methods: We engineered *L. reuteri* to express a prophage-derived gene encoding an antirepressor protein, which regulates prophage activation. To assess antirepressor functionality, we expressed the antirepressor protein and determined the optical cell density and phage production. We quantified the released recombinant protein fused to a luminescent tag, using a luminescent assay after antirepressor- or mitomycin C-induced cell lysis. To optimize therapeutic release, we fine-tuned the timing of antirepressor induction. Lastly, we determined the impact of antirepressor-mediated cell lysis on bacterial survival during gastrointestinal transit following oral administration.

Results: Induced antirepressor production in *L. reuteri* successfully triggered phage-mediated cell lysis at 3-h post induction. The lysis was mediated by the phages because antirepressor production in a *L. reuteri* derivative lacking phages did not result in cell lysis. Moreover, antirepressor induction leads to faster and more robust cell lysis compared to chemical induction with mitomycin C. Our approach also significantly improved protein release as we observed 4 times higher protein release percentage compared to chemical induction. In addition, fine-tuning the timing of antirepressor expression maximized released protein concentration. Finally, priming cells for lysis *in vitro* led to a significant reduction of *L. reuteri* survival during gastrointestinal transit, suggesting improved lysis efficiency.

Conclusions: Our approach demonstrates the potential of employing a lytic-switch for enhanced release of antimicrobials, advancing our quest for potent antimicrobial solutions against *S. suis* and other microbial pathogens.

Financial Support: This work was supported by Animal Health and Production and Animal Products: Animal Health and Disease [grant no. 2021-67015-34316] from the USDA National Institute of Food and Agriculture. The work is IN-PART also supported by NIH NIGMS (R01GM135483).

Notes:

P019 - Next-generation probiotics: Novel insights into controlling *Campylobacter* infection in humans and animals

Bibek Lamichhane[1], Illhem Messaoudi[2], Yosra 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 use, **2025-01-20, 6:00 - 8:00**

Objective: *Campylobacter* jejuni is one of the major causes of food-borne gastroenteritis in animals and humans. Food animals such as poultry are the primary reservoir and the main sourse of infection of *C. jejuni* in humans. *C. jejuni* infections are mainly transmitted through the consumption of contaminated food and water. Currently, antibiotics are used to treat *Campylobacter* infections in both animals and humans. However, the increase in antibiotic resistance to commonly used antibiotics underscores an urgent need for alternative treatment strategies. This study aims to assess the impact of next generation probiotics (NGPs) on the growth and survival of *C. jejuni* in vitro. Our goal is to develop probiotics as antibiotic-alternative therapies to control and treat *C. jejuni* infections in both animals and humans.

Methods: We screened 38 different probiotic strains to assess their effect on the growth of *C. jejuni* using an agar well diffusion assay. Probiotics demonstrating the highest growth inhibition of *C. jejuni* were selected for further in vitro studies. The selected probiotics were evaluated for their ability to prevent biofilm formation and disrupt pre-formed biofilms. Additionally, their effects on *C. jejuni* adhesion, invasion, and survival in human intestinal cells were examined. We also assessed the auto-aggregation and coaggregation properties of these probiotics. Similarly, RT-PCR was used to analyze the effect of the probiotics on the expression of virulence-associated genes including biofilm, invasion, motility and toxin production. All the experiments were conducted at least twice, and results were analyzed using two-way ANOVA for multiple comparisons between probiotic-treated and non-treated C. jejuni. We used Tukey to test for the significance.

Results: All probiotics showed inhibition for *C. jejuni* growth using agar well diffusion assay with different levels, however, we selected the best seven best probiotics strains that showed the highest efficacy. All the selected candidates significantly inhibited ($p<0.05$) the growth of *C. jejuni* by up to 5 folds within 24h and complete clearance by 48h of co-culture. They also inhibited the growth of other *Campylobacter* strains like *C. fetus*, *C. lari*, *C. hyointestinalis*, and *C. coli*. Four out of the seven candidates inhibited up to 100% of C. jejuni's biofilm formation and pre-formed biofilms. Interestingly, all the 7 candidates significantly ($p<0.05$) inhibited adhesion (up to 2 folds), invasion (up to 3.5 folds), and all 7 candidates cleared intracellular C. jejuni from human intestinal cells after 24 hours. Additionally, all 7 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 evaluating the impact of different concentrations of the probiotics on *C. jejuni* growth. We will focus on understanding how probiotics modulate their action on intestinal cells and focus on evaluating the best two probiotics 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 is supported by the U.S. Department of Agriculture (USDA) National Institute for Food and Agriculture (NIFA) (grant number 2022-09086), and the Center of Biomedical Research Excellence for Translational Chemical Biology (COBRE, NIH P20 GM130456).

Notes:

P020 - Effects of antimicrobial treatments on the microbiome and resistome of brood mares

Hannah L. Carter[1], Cory A. Wolfe[1], Enrique Doster[1], Charlotte Pipkin[2], Hannah Looman[3], Jessica Looman[3], Gregg O. Veneklasen[3], John L. Pipkin[4], Maggie M. Murphy[1], Eryah Mora[3, 4], Lee J. Pinnell[1], Paul S. Morley[1]

[1]Texas A&M University, VERO Program, [2]West Texas A&M University, [3]Timber Creek Veterinary Hospital, [4]Texas Tech University. hlc5746@tamu.edu

Session: Antimicrobial use, **2025-01-20, 6:00 - 8:00**

Objective: Endometritis is commonly associated with infertility in mares, and antimicrobial drugs are frequently administered by uterine infusion post-breeding to minimize infections. The goal of this study was to assess how post-breeding treatment of mares with antimicrobial drugs affects the resistome and microbiome.

Methods: Twenty-one reproductively healthy brood mares that were nongravid at least since the previous foaling season were randomly assigned to receive 1 of 3 treatments on the day after insemination: 1) 60 ml uterine infusion with saline and sodium bicarbonate containing 2 g gentamicin sulfate, 2) 60 ml uterine infusion with saline and sodium bicarbonate, and parenteral treatment with ceftiofur crystalline free acid (4g IM), or 3) 60 ml uterine infusion with saline and sodium bicarbonate, without any antibiotic treatment. Fecal samples, and uterine and vaginal swabs were collected at 3 time points: before breeding, 2 d post-treatment, and 5 d post-treatment. After DNA extraction from samples, target-enriched shotgun sequencing was used to characterize all antimicrobial resistance genes (ARGs) in samples, and 16S rRNA amplicon sequencing was conducted to characterize the microbiome. Bioinformatics were conducted using AMR++ pipeline and Qiime2, respectively, and statistical analyses were conducted using R. Microbial communities were compared among treatment groups using PERMANOVA and PERMDISP tests. Differences in richness and diversity were analyzed using Wilcoxon Ranked-Sum tests.

Results: As expected, microbial abundance was much greater in fecal samples than vaginal and uterine swab samples, which was reflected by similar trends for richness and diversity of microbial communities and ARGs. However, there were no statistically significant differences among treatment groups for richness, diversity or community structure for the microbiome or the resistome.

Conclusions: A single uterine infusion with gentamicin or parenteral ceftiofur treatment were not associated with significant impacts on ARG populations or microbial communities.

Finanical Support: Texas A&M University

Notes:

P021 - Quantifying the effects of three different metaphylactic drugs on the prevalence of Pasteurellaceae throughout the feeding period

Ethan Dudley[1], Robert Valeris-Chacin[1], John Dustin Loy[2], Rand Broadway[3], Kristin Hales[4]

[1]VERO Program, Texas A&M University, [2]University of Nebraska-Lincoln, School of Veterinary Medicine and Biomedical Sciences, [3]United States Department of Agriculture, Agricultural Research Service, Livestock Issues Research Unit, [4]Department of Animal and Food Sciences, Texas Tech University. edudley01@exchange.tamu.edu

Session: Antimicrobial use, **2025-01-20, 6:00 - 8:00**

Objective: Bovine Respiratory Disease (BRD) is a significant economic and animal welfare burden on the U.S. feedlot industry. A common prevention and control method for BRD is antimicrobial metaphylaxis: antimicrobial treatment given to animals upon arrival to the feedlot with the goal of preventing BRD from occurring. Many studies have shown that metaphylaxis can affect the isolation frequency of BRD pathogens, but do not encompass the entire feeding period. Therefore, the objective of this study was to evaluate the effects of three drugs commonly used for antimicrobial BRD metaphylaxis on the isolation frequency of members of the family Pasteurellaceae that are associated with BRD throughout the feeding period. Frequency of a non-BRD associated bacterial family were also assessed by examining isolation rates of Moraxella spp.

Methods: Cattle at high risk of BRD (n=246) were randomized at arrival to one of the four experimental groups (tulathromycin, ceftiofur, florfenicol, and negative control), and penned by group. Nasal swab samples were collected from both nostrils using a 5" flocked swab in liquid Amies transport media, longitudinally at arrival, after one month on feed, and immediately prior to slaughter (~245 days). Nasal swabs were streaked onto tryptic soy agar with 5% sheep's blood, chocolate agar, and MacConkey agar and incubated for 48 h at 37°C with 5% CO_2 supplementation. Samples were examined at 24 and 48 h after inoculation and colonies with morphology consistent with Mannheimia haemolytica, Pasteurella multocida, and Histophilus somni or Moraxella spp. were subjected to MALDI-TOF MS testing validated for definitive identification of these bacteria by an accredited laboratory. A generalized estimating equations multinomial logistic regression model, regressing the isolation of bacteria (collapsed as Pasteurellaceae, Moraxella, others, and no isolation) on metaphylaxis treatment, sampling event, the interaction between metaphylaxis treatment and sampling event, and block, were performed using STATA 18.5 MP. Robust standard errors were utilized to adjust the variance for the clustering at the animal level.

Results: Pasteurellaceae were isolated in 42.6% (308/723) of the samples, Moraxella in 24.48% (177/723) of the samples, other bacteria were detected in 5.67% (41/723), while 27.25% (197/723) of the samples had no bacterial isolation. An overall metaphylaxis effect was observed (p value<0.0001). At arrival, the florfenicol group had a significantly higher proportion of Pasteurellaceae ($p<0.05$) in comparison with the control and tulathromycin group (0.49 versus 0.31 and 0.32, respectively). After one month on feed, there was no statistically significant difference between the experimental groups (proportions ranging from 0.48 to 0.53; $p>0.05$). At the end of the feeding period, the florfenicol and tulathromycin groups had a significantly lower proportion of isolated Pasteurellaceae ($p<0.05$) compared with the ceftiofur group (0.32 and 0.28 versus 0.52, respectively). There was no statistically significant difference between the experimental groups in the proportion of Moraxella.

Conclusions: Our results suggest that the isolation frequency of Pasteurellaceae is significantly affected by time post administration and the drug used for metaphylaxis. Florfenicol and tulathromycin showed a long-term effect on the isolation of Pasteurellaceae by the end of the feeding period.

Financial Support: Funding was provided by the U.S. Department of Agriculture; National Institute of Food and Agriculture, Grant/Award Number: 2020-68015-30857.

Notes:

P023 - Effect of icing on proximate composition and microbial quality of *Hypophthalmichthys molitrix* during frozen storage

Shelly.S. Yaqub[1]

[1]Quality Control Lab. Shellyyaqub@gmail.com

Session: Aquaculture, **2025-01-20, 6:00 - 8:00**

Objective: The present study was to assess the effects of pre-freezing ice storage and duration of frozen storage on the proximate composition and microbial parameters of fish.

Methods: Thirty-six pond-raised *H. molitrix* were degutted and divided into three groups with three samples from each fish. Samples in Group 1 were immediately frozen at −18ºC, and those in Group 2 and Group 3 were stored in ice for 3 and 5 days, respectively, before freezing. Independent factorial design was used for the experiment with three levels for pre-freezing ice storage of fish (0 days, 3 days, and 5 days) and four levels for frozen storage (0 days, 30 days, 60 days, and 90 days). Dependent variables were proximate composition (moisture, ash, protein, and fat), (Association of Official Analytical Chemists, (AOAC)), 2012 and microbial quality (total coliform, aerobic plate count, *E. coli*, and fecal coliform of fish muscles, AOAC (2005).

Results: Microbial count and protein analysis was conducted for fish as measured in different groups. This included the initial Aerobic Plate Count (APC) of fresh fish increased significantly. In samples of Group 1, APC increased after 30 days of freezing with a subsequent decrease after 60 days and an increase after 90 days. In Group 1 and Group 3, the initial increase in Total Coliform, Fecal Coliform, and *E. coli* was observed after 30 days of frozen storage (27.0 MPN/g ± 22.6 MPN/g, 17.0 MPN/g ± 8.5 MPN/g & 17.0MPN/g ± 8.48MPN/g in Group 1 and 745.0 MPN/g ± 614.9 MPN/g, 742.67 MPN/g ± 618.92MPN/g&742.67 MPN/g ± 618.92 MPN/g in Group 3) was followed by a decrease after 60 days with subsequent increase after 90 days. The coliform count in Group 2 increased after 60 days. The protein content decreased after the 60-day period whereas it decreased significantly after the 90-day period. In samples of Group 3, the initial protein content (20.09% ± 0.44%) of fish muscles significantly decreased to 17.71% ± 0.87% ($p < .05$) after 60 days of frozen storage that further reduced to 17.10% ± 0.28% after 90 days. Samples in Group 1(21.10±0.36% to 19.22±0.58%) and Group 2 (19.88%±1.04% to 17.10±0.28) showed significantly reduced protein content after 90 days of freezing.

Conclusions: Results of the present study have led to the conclusion that an increase in pre-freezing ice storage enhances the degradation of protein in fish muscles. Frozen storage following ice storage cannot terminate the ongoing enzymatic and microbial spoilage. The results of muscle degradation in the form of low protein content have been manifested at an earlier stage if fish has been stored in ice for a longer period. Moreover, an increase in ice storage can also lead to increased microbial count in fish muscles during freezing.

Notes:

P024 - Assessment of recirculating aquaculture for embryonic and larval culture for burbot (*Lota lota*)

Luke Oliver[1], Trevor Strickland[1], Ryan Maxwell[1], Veronica Myrsell[1], Spenser Stenmark[1], Joseph Evavold[1], Kenneth Cain[1, 2]

[1]University of Idaho, [2]Noaa. loliver@uidaho.edu

Session: Aquaculture, **2025-01-20, 6:00 - 8:00**

Objective: As the aquaculture industry expands in the United States, investigation of the commercial aquaculture potential of novel species is imperative as this may allow producers to provide unique products for market. Burbot (Lota lota), demonstrates potential for commercial production in northwestern states, all life stages of this cool to cold-water species can be cultured in captivity and grow-out culture conditions are shared with rainbow trout (Oncorhynchus mykiss). Burbot offers a thick flake white fillet, delicacies such as roe and liver, and its skin can be used as a leather product. Finally, L. lota is refractory to many salmonid pathogens, offering an option to diversify existing trout production operations. Recent burbot research at the University of Idaho has evaluated commercial diet efficacy, reproductive control, and immunological monitoring; the results have provided insight to improve and expand burbot culture. Conventional burbot egg incubation and larval culture practices utilize single pass aquaculture methodology; however, recirculation technologies may be a viable method to improve sustainability and increase viable farming locations.

Methods: Thus, a set of experiments were conducted to characterize egg and larval survival within recirculating (RAS) and flow through (FT) systems. Six treatments were utilized: 1) flow-through (FT-NILL), 2) flow-through with hydrogen peroxide treatment (FT-H), 3) flow-through with UV treatment (FT-UV), 4) recirculating (RAS-NILL), 5) recirculating with hydrogen peroxide treatment (RAS-H), and 6) recirculating with UV treatment (RAS-UV). Initial experiments served to refine methods and yielded preliminary survival data.

Results: Highest egg survival was observed in treatments FT-H, FT-UV, and RAS-H, which were all statistically similar ($p = 0.05$). The highest larval survival was observed in FT-H, FT-UV, and RAS-NILL, which were all statistically similar ($p = 0.05$).

Conclusions: Together, the egg and larval survival indicate that RAS may be a viable approach for burbot early life history culture. Refined experiments will be conducted in spring of 2025 and will also include examination of culture microbiomes. The results from this project will be of interest to commercial cold-water aquaculture producers and agencies utilizing burbot for management applications, as this may serve to improve the water resource use for burbot early life history culture.

Finanical Support: This project was supported by Agriculture and Food Research Initiative Competitive Grant no. 2024-67012-41932 from the USDA National Institute of Food and Agriculture.

Notes:

P025 - Molecular mechanisms of interspecies interactions in mitigating vibriosis in bivalves

Arvie Grace Masibag[1], Rachel Cheney[1], Elise LePage[1], Ololade Gbadebo[1], Marta Gomez-Chiarri[2], David Rowley[1]

[1]Dept. of Biomedical and Pharmaceutical Sciences, University of Rhode Island, [2]Dept. of Fisheries, Animal and Veterinary Sciences, University of Rhode Island. drowley@uri.edu

Session: Aquaculture, **2025-01-20, 6:00 - 8:00**

Objective: Novel tools are needed to prevent disease outbreaks at aquaculture facilities. Marine bacteria from the genus Phaeobacter have emerged as promising additives to prevent diseases in larviculture. Phaeobacter inhibens S4 (S4), isolated from a healthy oyster (Crassostrea virginica), protects larval and seed oysters against bacterial infections and has proven safe for use in oyster hatcheries. Understanding the responsible mechanisms for favorable interspecies interactions between host, pathogen, and Phaeobacter is important for optimal delivery and effectiveness in hatcheries. Our investigation aims to elucidate the molecular mechanisms that enable disease prevention in oyster larvae by S4. Our previous studies have demonstrated that S4 protects oyster larvae against vibriosis by multiple mechanisms of action, including antibiotic production, biofilm formation, repression of virulence factor production, and immune modulation in the host. Here, we characterize membrane vesicles (MV) produced by S4 and the oyster pathogen Vibrio coralliilyticusRE22 and show that they reduce biofilm production by their competitor.

Methods: MVs were isolated from 24-hour cultures of V. coralliilyticus RE22 and P. inhibens S4 using ultracentrifugation and characterized by nanoparticle tracking particle analysis and electron microscopy. Biofilm assays were first conducted in 96-well plates using a crystal violet staining method. A second approach measured biofilm formation on sterile coverslips using confocal microscopy. For confocal fluorescence imaging microscopy, biofilms were stained with Film Tracer (live/dead viability), and photomicrographs (z-step: 2 μm) were acquired at 40× magnification using a Nikon Eclipse Ti2 inverted confocal microscope. Maximum intensity projections of the green (488 nm) and red (561 nm) channels were generated for each z-stack and merged using ImageJ. Biofilm Viability checker and COMSTAT1 were used to determine the percentages of live and dead cells and biofilm biomass, respectively.

Results: Biofilm formation by RE22 was reduced by ~40% ($p < 0.001$) during exposure to S4 MVs (2.5 x 10^8 particles/mL). Furthermore, live/dead staining showed that exposure to S4 MVs increased the proportion of dead cells by 10% ($p < 0.01$) compared to untreated controls. Biofilm formation by S4 was reduced by 40-60% ($p < 0.001$) during exposure to RE22 MVs (9.2 x 10^7 particles/mL). No changes were observed in the percentage of dead S4 cells versus an untreated control.

Conclusions: Biofilm production was substantially reduced when either bacterium was exposed to MVs produced by the other, suggesting a contributing mechanism for host protection and pathogenesis. Future studies will define the components of MVs that limit this critical host colonization phenotype.

Financial Support: This research was supported by project award number 2023-67016-39712 from the U.S. Department of Agriculture's National Institute of Food and Agriculture.

Notes:

P026 - Time series and risk factor analysis of valley fever in Arizona: Application of machine learning tools

D Areda[1]

[1]Ottawa University, demelash.biffa@gmail.com

Session: Artificial intelligence, **2025-01-20, 6:00 - 8:00**

Objective: Valley fever is a chronic respiratory disease of significant public health concern in Arizona. Over the past decade, the incidence of valley fever is believed to have risen, driven largely by demographic, environmental, and climatic factors. Capturing of an interplay of these factors entails use of advanced analytical tools as conventional epidemiological approaches have limitations to perform that. The objective of this study was to use machine learning (ML) tools to forecast valley fever cases from Arizona and identify key risk factors contributing to spread of the infection.

Methods: Data on Valley fever cases, incidence rate, climatic, demographic and health records were collected from various local, state and federal institutions. Data was analyzed using descriptive and analytical statistics. Time series forecasting of valley fever cases was performed using various machine learning tools namely Random Forest, Support Vector Regression**,** Linear Regression and SARIMA.

Results: The results demonstrated that ML models forecast an increasing trend in Valley Fever cases in AZ, supported by time series decomposition, which shows a clear upward rise over the years. Seasonality assessment revealed that while there are fluctuations, no consistent seasonal pattern is observed within a year**.** There was strong evidence of heteroskedasticity ($P<0.001$), indicating that the variability of Valley Fever cases changes over time, with periods of varying volatility. Autocorrelation (ACF) plot revealed significant autocorrelation, indicating that Valley Fever cases in a given month are influenced by cases in previous months. Based on key model comparison metrics (Mean Absolute Error (MAE), Root Mean Squared Error (RMSE) and Mean Absolute Percentage Error (MAPE), Ransom Forest model performance well with the data. Higher levels of PM10 were associated ($R^2=0.3$) with more valley fever cases. More severe droughts might slightly reduce valley fever cases ($R^2=-0.17$). older populations (>65 years) were increasingly vulnerable to valley fever ($R^2=0.85$), and the overall number of valley fever cases for all age groups over the study duration (2000 to 2022) was rising ($R^2=0.63$).

Conclusions: this study used machine learning (ML) models to forecast Valley Fever cases in Arizona and identify key risk factors, revealing an increasing trend over the years. Random Forest performed best among the models, and higher levels of PM10 were associated with more Valley Fever cases, while severe droughts were linked to a slight decrease.

Notes:

P027 - Artificial intelligence enhanced automated prevalence estimation of digital dermatitis affected cattle at harves

M. Baerwolf[1], M. Henige[1], F. Valle[1], D. Döpfer[1]

[1]University of Wisconsin, School of Veterinary Medicine. mbaerwolf@wisc.edu

Session: Artificial intelligence, **2025-01-20, 6:00 - 8:00**

Objective: Digital Dermatitis (DD), the second most treated condition in feedlot cattle, is the leading cause of lameness within the cattle industry. The painful lesions associated with this disease result in decreased animal welfare and poor productivity. The losses caused by decreased production rates and treatment expenses result in major economic challenges for the industry. Changes in management practices and environmental variables have been used to help prevent the incidence of DD, however, the prevalence of DD affected beef cattle at harvest is unknown. Without an accurate, real-time estimation of DD prevalence, it is difficult to determine the effectiveness of any preventative measures. The objective of this study is to develop a multi-step computer vision (CV) model that uses object detection and image classification to identify DD lesions in cattle at harvest in order to estimate DD prevalence in real time.

Methods: Video footage of beef cattle arriving at a Midwestern slaughter facility was collected over a nine-month period of time. The slaughter facility harvested an average of 480 head of cattle per day from roughly 460 different producers within approximately a 100-mile radius. All cattle were Black Angus mixed breeds and weighed between 1000 and 1800 lbs. Camera footage was obtained at ground level near the unloading ramp at the slaughter facility. Video clips were chopped into images at a rate of 15 frames per second (FPS) and all visible feet were annotated. A YOLOv5 model was trained for detection of feet and designed to extract images of each foot detection. The newly extracted images of feet detections were then sorted into three categories: presence of DD, no presence of DD, and non-foot object or poor-quality image. A resnet-50 image classification model was trained to classify images based on the three pre-defined categories. The two CV models were then pipelined together in order to create a single CV model capable of performing object detection on cattle feet and then directly feed images of each detection to the image classification model for detection of DD lesions.

Results: Successful application of the full CV model on video footage demonstrated its capability to make real-time detections and perform multiple CV tasks in a progressive, stepwise manner. The YOLOv5 object detection model achieved a mAP50 of 0.90 with a precision of 0.835 and recall of 0.89 for all classes. The resent-50 image classification model achieved a validation accuracy of 0.6491.

Conclusions: These results demonstrate the ability to create a multi-step, real-time computer vision model capable of the detection and classification of DD lesions in cattle at harvest. The addition of more images and various stages of DD will help improve the accuracy of our model. Once accuracy benchmarks have been achieved, prevalence estimations can be calculated from the detection results of the model. Once complete, this model could be implemented at a large scale to help determine the effectiveness of preventative measures against DD for dairy farms and feedlots globally.

Financial Support: Funding support for M.H. was provided by the National Institutes of Health through the Comparative Biomedical Sciences Training Grant T32OD010423. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the NIH.

Notes:

P029 - Artificial intelligence-assisted detection of water usage by sprinkler on a dairy farm

Fernando Valle[1], Kelly Anklam[1], Doerte Doepfer[1]

[1]University of Wisconsin – Madison. fvalle2@wisc.edu

Session: Artificial intelligence, **2025-01-20, 6:00 - 8:00**

Objective: Water conservation is a critical concern, particularly in the context of dairy farming. There has been increasing interest in leveraging artificial intelligence (AI) to optimize water usage, specifically concerning the use of sprinklers to cool cows in high-temperature environments. Our study delves into the potential of AI-assisted detection systems to monitor and manage water consumption for this purpose. We explore the feasibility of employing a computer vision (CV) model with object detection capabilities to identify the presence or absence of cows in areas where the sprinklers are active, to improve water efficiency and promote sustainable practices in dairy farming.

Methods: The dataset for training an object detection model originated from three fisheye cameras installed above a pen of 100 x 25 meters with 400 cows at a Midwestern commercial dairy farm in the USA. The video footage is 2 hours long each from the three different camera's locations with 33 meters between each camera at 10m height in the ceiling of the barn was used to train a Yolov5s model. The model was trained with 1700 epochs and a batch size of 16 for the data set of 200 label images. The sprinkler operated on the commercial dairy farm required 2.83 liters of water per minute during their operation. The sprinkler system from Seneca Dairy Systems has been designed to activate water sprinklers based on ambient temperature. Specifically, at temperatures exceeding 22.2°C, the system is programmed to run sprinklers for one minute at 15-minute intervals. At higher temperatures exceeding 32.2°C, the system adjusts the sprinklers to run for one minute at 5-minute intervals. The model was trained to identify the presence and absence of cows and manage the water usage based on the presence of cows.

Results: The successful application of the object detection model on video footage demonstrated that it is capable of real-time decision-making. The resulting YOLOv5s prediction performance after training the model for 1700 epochs had an overall mAP of 0.940. Given its high accuracy and real-time decision-making capabilities, the model has the potential to conserve up to 75% of water.

Conclusions: These results demonstrate the benefits of incorporating an AI-enhanced sprinkler solution. Additionally, we observed that cows could be guided to certain headgates with the help of AI. In this case, AI aids in making informed decisions regarding sprinkler usage and the availability of space for heat abatement of cows in the bar for future use on dairy farms. Customizing water dispensation to the cows' barn locations will save water while preventing heat stress. This saves resources, and costs, and prevents cows from suffering from heat stress.

Financial Support: We want to thank the producers, herdsmen and their dairy cows for supporting this project.

Notes:

P030 - Typing of clinical *Pasteurella multocida* isolates from goats from the midwestern United States

Andrea Haluch[1], Karen LeCount[1], Tammy Anderson[1], Alan Hassall[2], Brenda Morningstar-Shaw[1], Amanda Kreuder[2]

[1]National Veterinary Services Laboratory, [2]Iowa State University. andrea.haluch@usda.gov

Session: Bacteriology, **2025-01-20, 6:00 - 8:00**

Objective: Pasteurella multocida is an opportunistic bacterium found in the upper respiratory tract of most mammals, including goats, and can cause bacterial bronchopneumonia following stressful events or viral infections. The serotypes and genotypes of P. multocida affecting goats are not well documented in the literature. Goats are most frequently vaccinated using a cattle bacterin which helps protect against serovar 3. Understanding the diversity of Pasteurella multocida is vital to understanding disease dynamics and improving vaccine design, particularly in understudied species such as goats.

Methods: Weaned goats of mixed breeds were sourced from multiple states and shipped to a central Iowa feedlot as part of a randomized control trial for treatment of bacterial bronchopneumonia. Deep nasopharyngeal samples were collected at the time of respiratory disease diagnosis, and lung swabs were taken from euthanized animals deemed to be treatment failures. Out of 99 enrolled animals, 94 P. multocida isolates were identified (nasal and lung, 47 each) via routine culture followed by MALDI-TOF. Isolates from animals with P. multocida identified from both nasal and lung swabs (22 nasal and 27 lung isolates) were selected to be serotyped and genotyped. Serotyping was done using the gel diffusion precipitin test (GDPT) and classified by Heddleston's method (serovars 1-16); the assay was repeated a second time for isolates that were not typeable. Restriction endonuclease analysis (REA) was used to compare and analyze DNA fingerprints of each isolate using the enzyme Hhal I.

Results: 4/22 nasal isolates (18.2%) were found to be serovar 3; in contrast, 15/27 lung isolates (55.6%) were found to be serovar 3. The remaining 30 isolates (30/49; 61.2%) did not serotype using Heddleston's method. A total of 13 unique DNA profiles were found, with 20 (40.8%) of the isolates belonging to a single profile (internally designated as G1). Of these 20 isolates, 11 (55%) serotyped as serovar 3 while the remaining 9 were not typeable. When comparing nasal and lung isolates from the same animals, 11/22 (50%) nasal and lung pairs had identical DNA profiles.

Conclusion: Generating this information is vital for vaccine design and understanding the epidemiology of disease caused by P. multocida in goats. Serovar 3 was the most common serotype identified, although most isolates did not serotype as one of the 16 Heddleston types using the GDPT method. REA fingerprinting revealed similar profiles between nasal and lung isolates in half of the animals studied despite a lack of congruency in serotyping, warranting further study.

Financial Support: USDA National Veterinary Services Laboratory, and Iowa State University

Notes:

P031 - Characterizing the O-serogroups of Avian Pathogenic *E. coli* (APEC) associated with colibacillosis in Georgia poultry

Catherine Logue[1], Klao Runcharoon[1], Bella Garcia[1], Breck Peterson[1], Margaret Favro[1], Meaghan Young[1], Nicolle Lima Barbieri[1], Doug Waltman[2], Briget Flores[2], Emily Dinh[2]

[1]University of Georgia, [2]Georgia Poultry Lab Network. catherine.logue@uga.edu

Session: Bacteriology, 2025-01-20, 6:00 - 8:00

Objective: Avian pathogenic *Escherichia coli* (APEC), the causative agent of colibacillosis is a significant cause of poultry disease worldwide. Colibacillosis often presents as systemic or localized infections such as airsacculitis, septicemia, pericarditis, perihepatitis, salpingitis, and cellulitis in chickens. Serotyping based on somatic O-antigen detection is used to classify *E. coli* into different serogroups. Currently, there are approximately 180 O- serogroups described with O1, O2, and O78 being most commonly linked with disease. However, there is limited information on APEC O-serogroups in Georgia poultry. This study sought to identify APEC serogroups causing disease in Georgia poultry and characterize any new emerging APEC strains. Additional work has sequenced novel APEC genomes, assessed pathogenicity of novel strains in poultry disease models and their potential risk for human disease.

Methods: All APEC isolates examined (n=569) were recovered from poultry diagnosed with colibacillosis in the State of Georgia between March 2021 and March 2022. Isolates were collected from different bird types and lesion sites/tissues. Isolates were analyzed and screened for O-serogroups using multiplex polymerase chain reaction (PCR). Notably, a new Klao9 SeroPCR panel was developed to target common APEC serogroups from diagnostic cases and validated using a collection of isolates from 2023 (n=63). Moreover, genes associated with APEC virulence (*cvaC, iroN, ompT, hlyF, etsB, iss, iutA, ireA,* and *papC*) were assessed in all APEC using multiplex PCR.

Results: Overall, 339 isolates of 569 isolates (~60%) examined were classified into one of 32 single O serogroups. The most prevalent serogroups identified included O78, O2, O25, O8, O1, O86, O18, and O15. Each bird type had different APEC serogroups contributing to disease including serogroups O8, O2, O78, and O25 which constituted the majority of serogroups detected in broilers and broiler breeders; O1 was detected at a higher prevalence in broiler breeders. Only three serogroups (O2, O8, and O18) were detected in commercial layers. Of interest, O2, O25, and O8 were the most common serogroups detected in pet/hobby birds. Serogroup O1 had a significantly higher prevalence during Summer and Fall than in Spring, while O86 was more prevalent in the Winter than in the Spring ($P<0.05$). The prevalence of the VAGs detected, and genes associated with the ColV plasmid (*iroN*, *ompT*, *hlyF*, *iss*, and *aerJ*) were detected at a higher prevalence than other genes ranging from 80-96% among all isolates examined. The number of VAGs detected per isolate ranged from 0 (23.2%) to as many as 9 (0.88%). The Klao9 SeroPCR was able to identify common serogroups in about 36% of isolates targeting O1, O2, O8, O18, O25, O78, O88, O103, and O119.

Conclusions: This study identified 32 APEC serogroups among APEC isolates (n=569) from Georgia poultry. Many associations were observed between serogroups, phylogenetic groups, VAGs, bird types, and seasons. A new Klao9 SeroPCR was developed and validated, and serogroups were identified for 36% of isolates. This assay can be used as a rapid screening tool for APEC serogrouping. This study provides unique insight on APEC causing disease in one of the nation's largest poultry producing regions.

Financial Support: This research was supported by the National Institute of Food and Agriculture under Award Number #: 2022-67015-36878.

Notes:

P032 - Response of *Escherichia coli* O157 to bovine bile under aerobic and anaerobic conditions

Joel J Maki[1], Randy Ortiz[1, 2], Nathan Peroutka-Bigus[1, 3], Crystal L Loving[1]

[1]USDA-Agricultural Research Service, [2]Iowa State University, [3]Oak Ridge Institute for Science and Education. joel.maki@usda.gov

Session: Bacteriology, **2025-01-20, 6:00 - 8:00**

Objective: *Escherichia coli* O157 continues to be an important food safety pathogen responsible for significant human disease and economic impacts to the agricultural industry. *E. coli* O157 asymptomatically colonizes the gastrointestinal tract of ruminants, especially cattle. As part of the initial colonization process, *E. coli* O157 must sense and respond to various chemical stimuli within the bovine gut, priming it for successful establishment. One commonly encountered chemical signal is bile. Bile is an important indicator for many enteric bacteria, signaling arrival into the gastrointestinal tract and leading to shifts in cell metabolism, expression of colonization factors, and virulence. Another important signal is the oxygen availability in the cattle gastrointestinal tract, which varies both between and within gut compartments and regulates various cellular processes. Understanding the response of *E. coli* O157 to bovine bile under different levels of oxygenation can provide insights into cell responses in different sections within the bovine gastrointestinal tract and potentially lead to the development of targeted interventions to discourage *E. coli* O157 colonization in cattle and other ruminants. Here, we investigated the effects of bovine bile powder on *E. coli* O157 growth and transcriptomic profiles under aerobic and anaerobic conditions.

Methods: *Escherichia coli* O157 strain EDL933 was grown to mid-log phase at 39ºC in tryptic soy broth (TSB) with and without 0.2% (w/v) dried, unfractionated bovine bile (Millipore Sigma) under aerobic and anaerobic conditions. RNA was extracted (RNeasy kit; Qiagen) and submitted to the Iowa State University Genomics Core Facility for RNAseq on an Illumina NovaSeq 6000 instrument. Resultant reads were analyzed using in silico methods to identify genes and pathways that were enriched and/or depleted under the different conditions tested.

Results: The addition of bile powder to culture media impacted growth characteristics of *E. coli* O157 under aerobic and anaerobic conditions. Differentially expressed genes were observed for both the aerobic and anaerobic bile powder-supplemented conditions, mainly involving metabolism and cell response. Interestingly, genes involved in pilus formation and cell adhesion were significantly upregulated in the aerobic cultures exposed to bovine bile while metabolism and quorum sensing pathways were significantly upregulated in the anaerobic cultures exposed to bovine bile.

Conclusions: Growth and transcriptomic profiles of *E. coli* O157 were impacted by the presence of bovine bile, with metabolic and cell adhesion responses differing based on the presence or absence of oxygen. These observations provide important insights into the *E. coli* O157 response to bovine bile under different physiological conditions.

Financial Support: U.S. Department of Agriculture (USDA) Agricultural Research Service (ARS) CRIS project #5030-3200-225-00D, USDA-ARS Research Participation Program of Oak Ridge Institute for Science and Education (ORISE), and SCINet project of the USDA-ARS.

Notes:

P033 - Stimulation of phenotypic expression of macrolide resistance in *Rhodococcus equi* strains

E. D. Levitzki[1, 2], E. Martins[3], A. L. R. S. Pereira[2], B. A. Ottobeli[1], K. P. da Costa[1], L. T. Gressler[1], L. Huber[2]

[1]Laboratório de Microbiologia e Imunologia Veterinária, Instituto Federal Farroupilha Campus Frederico Westphalen, [2]Department of Pathobiology, Auburn University, [3]Laboratório de Bacteriologia Veterinária, Universidade Federal de Santa Maria. eduarda.2019011432@aluno.iffar.edu.br

Session: Bacteriology, **2025-01-20, 6:00 - 8:00**

Objective: *Rhodococcus equi* is a zoonotic soil Actinobacterium agent that causes pyogranulomatous bronchopneumonia in foals and can become endemic in farms. *R. equi* resistance to commonly used treatments in *R. equi* increases the odds of foal mortality and economic losses in the equestrian industry. Moreover, *R. equi* can cause disease in immunocompromised humans making it a public health threat and One health issue. This study aimed to evaluate the behavior and expression of resistance phenotypes by inducing antimicrobial resistance in sensitive strains in vitro.

Methods: We obtained 41 *R. equi* strains stored in lyophilized form, from clinical and environmental samples isolated between 1991 and 2023 from the South of Brazil. Initial antimicrobial susceptibility levels of wild strains were assessed by disk diffusion (DD) and minimum inhibitory concentration (MIC) assays in Tryptone Soy Agar (TSA), following CLSI 2017 guidelines. DD assays used erythromycin (15µg), azithromycin (15µg), clarithromycin (15µg), rifampicin (5µg), doxycycline (30µg), tobramycin (10µg) and gentamicin (10µg) and MIC assays used erythromycin, azithromycin and clarithromycin. For each antibiotic used in the MIC assays, strains were initially cultured in subinhibitory concentrations, based on mean MIC results. Resistance was induced by culturing in Tryptone Soy Broth with increasing antibiotic concentrations for 10 passings. Cultures were incubated with shaking until turbidity was observed. For each passing, purity was assessed by visual inspection of TSA plates and CAMP assays. After final treatment, DD and MIC susceptibility assays were repeated for 4 representative resistance-induced strains. Statistical comparisons between initial and final resistance were made with paired t-tests.

Results: All wild strains were sensitive to all antibiotics tested by DD assay. Most strains were classified as sensitive in the MIC assays, with average of 4, 0.125 and 1µg/mL for azithromycin, clarithromycin and erythromycin, respectively. However, some strains were classified as resistant (MIC >8µg/mL for macrolides), with 8 (19.51%) strains resistant to azithromycin and 16 (39. 02%), 29 (70.73%) and with intermediate resistance to azithromycin, erythromycin, respectively. After the final treatment, 9 strains (21.95%) grew at 40µg/mL for all antibiotics tested. The 4 representative strains had significant increase in MIC for all antibiotics tested, with an increase in average MIC of 120x for azithromycin (p=4.99e-08), 513x for clarithromycin (p=7.363e-10) and 744x for erythromycin (p=1.087e-10), for a final MIC concentration of 512, 64 and 512µg/mL, respectively. Higher concentrations were not tested. The treatments induced the same level of resistance to other antibiotics of the same class. However, treatments with macrolides did not induce resistance to other classes, except for Tobramycin (p=1.455e-05). Interestingly, the same was not observed for the aminoglycoside Gentamycin (p=0.37).

Conclusions: Sensitive *R. equi* strains were readily induced to develop notable levels of resistance by exposure to increasing concentrations of macrolides and rifampicin in vitro. Furthermore, exposure to one macrolide induced resistance to other antibiotics of the same class. However, induction of resistance across classes was not observed. Our results highlight the risks for rapid development of resistance due to overuse of antimicrobials, but further studies are needed to unveil the resistance mechanisms recruited by these strains.

Financial Support: Instituto Federal Farroupilha (Brazil); Department of Pathobiology, College of Veterinary Medicine, Auburn University (US).

Notes:

P034 - Antimicrobial susceptibility in bacteria from clinical samples of dogs from Texas

Saroj Gopali[1], Nancy Zimmerman[1], Marcelo Schmidt[1], Tianxi Ji[2], Babafela Awosile[1]

[1]Texas Tech University School of Veterinary Medicine, Amarillo TX, [2]Texas Tech University. babafela.awosile@ttu.edu

Session: Bacteriology, **2025-01-20, 6:00 - 8:00**

Objective: Knowledge of the common bacterial pathogens causing infections in companion animals and their antimicrobial susceptibility are important in antimicrobial stewardship and guiding optimal empirical and pathogen-specific therapy. Nevertheless, there is still a relatively limited understanding of antimicrobial resistance (AMR) dynamics in companion animal medicine, especially in rural and regional veterinary communities. Therefore, this study aimed to determine antimicrobial susceptibility in the most common bacteria isolated from the clinical samples of dogs from selected veterinary practices in Texas, United States.

Methods: Historical antimicrobial susceptibility data were retrieved from 20 veterinary practices in Texas and associated diagnostic laboratories serving these practices over 15 years (2010-2024). The clinical samples submitted, common bacterial isolates, and associated antimicrobial susceptibilities were presented as proportions using graphical plots.

Results: Bacteria were commonly isolated from ear swabs (38.41%), urine (21.32%), skin (13.11%), and abscesses (5.43%). These clinical samples accounted for 78.27% of the total clinical samples (n=755) submitted for culture and susceptibility testing. Staphylococcus pseudintermedius, Escherichia coli, and Pseudomonas aeruginosa were the most isolated bacteria from the ear swab, urine, and skin samples. Antimicrobial susceptibilities varied within and between the antimicrobial classes and the bacteria tested. Percentage susceptibility in Staphylococcus pseudintermedius (n=195) varied from 0-34.36% to aminoglycoside, 0-45.64% to beta-lactam inhibitors, 0-49.74% to cephalosporins, 0- 46.67% to fluoroquinolone, and 6.67-42.05% to tetracycline. Percentage susceptibility in Escherichia coli (n=113) varied from 0-23.89% to aminoglycoside, 0-35.40% to beta-lactam inhibitors, 0-32.74% to cephalosporins, 0- 23.01% to fluoroquinolone, 0-42.48% to penicillin, and 0-34.51% to tetracycline. Percentage susceptibility in Pseudomonas aeruginosa (n=65) varied from 0-36.92% to aminoglycoside, 0-3.08% to beta-lactam inhibitors, 0-9.23% to cephalosporins, 0- 24.62% to fluoroquinolone, and 0-10.77% to penicillin. Percentage susceptibility in Proteus mirabilis (n= 52) varied from 0-36.54% to aminoglycoside, 13.46-46.15% to beta-lactam inhibitors, 0-34.62% to cephalosporins, 0- 40.38% to fluoroquinolone, 0-36.54% to penicillin, and 0-59.62% to tetracycline.

Conclusions: This study provides information on antimicrobial susceptibility in bacteria isolated from companion animals that are often limited. This is necessary to bridge the gap in knowledge of AMR dynamics and provide the necessary data to support antimicrobial stewardship in regional veterinary settings.

Financial Support: This project was supported by the grant funding from USDA-APHIS under the cooperative agreement for AMR Dashboard Development.

Notes:

P035 - Cannabidiol reduces environmental stress tolerance and downregulates stress-response proteins in *Listeria monocytogenes*

Divya Joseph[1], Leya Susan Viju[1], Kumar Venkitanarayanan[1]

[1]University of Connecticut. divya.joseph@uconn.edu

Session: Bacteriology, **2025-01-20, 6:00 - 8:00**

Objective: *Listeria monocytogenes* (LM) is a pathogen causing foodborne illness in humans. Its resilience to environmental stresses, including cold, low pH, high salt, and heat enables LM to persist in animal food products, posing public health risks. This study determined the efficacy of cannabidiol (CBD), a non-psychoactive compound present in Cannabis sativa, in reducing LM tolerance to aforementioned stresses. Proteomic analysis was conducted to identify the effect of CBD on LM molecular mechanisms mediating stress protection.

Methods: *L. monocytogenes* Scott A (6 log CFU/ml) was cultured in Tryptic soy broth containing 0.6% yeast extract (TSBYE) supplemented with CBD at subinhibitory concentration (SIC: 11.92 µM), 1/32x MIC (71.54 µM), 1/4x MIC (572.39 µM) and MIC (2289.56 µM), along with a control (no CBD) and DMSO solvent control. Stress conditions included salt (5% and 10% NaCl), pH (3 and 5), cold (4°C), and high temperatures (55°C and 65°C). Viable LM populations were enumerated at 8 and 24 hours under salt and pH stresses, on days 1, 3, 5, and 7 under cold stress, and at 2, 15, 30, and 60 minutes after heat stress. Samples yielding no LM were enriched in TSBYE (0.6%) at 37ºC for 24 hours. The experiment was repeated three times with duplicate samples (n=6) and data were analyzed by GraphPad Prism using two-way ANOVA. The effect of CBD on LM proteome (n=3) was analyzed by LC-MS/MS following ultra-high-performance liquid chromatography (UPLC) coupled with Orbitrap mass spectrometry and was statistically analyzed by Limma (R).

Results: Cannabidiol significantly reduced LM survival under all stress conditions in a dose-dependent manner ($P < 0.05$). In presence of 5% and 10% NaCl, CBD at 1/4xMIC and MIC reduced LM counts by > 5 log CFU/ml at 8 hours and to undetectable levels (enrichment negative) at 24 hours ($P < 0.05$). At pH 3, LM counts in control were reduced by 3 log CFU/ml at 8 hours and to undetectable levels at 24 hours. However, all CBD concentrations decreased LM to undetectable levels at 8 hours and 24 hours of incubation ($P < 0. 05$). At pH 5, LM counts were undetectable in the samples containing CBD at 1/4xMIC and MIC. Under cold stress, LM populations in control remained the same throughout the sampling period. On the other hand, LM in the presence of CBD was decreased in a dose-dependent order ($P < 0.05$). Under 55°C, LM counts in controls were decreased only by 1 log CFU/ml after 60 minutes, whereas all concentrations of CBD except SIC reduced LM to undetectable levels. At 65°C for 15 minutes, control samples yielded ~3 log CFU/ml LM. However, in the presence of CBD except at SIC, no viable LM was recovered. Proteomic analysis of LM revealed downregulation of multiple stress-related proteins ($P < 0.05$), including those associated with salt tolerance and osmotic stress, acid tolerance, heat tolerance and cold tolerance.

Conclusions: CBD effectively reduced *L. monocytogenes* tolerance to environmental stresses by downregulating major stress-related proteins, thereby suggesting its potential use for controlling LM virulence in the host.

Notes:

P036 - Prevalence of major bacterial pathogens in liver abscesses of beef-on-dairy cattle

H. Alneaemy[1], R.G. Amachawadi[1], H. Salih[1], T. Mahmood[1], R. Wilson[2], D. Woerner[2], T.G. Nagaraja[3]

[1]Department of Clinical Sciences, Kansas State University, [2]Department of Animal and Food Sciences, Texas Tech University, [3]Department of Diagnostic Medicine and Pathobiology, Kansas State University. alneaemy@vet.k-state.edu

Session: Bacteriology, **2025-01-20, 6:00 - 8:00**

Objective: Liver abscesses continue to be of considerable economic concern to the beef cattle industry. The causative agents, believed to originate from the rumen, include two subspecies of Fusobacterium necrophorum, necrophorum and funduliforme, Trueperella pyogenes, and *Salmonella enterica*. The use of beef cattle semen to breed dairy cows to produce calves, called beef-on-dairy crosses, for beef production has greatly increased in the past 5 years. The practice increases the value of calves produced from dairies, but a major limitation is high incidence of liver abscesses and associated economic losses. The incidence is reported to be 2 to 3 times higher than the conventional feedlot cattle. The reason for the high incidence is not known. Liver abscesses are complex and multifactorial, therefore, demand comprehensive analysis of factors that contribute to the high incidence. Our objectives were to investigate the prevalence of major bacterial pathogens and evaluate risk factors, diet, management, associated with liver abscesses in beef-on-dairy cattle.

Methods: Liver abscesses (n=130) of beef-on-dairy cattle, originating from 5 feedlots, were collected at slaughter. Samples were subjected to anaerobic and aerobic bacterial isolations utilizing enrichment and selective media.

Results: Overall, prevalence of subsp. necrophorum, subsp. funduliforme, T. pyogenes and S. enterica in liver abscesses were 82.3% (107/130), 28.4% (37/130), 30% (39/130) and 7.7% (10/130), respectively. Eighteen (13.8%) samples were positive for both subsp. necrophorum and subsp. funduliforme. Eleven samples (8.4%) were positive for subsp. necrophorum, subsp. funduliforme, and T. pyogenes. We didn't find any statistical difference between feedlots in relation to the prevalence of bacterial species ($P > 0.05$).

Conclusions: The subsp. necrophorum was the most dominant pathogen, which is similar to conventional beef cattle. The prevalence of *Salmonella* was low and were isolated only after enrichment of the samples. Efforts are ongoing to collect additional liver abscess samples and feeding and management data to relate prevalence to feeding and management practices.

Financial Support: This work is supported by the International Consortium for Antimicrobial Stewardship in Agriculture (ICASA) - Foundation for Food & Agriculture Research (FFAR), project award number 22-000448.

Notes:

P038 - Changes in risk tolerance: Behavioral dynamism in a biosecurity adoption game

Jackson Dean[1], Richmond S Baye[1], Eric Clark[1], Scott Turnbull[1], Samuel 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 and infection control, **2025-01-20, 6:00 - 8:00**

Objective: This study investigates livestock producers' complex decision-making over whether to invest in biosecurity measures. Animal disease is responsible for billions of dollars in costs to the livestock industry annually. Computational models offer insights into the most effective ways to invest in biosecurity. Such models often rely on simplifying assumptions about producers. In the real world, the tolerance of producers for risk changes over time based on their observations and also their learning. When considering policy and other interventions to prevent the outbreak of animal disease, behavioral dynamism should be carefully considered. We investigate these dynamics in a biosecurity-adoption game where players must make decisions to invest in biosecurity. The game simulates real-world challenges by incorporating financial costs for investments and the potential for indemnity payments if infection occurs. With 20 distinct treatment conditions, determined by varying levels of indemnity payments, biosecurity thresholds, and trucking network connectivity, this study examines how these factors influence decision-making and learning. By analyzing players' investment behaviors and how they change over time, especially before and after infection events, the study aims to uncover patterns in biosecurity adoption that could inform policy strategies to enhance biosecurity practices and better manage disease risks in agriculture and animal health industries.

Methods: We gathered data from a serious game where players make biosecurity investment decisions and balance costs against the risk of infection. There were 20 treatment conditions created by combining three levels of conditional indemnity payments, one unconditional indemnity condition, and three levels of biosecurity thresholds for indemnity eligibility. Each of these conditions is further divided by high and low trucking network connectivity. Players could either be infected with a high- or low- consequence disease. Player behavior was tracked in continuous time, particularly focusing on when players decided to invest in biosecurity. We analyze the data using techniques from reinforcement learning to determine how players are learning over time**.**

Results: The data show that although most (63.63%) of participants stayed within the same biosecurity investment index range between rounds, the remaining 36.37% of participants decided to change their level of biosecurity investment based on the treatment condition, the spread of disease, or their own learning. This was moderated by whether the participant was infected in the last round. In rounds after they were infected with the common disease, they changed their biosecurity investment strategy 45.71% of the time. We also found that players demonstrated learning by investing in more biosecurity in rounds after being infected and behaved more similarly to the optimal strategy.

Conclusions: This study reveals behavioral dynamism in a biosecurity adoption game. The results contribute to understanding human behavior in animal disease control and suggest actionable insights for improving biosecurity policy and reducing the spread of infectious diseases. Future work should investigate more sophisticated models of human behavior and apply these findings to other models of biosecurity investment.

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

Notes:

P039 - Safeguarding the future of academic research: Innovations in biosecurity and biosafety education for academic excellence

Rachael Weiderman[1, 2], Laura Miller[2, 3], Joseph Whitlock[1, 2]

[1]Department of Environmental Health and Safety, [2]Kansas State University, [3]Department of Diagnostic Medicine and Pathobiology. warhurst@ksu.edu

Session: Biosecurity and infection control, **2025-01-20, 6:00 - 8:00**

Objective: Over half of the research and development conducted at academic institutions is devoted to biological and life sciences discoveries. To mitigate research risks while celebrating discovery, Kansas State University (KSU) Environmental Health and Safety (EHS) Research and Laboratory Safety aims to provide high-quality, value-added education, training, and networking opportunities through two hybrid annual events. These events feature live discussion, poster presentations, and vendor fairs, offering valuable resources and promoting laboratory safety and security for the K-State community and researchers worldwide.

Methods: The Laboratory Safety Bootcamp and Biosafety and Biosecurity Summit are collaborative events focused on providing information, training, and resources related physical and psychological safety, information security, and updates on regulations and emerging research technologies. These hybrid events are held on Kansas State University campus twice per year. Presenters include representatives from government and state regulatory agencies, private industry subject-matter experts, K-State collaborators, partner organizations, and campus faculty and staff. The first event was held in February 2024 and the second event is scheduled for October 2024.

Results: The Laboratory Safety Bootcamp and Biosafety and Biosecurity Summit provide thousands of contact hours of training annually on topics related to laboratory safety, security, and regulatory compliance. The events attract attendance locally and nationally for education and collaboration. Qualitative post-event survey results reveal that faculty, staff, students, industry attendees and community members find the event beneficial, with 100% of respondents indicating expressed interest in attending a future event and a positive net promotor score. Additionally, 50% of attendees expressed interest in similar course offerings for continuing education and university credit. Quantitative post-event metrics include an increase in near-miss reporting, voluntary laboratory audit requests, and increased attendance at post-event safety training offered to campus.

Conclusions: In academia, biological research accounts for greater than 50% of research and discovery. Collectively, we can mitigate the risk of research while still celebrating the necessity and excitement of research discoveries and contributions to our world. At K-State, providing education and resources are key to promoting a safety culture that reduces the risk of injury and infection to researchers, students, and laboratory staff. By furthering knowledge about biosecurity risks and threats, we encourage continued learning and awareness in research laboratories, enabling informed decisions to protect human life and health, security of biological research, and the environment.

Notes:

P040 - Enhancing biosecurity measures to protect Puerto Rico from emerging swine diseases

Kara E. Flaherty[1, 2], B. Alexander Fonseca-Martinez[1], Juan F. Hernandez-Cuevas[1], Andreia G. Arruda[1]

[1]Department of Veterinary Preventive Medicine, The Ohio State University, [2]Department of Animal Sciences, The Ohio State University. flaherty.177@osu.edu

Session: Biosecurity and infection control, **2025-01-20, 6:00 - 8:00**

Objective: African Swine Fever (ASF) was first detected in Haiti and the Dominican Republic in September 2021, leading to the USDA's Animal and Plant Health Inspection Service (APHIS) to designate Puerto Rico (PR) as a protection zone. The Puerto Rican swine industry is primarily composed small-scale, non-commercial, backyard producers. These producers often lack the knowledge needed to implement effective biosecurity practices. There is a need for large-scale, culturally and contextually tailored biosecurity education efforts in order to enhance biosecurity practices across PR. The objectives of this project were to adapt existing resources and develop new materials to guide and educate Puerto Rican swine industry stakeholders on biosecurity practices, and to conduct outreach sessions across PR to create biosecurity plans and gather feedback on the sessions and difficulty of tasks.

Methods: Six 3-hour in-person workshops were conducted in collaboration with USDA staff at various locations across Puerto Rico. Workshop sessions covered initial presentations on emerging swine diseases including ASF and on-farm biosecurity measures. This was followed by hands-on development of standardized biosecurity plans, conducted using the Secure Pork Supply (SPS) framework and adapted to local cultural and contextual specifics. Complete biosecurity plans included a written plan and biosecurity checklist specifying premise characteristics, and a premise map containing 13 key components. Feedback surveys were collected from participants following the workshops. Data was descriptively analyzed.

Results: Ninety-two people attended the three workshops, including 64 producers, 13 animal health officials, and 15 "other". Implemented biosecurity measures were analyzed from 36 written biosecurity plans. The average number of pigs per site was 59 (range: 3-259). 58% of farms reported waste-feeding and 30% of farms reported having multiple species on the premise. Of the site workers, 80% lacked previous biosecurity training. Among all participants, 87.5% did not have a prior biosecurity plan, 36% did not protect property entry points, and 36% possessed a marked and equipped cleaning and disinfection station on the site. Only 6% of biosecurity plans reported having euthanasia equipment for emergencies. Most maps (90-100%) identified a site entry, line of separation, perimeter buffer area, carcass disposal location, parking area, and vehicle movements. 49% of maps marked an animal loading area and only 33% marked a complete carcass removal pathway. According to 20 completed feedback surveys, the most challenging aspect of map completion was drawing the perimeter buffer area (30%). This was followed by identifying the line of separation (10%) and noting access points (10%).

Conclusions: This study identified substantial gaps in biosecurity knowledge and subsequent implementation among Puerto Rican swine producers. Many respondents lacked formal biosecurity training and essential biosecurity infrastructure on site. The training programs were a positive initial step for improving biosecurity knowledge and planning, however, ongoing education and support are essential in improving biosecurity practices across Puerto Rico. Written biosecurity plans and premise maps continue to be collected to understand continued efforts to be utilized for prevention and management of ASF and other emerging biosecurity threats.

Financial Support: We would like to thank our USDA collaborators in Puerto Rico.

Notes:

P041 - Characterization of the respiratory microbiome and virome associated with Bovine Respiratory Disease Complex

Tara McDaneld[1], Bernadette Earley[2], Sara 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], Kenneth Lemon[3], Michael J McMenamy[3], Victoria Smyth[3], Patrick John Collins[3], Paula Lagan[3], Veronica Casement[3], James Devlin[3], Timothy Smith[1]

[1]US Meat Animal Research Center, [2]Teagasc, [3]Agri-Food and Biosciences Institute, [4]North Carolina State University. tara.mcdaneld@usda.gov

Session: Bovine Respiratory Disease, **2025-01-20, 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 2024 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 after infection of calves with BRSV and BHV-1.

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:

P042 - Molecular epidemiology of field *Mannheimia haemolytica* isolates in beef cattle

Nicholas Hacker[1], Matthew Scott[1], Lee Pinnell[1], William B. Crosby[2], Amelia R. Woolums[2], Enrique Doster[1], Paul S. Morley[1], Robert Valeris-Chacin[1]

[1]VERO Program, Texas A&M University, [2]Mississippi State University. nhacker@tamu.edu

Session: Bovine Respiratory Disease, **2025-01-20, 6:00 - 8:00**

Objective: Bovine respiratory disease (BRD) remains a major hurdle facing the North American beef industry. *Mannheimia haemolytica* is currently recognized as the most relevant bacteria in acute BRD. Understanding the genetic determinants that may facilitate BRD pathogenesis is imperative to control and treat BRD in beef cattle. This study used whole genome sequencing (WGS) to characterize the virulence and antimicrobial resistance genes in field *M. haemolytica* isolates from beef cattle.

Methods: Archived *Mannheimia haemolytica* isolates (n = 70) from prior studies were selected for use in this project. DNA extraction from isolates was performed using DNeasy UltraClean microbial kit. Library preparation was performed using the Oxford Nanopore Technologies native barcoding kit v14. Sequencing was carried out using the R10.4.1 flow cells on a Mk1C device. Long read quality was assessed using LongQC. Assemblies were generated using Flye and polished using Medaka. Annotated genomes were obtained with Prokka from the polished assemblies. The pangenome was estimated using Roary. The presence of single nucleotide polymorphisms (SNPs) in the core genome was inferred using Snippy. Antimicrobial resistance and virulence genes were detected using ABRicate and the MEGARes and VFDB databases, respectively. Scoary was used to assess the association between the presence of antimicrobial resistance and virulence genes in *M. haemolytica* isolates and the host characteristics.

Results: We found a wide array of virulence and antimicrobial resistance genes in *M. haemolytica* isolates from beef cattle. In addition, we observed that *M. haemolytica* gene prevalence differs by host characteristics.

Conclusions: This study broadens our knowledge of the genetic determinants of virulence and antimicrobial resistance associated with *M. haemolytica* isolates from beef cattle, further expanding our understanding of *M. haemolytica*'s influence on BRD pathogenesis.

Financial Support: This research was funded by Texas A&M Agrilife Research, Animal Health and Disease Research Capacity Funding FY 24-25.

Notes:

P043 - Gene network analysis of cattle co-infected with BVDV-2 and BHV-1 following vaccination and mineral treatment

Matthew Scott[1], Roberto Palomares[2], M'Lehne Linson[1], Erika Altamiranda[2, 3], Alejandro Hoyos-Jaramillo[2], Joao Bittar[2, 4], Adriana Rodriguez[2], Jose Urdaneta[2], Francesca Granberry[2]

[1]Texas A&M University Veterinary Education, Research, and Outreach Program, [2]Group for Reproduction in Animals Vaccinology & Infectious Diseases (GRAVID), University of Georgia, [3]Consejo Nacional de Investigaciones Científicas y Tecnicas (CONICET), Instituto Nacional de Tecnología Agropecuaria (INTA EEA Balcarce), [4]Department of Large Animal Clinical Sciences, University of Florida. mlehne.krii01@gmail.com

Session: Bovine Respiratory Disease, **2025-01-20, 6:00 - 8:00**

Objective: Common management practices in controlling bovine respiratory disease (BRD) in dairy and beef cattle involve the administration of modified-live virus (MLV) vaccines and/or injectable trace minerals (ITM). While widely utilized, the influence these tools have on immunomodulation and cellular mobilization within bovine lymphoid tissue is largely unknown. This study comprehensively evaluated gene expression patterns from cattle administered MLV vaccines and ITM followed by BVDV2 and BHV1 challenge.

Methods: Twenty-nine male dairy calves (age: ~1 month) previously enrolled in a larger randomized clinical trial were utilized. Twenty-two calves received an MLV intranasal (IN) vaccine containing BHV1, BRSV, and BPI3V and subcutaneous (SC) ITM (Se, Cu, Zn & Mn; ITM, n = 12) or saline (SAL, n = 10). Ten weeks later, calves received a second dose of ITM, or saline, according to previous groups and were randomly assigned to receive the same IN vaccine [ITM-IN (n = 6), SAL-IN (n = 5)] or a SC MLV vaccine containing BHV1, BRSV, BPI3V, BVDV1 & 2 [ITM-SC (n = 6), SAL-SC (n = 5)]. Seven calves did not receive vaccine nor mineral treatment and served as a negative control (UNVAC, n = 7). Forty-nine days after booster, all calves were challenged with BVDV2, and seven days later with BHV1. Calves were euthanized seven days after BHV1 challenge and lymphoid tissues (spleen, tonsil, buffy coat) were preserved in RNAlater and stored at -80°C until processing. Total RNA was extracted from lymphoid samples using RNeasy Mini Kits according to manufacturer instructions. RNA sequencing (NovaSeq 6000; ~50M PE reads/sample) was performed from isolated RNA. Following HISAT2-StringTie2 reference-guided gene assembly, weighted gene co-expression network analysis (WGCNA) was utilized for expression network and gene module construction. Filtered genes were constructed into co-expression modules according to signed biweight midcorrelation, with a minimum of 40 genes in each module, merging modules with intermodular correlations above 0.70, and assigning unique color identifications to each expression module. Module-trait Kendall's tau coefficients were identified between gene expression modules and clinical traits, further considered as weak or strong at $p<0.1$ and $|R|>0.20$ or $p<0.05$ and $|R|>0.30$, respectively. Significantly correlated modules were evaluated for functional enrichments within g: Profiler (FDR<0.05). Hub genes ($|R|>0.30$) driving correlations were evaluated for predicted protein-protein interactions via STRING v12.0 (confidence score>0.500).

Results: One expression module ("yellow") was positively associated with vaccination in splenic tissue; this module possessed genes related to cellular filament organization and development, Notch signaling, and collagen biosynthesis. One expression module ("purple") was negatively associated with trace minerals administration, related to the regulation of T-cell activation, antigen processing and presentation via MHC class II, complement regulation, interferon-γ signaling, and neutrophil degranulation. Several expression modules were associated with clinical illness scores over time, depicting a predicted protein-protein interaction complex centered around ubiquitin C.

Conclusions: These results illustrate associations between lymphoid gene expression patterns following BVDV2 and BHV1 infections and common management practices used to control BRD. These patterns may be leveraged to improve our understanding of immunomodulation and acquired immune response against viral infections involved in BRD.

Financial Suport: This work was financially supported by Axiota Animal Health, the University of Georgia, and Texas A&M University.

Notes:

P044 - The single-cell RNA sequencing of bovine airway cells demonstrates signatures of pneumonia and viral infection

Grace Jakes[1], Dylan Ammons[1], Randy Hunter[2], Ediane Silva[3], Steven Dow[1, 4], Sarah Raabis[4]

[1]Dept. of Microbiology, Immunology, and Pathology, Colorado State University, [2]Hunter Cattle Company, [3]U.S. Department of Agriculture, Agricultural Research Service, National Bio and Agro-Defense Facility, [4]Dept. of Clinical Sciences, Colorado State University. grace.jakes@colostate.edu

Session: Bovine Respiratory Disease, **2025-01-20, 6:00 - 8:00**

Objective: Despite significant efforts to reduce bovine respiratory disease (BRD) prevalence, it remains the leading cause of morbidity and mortality in United States beef cattle. Understanding the pulmonary mucosal immune response to BRD is integral to the development of improved diagnostics and therapeutics. Strategies to study and mitigate BRD are limited by the relative lack of species-specific reagents, and the ability of available methods to characterize the role of individual cell populations. Single-cell RNA sequencing (scRNA-seq) enables the evaluation of transcriptional profiles of individual cells, supporting the identification of unique cell populations and novel cell-type markers. The objective of this study was to evaluate the transcriptomes of airway cells from healthy cattle and cattle with pneumonia using scRNA-seq.

Methods: Stocker calves were purchased at auction and transported to a commercial backgrounding facility. Calves that were exhibiting BRD signs as identified by pen riders (n=5; BRD) were selected for bronchoalveolar lavage (BAL) sampling based upon rectal temperature (RT) >103.5 °F and evidence of lung consolidation on thoracic ultrasound. Healthy calves (n=5; HEALTHY) were selected based on absence of clinical signs of illness, RT <103.0 °F, and absence of appreciable lung consolidation by ultrasound. BAL sampling was performed at the backgrounding facility, and BAL samples were processed using a 10X Genomics Chromium iX platform, with 5,000 cells targeted per sample. Raw sequencing data was aligned to a customized reference including the bovine genome (Ensembl release 113, Bos_taurus.ARS-UCD1.3.113), and viral genomes including bovine respiratory syncytial virus (BRSV), bovine herpesvirus, bovine coronavirus, and bovine viral diarrhea virus using Cell Ranger (version 7.1.0). Downstream processing and analysis were completed in R using the Seurat package (version 5.0.1) with differential expression analysis completed using DESeq2.

Results: In total we profiled 59,665 cells, which included 5 major cell types: macrophages (n=40,105), neutrophils (n=9,583), T cells (n=5,200), actively proliferating macrophages (n=3,886), and B cells (n=891). Using unsupervised clustering of macrophages, we identified 11 cell clusters, which revealed more monocytes in BRD calves (5.1 $\log_2$ fold increase, P<0.01), and demonstrated relevant differentiating surface markers between macrophage clusters including CD40, CD9, CD74 and CXCR4. Unsupervised clustering of T cells revealed 10 cell clusters, including two distinct populations of γδ T cells, which have important immunological roles in the response to BRD, that could be subdivided based on IFNG, IL23R, and WC1.3 expression. Additionally, a previously undescribed T cell population (HSP+ T cells) displaying a distinct heat-shock protein cellular stress signature (DNAJA1 and HSPA1A expression) was significantly increased in BRD (2.0 $\log_2$ fold increase, P=0.003). Three BRD calves expressed BRSV viral transcripts, concentrated in a subpopulation of neutrophils. Virally infected calves displayed blunted inflammatory signatures (greater than 2.5 $\log_2$ fold reduction of inflammatory genes including NOS2 [P<0.01], IL12B [P<0.01], and VCAN [P=0.04]) in macrophages relative to calves with bacterial pneumonia.

Conclusions: Using the power of scRNA-seq, we were able to demonstrate the complex cellular heterogeneity present in airway cells of healthy and diseased stocker calves. These findings provide important insights into the cellular dynamics of early respiratory immune responses to BRD.

Financial Support: Funding for this project was provided by USDA Non-Assistance Cooperative Agreement #: 58-3022-3-023. Research reported was supported by the National Institutes of Health, NIGMS Award #: T32GM136628.

Notes:

P045 - Effect of intranasal mineral administration on immune and clinical outcomes of dairy calves challenged with BHV-1

Jessica Prim[1], Manuel Chamorro[1], Jenna Stockler[1], Jessica Rush[1], Florencia Meyer[2], Thomas Passler[1], Roberto Palomares[3], Shollie Falkenberg[1]

[1]Auburn Univeristy, [2]Mississipi State University, [3]University of Georgia. jgp0048@auburn.edu

Session: Bovine Respiratory Disease, **2025-01-20, 6:00 - 8:00**

Objective: Bovine Respiratory Disease Complex (BRDC) remains a significant cause of economic loss and animal welfare concerns in the cattle industry. Minerals such as zinc, copper, and magnesium play crucial roles in immune function, potentially reducing the incidence and severity of BRDC. Results from previous studies suggest that injectable trace minerals (ITM) enhance immune responses following modified-live virus (MLV) vaccination in calves. Additionally, reduction of clinical scores following experimental infection with respiratory viruses has been reported in calves previously treated with ITM; however, the effect of intranasal mineral administration on immune responses and clinical outcomes following experimental infection with respiratory viruses in calves is unknown. This study explores the effect of a mineral formulation administered intranasally on immune and clinical outcomes in dairy calves following challenge with Bovine Herpesvirus 1 (BHV-1).

Methods: Fourteen 3-month-old dairy calves were randomly assigned to one of two treatment groups. The INM group (n=7) received 4.4 mL of a mineral solution containing 14 mM Zn, 0.25 mM Cu, and 1.5 mM Mg intranasally (IN). The Control group (n=7) received 4.4 mL of phosphate-buffered saline (PBS) IN. Treatments were administered to calves from both groups on days -2, 2, and 6 relative to BHV-1 challenge (day 0 of study). On day 0, all calves were challenged with 2 mL of a BHV-1 inoculum ($3x10^7$ $CCID_{50}$/mL) intranasally. Clinical signs were assessed on days -2, 0, 2, 6, 10, 14, 21, and 28 using a respiratory score sheet. Nasal swabs (NS) were collected for virus isolation and serum samples were collected to evaluate BHV-1 neutralizing antibody titers. Data were analyzed by ANOVA for repeated measures, with statistical significance considered when $p < 0.05$.

Results: Clinical parameters before and after virus challenge were similar between groups. All calves were negative to BHV-1 in NS and seronegative to BHV-1 before challenge. Detection of BHV-1 in NS after challenge was similar between groups. A numerically greater mean ± SEM Log2 serum neutralizing antibody titer was observed in the Control group compared with the INM group on day 28 (32.9 ± 47.9 vs. 10 ± 11, respectively); however, this was not significantly different ($p = 0.1$).

Conclusions: Based on results from this study, treatment with INM did not have a significant effect on health outcomes, antibody responses, or virus isolation in dairy calves experimentally challenged with BHV-1.

Notes:

P046 - Antimicrobial resistance profiles of *Mannheimia haemolytica* and *Pasteurella multocida*: Review of diagnostic laboratory data

S.N. Dasari[1], R.G. Amachawadi[1], H. Wang[2], H. Kittana[3, 4], M. M. Chengappa[3], T. G. Nagaraja[3]

[1]Department of Clinical Sciences, Kansas State University, [2]Department of Statistics, Kansas State University, [3]Department of Diagnostic Medicine/Pathobiology, Kansas State University, [4]Kansas State Veterinary Diagnostic Laboratory, Kansas State University. sdasari@vet.k-state.edu

Session: Bovine Respiratory Disease, **2025-01-20, 6:00 - 8:00**

Objective: Antimicrobial resistance (AMR) is a significant global public health threat. A major driver of antimicrobial use in feedlot cattle is bovine respiratory disease (BRD), a complex and economically important disease. Medically important antimicrobials are widely used in the treatment and prevention of BRD. Therefore, BRD treatment and control are a major concern for emergence and dissemination of AMR to these antimicrobials. Passive surveillance through veterinary diagnostic laboratories for major BRD pathogens, *M. haemolytica* and *P. multocida*, is essential to build a comprehensive AMR surveillance program. Monitoring AMR patterns in these pathogens not only informs therapeutic strategies but also helps identify emerging resistance trends that could impact both animal and public health. Treatment failures in BRD presents a significant concern, as previous studies indicate that approximately 33% of treated cattle experience initial therapy failure, requiring additional treatment or resulting in mortality. These findings underscore the critical need for comprehensive AMR monitoring to support therapeutic decision-making and prevent disease progression. Our objectives were to describe the prevalence and trends in AMR for major BRD bacterial pathogens isolated from nasal swabs and lung samples submitted to the Kansas State Veterinary Diagnostic Laboratory between 2010-2024.

Methods: A total of 3,651 isolates of *M. haemolytica* [64.6%: lung (2,028) and nasal (332)], and *P. multocida* [35.4%: lung (906) and nasal (385)] were included in the analyses. Overall, 22 antimicrobials from 10 antimicrobial classes were examined. All the data analyses (descriptive and inferential) were calculated using R programming software (Version 2024.09.0+375).

Results: A total of 3,651 *M. haemolytica* and *P. multocida* isolates were tested at the diagnostic laboratory from April 2010 to June 2024. During this period, twenty-two antimicrobials were tested. Both *M. haemolytica* and *P. multocida* exhibited high level resistance to clindamycin, tylosin tartrate and trimethoprim/sulphamethoxazole compared to other antimicrobials. In the case of BRD-specific antimicrobials, the percentage of resistant isolates was highest in tilmicosin (64%), tetracycline (63%), enrofloxacin (60%), and tulathromycin (57%) in *M. haemolytica*. In *P. multocida*, tilmicosin (52%), tetracycline (35%), gamithromycin (32%), tildipirosin (31%), and tulathromicin (19%) had high levels of resistance. In general, isolates from lung samples had higher resistance ($P < 0.05$) than isolates from nasal samples for both bacterial species. More *M. haemolytica* (n=1,759; 77.8%) isolates were multidrug resistant compared to *P. multocida* (n=497; 42.1%). The high prevalence of AMR observed in this study might be attributed to BRD treatment failure, which results in persistent infections and increased treatment costs. Treatment failure in BRD cases often requires multiple or alternative antimicrobial therapies, which can further drive the development and spread of resistant bacterial strains.

Conclusions: Future studies are directed towards studying temporal trends and their association with the prevalence of MDR isolates in both lung and nasal samples, including comparative analysis, antimicrobial resistance profiling and survival analysis. Expanding passive surveillance will be essential in providing veterinarians with the data needed to make informed treatment decisions, ultimately helping to mitigate the impact of AMR in cattle industry and beyond.

Financial Support: This work is supported by the United States Department of Agriculture, project award no. 2023-68015-39985, from the U.S. Department of Agriculture's National Institute of Food and Agriculture.

Notes:

P047 - Bacteriological investigation and biotyping of *Brucella* species isolated in Georgia from human and animal samples

Marine Ramishvili[1], Natalia Abazashvili[1], Marina Grdzelidze[1], Ketevan Sidamonidze[1], Paata Imnadze[1], Jeffrey Foster[2]

[1]National Center for Disease Control and Public Health of Georgia, [2]Pathogen and Microbiome Institute, Northern Arizona University. m.ramishvili@ncdc.ge

Session: Brucellosis, **2025-01-20, 6:00 - 8:00**

Objective: Brucellosis is a zoonotic infection associated with reproductive failure in animals and febrile disease in humans. It is one of the most widespread zoonotic pathogens and is responsible for enormous economic losses for livestock production. Brucellosis continues to be a major public and animal health problem in Georgia, where livestock are a major source of food and income. Laboratory testing is essential for the diagnosis of brucellosis. Serological and bacteriological tests are widely used for the detection of infected animals and humans. The goal of this study was to molecularly type *Brucella* strains that we had collected during 2021-2024 to understand the species circulating in humans and animals in Georgia.

Methods: From 2021 to 2024, *Brucella* isolates were obtained from blood cultures of human volunteers suspected to have brucellosis and milk or aborted fetuses of domestic ruminants. Samples were cultured on the *Brucella* selective agar (Farrell's media) and Chocolate agar for the presence of *Brucella* species, and incubated in anaerobic conditions with 5% CO_2. Isolated colonies suspected as *Brucella* by microbiological testing (gram staining, agglutination with *Brucella* specific sera) were then confirmed and typed by Bruce-ladder PCR assay.

Results: Ninety-four strains (84 human, 10 animal isolates) were confirmed as *Brucella* by Bruce-ladder PCR, which supported the bacteriological test results of 79 *B. melitensis* and 15 *B. abortus* strains. Out of 221 human blood samples, 38% (84/221) isolates, with 79 strains were typed as *B. melitensis* and 5 isolates as *B. abortus*. Of the 10 animal isolates submitted for confirmation, all 10 were typed as *B. abortus*.

Conclusions: Prevention of human brucellosis is best achieved by control or eradication of the disease in animals. Thus, it is very important to determine which species are dominant in animals compared to those identified from clinical samples. Based on molecular typing data carried out *B. melitensis* and *B. abortus* strains are the species most commonly circulating in Georgia. Genomic analyses are ongoing, which will provide a more detailed characterization of these isolates to better understand the genetic diversity and movement of *Brucella* in the region.

Financial Support: The authors express their sincere gratitude to the U.S. Defense Threat Reduction Agency (DTRA) for financial support.

Notes:

P048 - Comprehensive whole genome sequencing and comparative analysis of *Brucella melitensis* and *Brucella abortus* isolates from humans and livestock in Georgia

Ketevan Sidamonidze[1], Jeff Foster[2], Paata Imnadze[1], Ekaterine Khmaladze[1], John Gillece[2]

[1]National Center for Disease Control (NCDC) and Public Health/R. Lugar Center, [2]Pathogen and Microbiome Institute, Northern Arizona University. ksidamonidze@gmail.com

Session: Brucellosis, **2025-01-20, 6:00 - 8:00**

Objective: Brucellosis stands as a prevalent global zoonotic disease, annually reporting millions of new human cases worldwide. It remains endemicity in Georgia, leading to considerable human morbidity and substantial economic losses in the agricultural sector. The limited genetic resolution provided by many genotyping methods has posed challenges in unraveling the evolutionary history of this pathogen and tracking its global dissemination.

Methods: Whole genome sequencing (WGS) offers a comprehensive means to elucidate the phylogenetic connections between bacterial strains. To evaluate genetic diversity within *Brucella* spp. present in Georgia, we selected 124 isolates from humans and animals (human n=61 and animal n=63) from the repository at NCDC for sequencing. DNA was extracted from each of the cultured samples using a Qiagen extraction method. Preliminary analysis of the DNA with PCR methods suggested that there were 65 *B. melitensis* and 59 *B. abortus*. DNA fragment library preparation and sequencing were performed on a MiSeq platform at Northern Arizona University. A phylogenetic tree was constructed for each species using single nucleotide polymorphisms (SNPs) and comparisons to genomes on GenBank.

Results: Our analysis revealed that the Georgian strains of both *B. abortus* and *B. melitensis* predominantly constitute distinct clades, clearly distinguished from most publically available genomes.

Conclusions: This is the first WGS and phylogenetic comparison effort for archival Brucella strains performed in the country of Georgia, which is an approach that will be incorporated in future surveillance efforts. Overall, WGS significantly enhances the capacity for brucellosis surveillance, making it a vital tool in public health efforts to control this zoonotic disease.

Notes:

P050 - Zoonotic bacterial pathogens in bat samples in Georgia

Ekaterine Zhgenti[1], Lela Urushadze[1], Tea Tevdoradze[1], Gvantsa Brachveli[1], Ioseb Natradze[2], Andres Velasco-Villa[3]

[1]National Center for Disease Control and Public Health/R. Lugar Center, [2]Ilia State University, [3]CDC Atlanta. ezhgenti@gmail.com

Session: Brucellosis, **2025-01-20, 6:00 - 8:00**

Objective: Bats are considered to be carriers of many important zoonotic pathogens. Bats as a possible reservoir of *Brucella* were first identified in 2018 in Georgia, where brucellosis is endemic. Leptospirosis is also widespread in the country, with increasing morbidity in recent years. Understanding the prevalence of these zoonotic pathogens in local bats population could be important from a veterinary and public health perspective.

Methods: A total of 103 bats representing five different species (*Myotis blytii*, *Rhinolophus euryalle*, *Rhinolophus ferrumequinum*, *Rhinolophus hipposideros*, *Miniopterus schreibersii*) collected from three locations (Chiatura-Samertskle klde, Chiatura Gundaeti, Tskaltubo) in late autumn, winter and early spring of 2023-2024 were studied. In addition, kidney samples (n=51) obtained from two bat species (*M. schreibersii* and *R. ferrumequinum*) captured in the Tskaltubo area in the summer of 2018 were included in the study. Real-time PCR targeting the lipoprotein 32 kDa gene (lipL32) was used for detection of pathogenic *Leptospira* spp. in kidney (n =78) and IS*711* for *Brucella* spp. in liver (n =103) and spleen samples (n = 101).

Results: Pathogenic *Leptospira* DNA was detected in four of the 78 kidney samples tested, giving an overall prevalence of 5%. All Leptospira infected-bats were *M. schreibersii* collected from the same location (Tskaltubo-Khomuli cave) and time (Summer, 2018). *Leptospira* DNA was not detected in any kidney samples from bats collected in early spring 2024 (n=27). No Brucella DNA was found either in the spleen or liver samples of bats.

Conclusions: Our results indicate that bats in Georgia are potential carriers of Leptospira spp., at low prevalence and varies depending on the bat species, location and seasonality. Species-specific differences in bacterial infection may indicate that some bat species may be more susceptible or more affected by *Leptospira* than other bat species. Negative results for *Brucella* spp. may be related to sample size, species tested, or timing of sampling, which should be considered in future studies.

Financial Support: The research was funded by DTRA as a part of the BAA program.

Notes:

P051 - Comparison of bison and elk to *Brucella* vaccination and experimental challenge with *Brucella abortus* strain 2308

Paola Boggiatto[1], Ellie Putz[1], Steven Olsen[1]

[1]USDA/ARS/National Animal Disease Center. Steven.olsen@usda.gov

Session: Brucellosis, **2025-01-20, 6:00 - 8:00**

Objective: A metanalysis study was conducted to compare susceptibility of pregnant bison and elk to experimental challenge with virulent *B. abortus*.

Methods: Bison and elk that were naive (n=82 and 67, respectively) or vaccinated with *Brucella abortus* strain RB51 (n-99 and 29, respectively) were experimentally challenged with a virulent B. abortus strain during pregnancy. Viability of calves at birth was determined by observation. Samples from lymphatic and other tissues were obtained from dams and calves at necropsy and cultured by microbiologic techniques to assess recovery of the challenge strain. Differences in responses were compared using a generalized linear model.

Results: The incidence of abortion, fetal infection, uterine or mammary infection, or infection in maternal tissues after experimental challenge was greater ($P<0.05$) in naïve and vaccinated bison when compared to similar groups in elk. Bison vaccinated with RB51 had lower ($P<0.002$) abortion rates and recovery of *Brucella* from fetal or uterine/mammary tissues when compared to naïve bison. Vaccinated elk had reduced ($P<0.01$) rates of maternal infection, but rates of abortion and fetal or uterine/mammary infection did not differ ($P>0.05$) from naïve elk. In elk or bison that aborted, time between experimental challenge and parturition was less ($P<0.05$) than in animals delivering full-term calves. In both naïve and vaccinated bison mean colonization in placentomes, and parotid and supramammary lymphatic tissues was greater ($P<0.05$) than similar tissue/treatment combinations in elk. In elk or bison that aborted, mean colonization in placentome tissues were typically more than 5 logs higher than in animals that did not abort.

Conclusions: The results of our study suggest differences in disease pathogenesis between the two wildlife reservoirs of brucellosis in Yellowstone National Park and surrounding areas. These observations suggest brucellosis intervention strategies for bison and elk may need to be species-specific.

Notes:

P052 - Characterization and molecular typing of *Brucella* strains isolated from humans and animals in Georgia 2020-2024

David Tsaguria[1], Ketevan Sidamonidze[1], John Gillece[1], Paata Imnadze[1], Jeff Foster[1]

[1]National Center for Disease Control and Public Health. tsaguriadavid235@gmail.com

Session: Brucellosis, **2025-01-20, 6:00 - 8:00**

Introduction: Brucellosis, a highly contagious zoonotic disease, is caused by the Brucella genus and remains endemic in Georgia. The genus Brucella comprises ten species of aerobic Gram-negative rods: *B. abortus*, *B. suis*, *B. ovis*, *B. melitensis*, *B. canis*, *B. neotomae*, *B. pinnipedialis*, *B. ceti*, *B. microti*, and *B. inopinata*. Some Brucella species are responsible for significant human morbidity and substantial economic losses in agriculture. Moreover, Brucella species are regarded as potential biological threat agents. Among humans, B. melitensis is primarily responsible for the disease due to its high pathogenicity. The global epidemiology of human brucellosis, recognized as the most prevalent zoonotic infection on a worldwide scale, has undergone significant transformations in the past decade. These changes can be attributed to a range of factors, including improvements in sanitation, shifts in socioeconomic conditions, evolving political dynamics, and the rise in international travel.

Methods: Since 2020, 301 patients with fever and clinical signs of Brucellosis have applied to the Institute of Parasitology, where they took blood and sent it for testing to NCDC/LC. Isolates from human blood were identified by a bacteriological algorithm in BSL-3 facility. Isolates were then subjected to Bruce-ladder PCR for molecular typing.

Results: Culture was isolated in 131 samples from the blood of 301 patients. Out of 131 Brucella isolates 124 were B. melitensis and 7 B. abortus. The majority of cases were found in East Georgia. Men (92%) were more often infected than women (8%); the most affected age group was 22-55 years of age. As the Bruce-ladder PCR assay consistently and effectively confirmed the Brucella species in Georgian isolates, it demonstrates its potential for ongoing use in future surveillance initiatives.

Conclusion: The ongoing presence of infections caused by *B. abortus* and *B. melitensis* is crucial to monitor, as these pathogens not only threaten public health but also have significant economic implications for the livestock industry, affecting agricultural productivity and food security in Georgia.

Notes:

P053 - Epizootic situation and laboratory studies of *Brucella* in western Georgia

Nugzari Makhatadze[1]

[1]LEPL State Laboratory of Agriculture (SLA), Kutaisi, Georgia. nugzar.makhatadze@sla.gov.ge

Session: Brucellosis, **2025-01-20, 6:00 - 8:00**

Objective: Brucellosis is a particularly dangerous zoonotic disease that is widely spread among animals and humans. Although, more than 120 years have passed since the discovery of the pathogen, and this disease has been well studied, the problem of brucellosis still remains in many parts of the world. Based on the data of the National Center for Disease Control and Prevention (NCDC), from 2010 to 2023 in Georgia 2,763 people were infected with brucellosis. Our objective examines received samples for brucellosis in order to subsequently prevent the spread of the disease.

Methods: For the diagnosis of animal brucellosis, the following tests were used in the Kutaisi Zonal Diagnostic Laboratory (ZDL) of the Ministry of Environment Protection and Agriculture of Georgia**:** Serology - 1. Detection of immunoglobulins (the Rose Bengal Test)-antibodies generated against in the blood serum against brucella. 2. Fluorescent Polarization Assay (FPA) antibody detection test for *Brucella abortus* and *B. melitensis*. 3. Fast, indirect, immunofermental reaction method of detection of antibodies of *Brucella* species (ELISA); 4. Milk study by a Ring Test. The positive results for the brucellosis, revealed by Rose Bengal test, the specific immunofermental analysis and fluorescent polarization test, are similar. 5. Bacteriology - the goal is to cultivate microorganisms from alive and slaughtered bodies.

Results: During the period of 2010-2023 Kutaisi ZDL has received and investigated 206,212 blood samples of large livestock (cattle, cow, buffalo) with 5,342 positive results (3,86% positive); small livestock (goat, sheep) -17,096 blood samples with 503 positive results (3,39% positive); 1 sample of pig with negative result; 1,489 samples of large cattle (cow) milk samples with 167 positive results (8,4% positive). 15 samples of aborted fetuses received for bacteriological testing were negative. The RT-PCR test was not used because the bacteriological test gave a negative result.

Conclusions: An undiagnosed or undertreated disease can quickly spread throughout a herd or flock. Timely and correct diagnosis of the disease increases livestock productivity and prevents the spread of the disease. We hope that the cattle vaccination campaign in Georgia will bring its success and free our country from this disease. This is important for the country's economy.

Notes:

P054 - Genomic characterization of *Brucella* species in Italy over a 10-year period (2011 – 2021)

Anna Janowicz[1], Fabrizio De Massis[1], Sara Serrani[1], Michela Toro[1], Alessandra Sferrella[1], Nausica D'Aurelio[1], Lisa Di Marcantonio[1], Eugenio Felicioni[1], Cecilia Villani[1], Teresa Romualdi[1], Anna Abass[1], Katiuscia Zilli[1], Giuliano Garofolo[1]

[1]National, WOAH and FAO Reference Laboratory for Brucellosis, Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise "G. Caporale". s.serrani@izs.it

Session: Brucellosis, **2025-01-20, 6:00 - 8:00**

Objective: Brucellosis is a globally widespread disease that, although largely eradicated in much of the developed world, remains endemic in the developing countries. Nevertheless, in Italy, it continues to be present in animal populations at very low prevalence, despite over thirty years of control and eradication efforts. Brucellosis is a disease with a significant economic impact, affecting livestock and also relevant for wildlife species and marine mammals. Zoonotic transmission occurs primarily through the consumption of contaminated dairy products; however, direct contact with highly infectious materials, such as animal abortions, also presents a substantial risk. This study aimed to investigate the genomic diversity and spatiotemporal distribution of the most relevant Brucella species found in terrestrial animal populations in Italy in the 10-year period 2011-2021. The research evaluated the impact of control and eradication measures in high-risk areas, examining the risk of introduction of external clones or the survival of autochthonous clones.

Methods: The eradication plan, based on a test-and-slaughter strategy for livestock testing positive, allowed for the analysis of livestock populations suspected of brucellosis through microbiological examinations of targeted organs and tissues. For wildlife, testing was conducted as part of specific wildlife control plans or through research and isolation in cases of passive surveillance. Human-origin strains, however, were also included for specific laboratory investigations for tracking purposes. In total we applied WGS based typing methods on 1,326 Brucella strains isolated from animal and human samples including 681 strains of *B. abortus*, 498 strains of *B. melitensis* and 147 strains of B. suis.

Results: In Italy, human brucellosis is caused primarily by B. melitensis, originating either from Italian livestock or to livestock from abroad, suggesting it can also be travel-associated. The disease in livestock is observed into specific geographical areas defined as clusters. The persistence of brucellosis in these areas appears to be due to the circulation of the same clone, with no evidence of new strains introductions. Infection transmission between infected and brucellosis-free farms may occur due to extensive and traditional farming practices. In contrast, spillover infections in brucellosis-free regions are likely driven by unauthorized animal movements, perpetuating the risk of transmission and thus hindering the eradication efforts.

Conclusions: Whole-genome sequencing (WGS)-based typing methods are enabling the acquisition of data, significantly enhancing the level of detail compared to traditional typing methods. While traditional methods can identify few biovars in the whole Italy, WGS can distinguish hundreds of variants, thereby increasing the level of discrimination. The application of these techniques is essential for achieving eradication goals in the "endgame" phase of the eradication plan. The implementation of WGS, integrated with big data on animal movement and animal identification, allows real-time monitoring of disease spread, supporting effective veterinary actions for traceback and trace-forward infection tracking.

Notes:

P055 - Exploratory screening for micro-RNA biomarkers in canine multicentric lymphoma

Sabine E. Hammer[1], Stefanie Burger[2], Martin Hofer[2], Ilse Schwendenwein[3], Barbara C. Rütgen[3]

[1]Center of Pathobiology, University of Veterinary Medicine Vienna, [2]Genomics Core Facility, University of Veterinary Medicine Vienna, [3]Clinical Pathology Unit, Department of Biological Sciences and Pathobiology, University of Veterinary Medicine Vienna. sabine.hammer@phylo-dat.net

Session: Companion animal health, **2025-01-20, 6:00 - 8:00**

Objective: Micro-RNAs (miRNA, small non-coding RNAs) are pivotal in gene regulation fine tuning and their aberrant expression is expected in cancer. miRNAs might serve as diagnostic biomarkers and assist predicting therapeutic response and clinical outcome. Aim of this study was to investigate dysregulated miRNAs in lymphomatous lymph node tissues in comparison to normal lymph node material and peripheral blood mononuclear cells (PBMC) from healthy control dogs. Differences in miRNA expression between four lymphoma entities were assessed.

Methods: A panel of 89 canine target miRNAs were profiled by a customized PCR array. Quantification was performed by quantitative real time PCR and relative expression was determined by the delta-delta Ct method using the GeneGlobe Data Analysis Center (Qiagen, www.qiagen.com). Fold changes were evaluated by student's t-test and p-values less than 0.05 were considered significant.

Results: In total, 85 out of 89 miRNAs were successfully amplified and many were differently expressed in the lymphoma entities. In the 14 diffuse large B-cell lymphoma (DLBCL) patients, 28 miRNAs were significantly dysregulated in lymph node material and 24 miRNAs showed significant aberrations in PBMC. The six peripheral T-cell lymphoma (PTCL) samples showed 24 (lymph node) and 25 (PBMC) dysregulated miRNAs when compared to the healthy controls. A combined analysis of DLBCL and PTCL samples revealed seven shared and 19 differently expressed miRNAs.

Conclusions: The miRNA-17-92 cluster, miRNA-34a, miRNA-150, and miRNA-181-family might serve as biomarkers in canine T- and B-cell lymphoma. A panel of 26 significantly dysregulated miRNAs will be applied to confirm and validate these miRNAs together with those with unknown function and still missing literature record.

Notes:

P056 - Molecular characterization of *Salmonella* infections in companion animals: A One Health perspective

Golam M. Faisal[1], Ajran Kabir[1], Bibek Lamichhane[1], Rosbelly Rios[1], Charlotte Vaillant[1], Elizabeth Isenhower[1], Sydney Epley[2], Tasmia Habib[1], Megan Romano[2], Melissa Morgan[3], Yosra. A. Helmy[1]

[1]Maxwell H. Gluck Equine Research Center, University of Kentucky, [2]Veterinary Diagnostic Laboratory, University of Kentucky, [3]Department of Animal and Food Sciences, University of Kentucky, Lexington, KY. gm.faisal@uky.edu

Session: Companion animal health, **2025-01-20, 6:00 - 8:00**

Objective: *Salmonella* is a significant foodborne pathogen that can affect both humans and animals. Companion animals (dogs, cats) play an important role in transmitting the infection to their owners through direct or indirect contact. Despite the recognized zoonotic risks posed by companion animals, limited data exist on the prevalence and antimicrobial resistance (AMR) of *Salmonella* in this population in Central Kentucky. This study investigated the occurrence of *Salmonella* in apparently healthy dogs and cats in this region and characterized their virulence and antimicrobial resistance (AMR) profiles using both phenotypic and genotypic methods, contributing to a One Health understanding of the pathogen's risk.

Methods: A total of 206 fecal samples were collected from cats (n=66) and dogs (n=140) from pet clinics and animal shelters located in Central Kentucky. Fecal samples were enriched in tetrathionate broth and cultured on XLT4 agar plates. DNA was extracted using the boiling method, and a polymerase chain reaction (PCR) targeting the invA gene was performed. Antimicrobial resistance profiles of the isolates were determined by broth microdilution assay. Genotypic AMR and virulence profiles were characterized by PCR. Swarming and swimming motility assays were conducted to evaluate the motility of the isolates.

Results: The overall prevalence of *Salmonella* in the collected samples was 11.6% (24 out of 206; 95% CI: 7.61-16.84). PCR confirmed the presence of the invA gene within all the isolates. Other virulence genes included hilA (83.3%), sopB (66.6%), spi4D (58.3%), spiA (75%), and spvC (33.3%) were also detected. Multidrug resistance was detected in all of the isolates. Resistance rates were highest against macrolides (azithromycin: 100%) and tetracyclines (79.1%), followed by phenicols (chloramphenicol: 75%), aminoglycosides (gentamicin: 66.6%), and beta-lactams (ampicillin: 58.3%, ceftriaxone: 25%, cefotaxime: 29.1%). Lower resistance was observed for carbapenems (meropenem: 20.8%), fluoroquinolones (ciprofloxacin: 20.8%, levofloxacin: 16.6%), and folate pathway inhibitors (trimethoprim/sulfamethoxazole: 4.1%). Additionally, the beta-lactamase producing genes blaCTX (66.6%), sulfonamide resistance gene sul2 (54.1%), macrolide resistance gene ermB (25%), and chloramphenicol resistance gene floR (58.3%) were detected. In motility assays, 45.9% of isolates demonstrated strong swarming motility, while 54.1% exhibited weak or no swarming. In the swimming assay, 58.3% showed strong swimming motility, while 41.7% exhibited weak motility.

Conclusions: This study highlights the role of companion animals as potential reservoirs for the transmission of multidrug-resistant *Salmonella* to their owners. These findings demonstrate the need for a One Health approach, integrating enhanced surveillance and monitoring efforts to control the spread of *Salmonella*. Rigorous hygiene practices, veterinary check-ups, and safe handling of pet food will help to mitigate the risk of *Salmonella* infections in companion animals and their owners.

Notes:

P057 - Canine infectious respiratory disease pathogens in shelter dogs

Crystal LeRoy[1], Tiffany Pulliam[1], Jennifer Banach[1], Ashutosh Verma[1], Rebecca Randall[1]

[1]Lincoln Memorial University. crystal.leroy@lmunet.edu

Session: Companion animal health, **2025-01-20, 6:00 - 8:00**

Objective: Canine infectious respiratory disease (CIRD) is a common pathology in dogs caused by one or more viral and bacterial pathogens. Multiple bacteria (Bordetella bronchiseptica, Mycoplasma canis, Mycoplasma cynos, Streptococcus equi subsp. zooepidemicus) and viruses [canine adenovirus, canine herpesvirus, canine influenza A virus, canine respiratory coronavirus, canine distemper virus and canine parainfluenza virus] are associated with CIRD in dogs. CIRD occurs more commonly in shelters, kennels, or other settings where large groups of dogs are housed with the frequent addition of new animals. The objective of this study was to determine the prevalence of CIRD pathogens in the respiratory tract of the shelter dog populations and their transmissibility to other dogs sharing a common facility.

Methods: Nasopharyngeal swabs were collected from a total of 34 dogs from 5 shelters across the Appalachian region of TN, KY, and VA, and tested for the presence of the above-mentioned respiratory pathogens using conventional or real-time PCR assays.

Results: The highest percent positivity was detected for M. cynos (52.9%, 18/34), followed by M. canis (50%, 17/34), and coronavirus (32.4%, 11/34). Streptococcus equi subsp. zooepidemicus, adenovirus, herpesvirus and influenza A were not detected in any of the tested dogs. All shelters from which dogs were tested had at least one dog positive for two pathogens. Co-infection with 2 pathogens was detected in 16 dogs (47%). Co-infection with 3 pathogens was detected in 4 dogs (11.8%). One dog was positive for 4 pathogens (2.9%). Only 8.8% (3/34) of dogs displayed symptoms of CIRD at the shared facility. After being housed in the shared facility with dogs from other shelters, 61.3% (19/31) of the dogs tested positive for a pathogen they were not positive for on the previous swab. The prevalence of M. cynos (58.1%) remained stable however 19.4% of dogs that tested positive after being housed in a common facility were not positive for M. cynos prior to entering the facility. The prevalence of M. canis (51.2%) also remained stable but 19.4% of dogs that tested positive after sharing the facility were not positive prior to being housed in the facility. The prevalence of coronavirus increased to 58.1% after sharing the facility but 35.5% of dogs that tested positive were dogs that had been negative on the initial swab.

Concluions: These data show that dogs housed in shelters can asymptomatically carry multiple respiratory pathogens associated with CIRD which can be shed and potentially infect other animals in foster or adoption situations. The relative importance of these pathogens, alone or in combination, in respiratory infections in shelter settings, should be further investigated.

Notes:

P58 - Antimicrobial susceptibility of *Escherichia coli* from canine pyometra diagnostic samples, 2007-2024

Kassie N. Marino[1], Cassandra MB Guarino[1], Nicole Buote[2], Kurtis Sobkowich[3], Casey L Cazer[2,4],

[1]Department of Population Medicine and Diagnostic Sciences, Cornell University, [2]Department of Clinical Sciences, Cornell University, [3]Department of Population Medicine, Ontario Veterinary College, [4]Department of Public and Ecosystem Health, Cornell University. knm59@cornell.edu

Session: Companion animal health, **2025-01-20, 6:00 - 8:00**

Objective: This study aims to examine *Escherichia coli* susceptibility to commonly prescribed antibiotics in canine pyometra diagnostic samples from 2007-2024 at a single veterinary diagnostic laboratory.

Methods: Retrospective data of isolates cultured from canine uterine samples between 2007 and 2024 were extracted from the Cornell University Animal Health Diagnostic Center SWIN database *E. coli* was tested for antimicrobial susceptibility using Thermo Scientific™ Sensititre™ Complete Automated AST System with the Sensitire Vet Companion Animal Gram Negative plate (COMPGN1F). Antimicrobials from other AST plates were excluded from analysis. MIC values were interpreted with current breakpoint values from CLSI VET01S ED7 using RStudio. MIC values that could not be interpreted with current breakpoints were excluded from further analysis. Isolate susceptibility results were tabulated and visualized using a custom RShiny dashboard.

Results: A total of 353 isolates were extracted from canine uterine samples, revealing 172 distinct organisms. The predominant isolate was *E. coli/E. coli beta* (n=205 isolates from 169 samples). Other organisms, such as *Staphylococcus* and *Klebsiella* species, were also isolated but in lower quantities. With updated breakpoints, only 0.5% of *E. coli* isolates were susceptible to amoxicillin-clavulanic acid (1/192), and most enrofloxacin MICs were not interpretable. For example, 171/205 isolates (83.4%) had an enrofloxacin MIC that includes the susceptible and/or susceptible-dose-dependent category (e.g., $\leq$ 0.12). Susceptibility rates were high for other fluoroquinolones: marbofloxacin (80%, 112/140) and orbifloxacin (88%, 128/144). Third-generation cephalosporins also showed high susceptibility: cefovecin (88%, 169/192), cefpodoxime (88.7%, 180/203), and ceftazidime (95.5%, 127/133). First-generation cephalosporins showed varying results: cefazolin (74.7%, 115/154) was moderately effective, while cephalexin (2.3%, 3/133) showed very low susceptibility.

Conclusions: In veterinary medicine, enrofloxacin and amoxicillin-clavulanic acid are commonly used for broad-spectrum coverage, including for treating canine pyometra pre- and post-operatively. However, updated susceptibility breakpoints indicate amoxicillin-clavulanic acid is ineffective against *E. coli* outside the urinary tract, and enrofloxacin breakpoints are incompatible with current AST plates, complicating clinical guidance. This leads to non-interpretable data and challenges in therapy decisions. Despite this, other fluoroquinolones remain effective, and cephalosporins may serve as a suitable empiric choice for pyometra. Effective communication between microbiology labs and clinicians is critical for optimizing patient care amidst evolving AST guidelines.

Notes:

P059 - Higher RT-qPCR Ct values in vaccine-like strains compared to wild-type strains in PRRSV ORF5 lung and serum samples

Rabsa Naseer[1], Michael A. Zeller[2], Phillip C. Gauger[1], Giovani Trevisan[1]

[1]Department of Veterinary Diagnostic & Production Animal Medicine, Iowa State University, [2]Veterinary Diagnostic Laboratory, Iowa State University. rnaseer@iastate.edu

Session: Diagnostic testing, 2025-01-20, 6:00 - 8:00

Objective: Various strains of porcine reproductive and respiratory syndrome virus (PRRSV) may cause differences in the severity of clinical signs, adverse animal health impacts, and economic losses in swine populations. Understanding the relationship of cycle threshold (Ct) values between wild-type and vaccine-like strains is essential for assessing viral dynamics, and identifying samples to investigate potential recombination events that may pose future clinical risk.

Methods: Data was sourced from the Iowa State University Veterinary Diagnostic Laboratory (ISU VDL), encompassing years of PRRSV testing records. This repository offers access to historical and current PRRSV diagnostic results, including specimen type, Ct value, collection date, sequence lineage, and associated metadata. The dataset included only Lineage 5A (L5A) and Lineage 1D (L1D) sequences from 2018 to 2023, as these lineages possess robust diagnostic data and associated vaccine strains; Ingelvac MLV (Modified Live Virus) and Prevacent PRRS, respectively. The data was divided by sample type, with an emphasis on lung and serum samples. Analyses focused on positive Ct values, defined by a cutoff of < 38. L5A had over 200 sequences recovered from lung and over 170 from serum. L1D had over 800 sequences recovered from lung and over 750 from serum. Alignments and nucleotide identity were utilized to classify sequences as vaccine-like or wild-type based on a 98% threshold comparison to the relevant vaccine strains. Statistical analyses performed in R compared Ct value distributions and their correlation regarding classification and lineage.

Results: Statistically significant differences in median Ct values between vaccine-like and wild-type samples were identified at a 0.05 significance level. For wild-type samples, median Ct values were 21.3 (lung) and 24.4 (serum) in L5A, and 21.8 (lung) and 22.6 (serum) in L1D. Conversely, vaccine-like samples exhibited median Ct values of 21.6 (lung) and 28.6 (serum) in L5A, and 23.3 (lung) and 29.6 (serum) in L1D. In L5A, mean Ct differences between vaccine-like and wild-type strains were 1.04 (lung) and 3.01 (serum), while in L1D, they were 2.14 (lung) and 5.01 (serum). Annotated phylogenetic trees showed distinct clusters for wild-type and vaccine-like sequences, indicating phylogenetic differentiation. Wild-type sequences had lower Ct values, suggesting higher viral loads. However, although unexpected, some vaccine-like strains also had low Ct values, warranting further investigation and surveillance. Next steps include whole genome analysis of samples with vaccine-like strains and a review of relevant clinical data to evaluate differences in disease severity and identify potential recombination events.

Conclusions: Understanding the implications of Ct value distribution could help inform the early detection of strains with more severe clinical implications and viral behavior, enabling the rapid development of effective biosecurity and management strategies against PRRSV in swine.

Financial Support: We would like to acknowledge the Iowa State University Veterinary Diagnostic Laboratory for the funding of this research.

Notes:

P060 - A rapid and cost-effective field deployable diagnostic platform for detecting SARS-CoV-2 in white-tailed deer

Aneesh Kshirsagar[1], Anthony J. Politza[2], Santhamani Ramasamy[3], Lindsey C. LaBella[3], Kurt J. Vandegrift[4, 5], Suresh V. Kuchipudi[3], Weihua Guan[1, 2]

[1]Department of Electrical Engineering, The Pennsylvania State University, [2]Department of Biomedical Engineering, The Pennsylvania State University, [3]Department of Infectious Diseases and Microbiology, University of Pittsburgh, [4]Department of Biology, The Pennsylvania State University, [5]The Center for Infectious Disease, Huck Institutes of the Life Sciences, The Pennsylvania State University. sar465@pitt.edu

Session: Diagnostic testing, **2025-01-20, 6:00 - 8:00**

Objective: Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) has the remarkable ability to infect a wide range of animal species, including white-tailed deer, which could potentially serve as a viral reservoir. This increases the risk of spillover into U.S. livestock, such as cattle, posing a potential threat to agricultural industries. Continued circulation and evolution of SARS-CoV-2 in white-tailed deer, along with the possibility of spillover to livestock, highlights the urgent need for surveillance. Screening animal clinical samples for viruses like SARS-CoV-2 requires addressing key factors such as cost, distinguishing the virus from other endemic pathogens, and portability for field-level testing. To meet these needs, rapid, portable, and cost-effective molecular tools are necessary for detecting SARS-CoV-2 in farmed and wild animal populations. In this study, we aimed to develop and validate a rapid and portable reverse transcription loop-mediated isothermal amplification (RT-LAMP) system for SARS-CoV-2 surveillance in deer.

Methods: We developed a field-deployable system that uses modified column-based RNA extraction with a battery-powered centrifuge for nasal swabs, followed by RT-LAMP on a handheld, battery-powered analyzer. The analyzer performs eight simultaneous RT-LAMP reactions and features a thermal module for precise temperature control and an optical module with a tri-wavelength RGB LED and CMOS spectral sensor for fluorescence detection, all controlled by a Raspberry Pi Zero W. A mobile app communicating via Bluetooth allows users to input experiment details, start testing, and monitor amplification curves and results in real time. The RT-LAMP mix includes 1x isothermal buffer, 0.5 U/µL Bst 2.0 DNA polymerase, 0.3 U/µL WarmStart reverse transcriptase, six primers, 0.6 M Betain, 5 mM $MgSO_4$, 3 µM syto-9 dye, 1.4 mM dNTPs, and either 1.5 µL purified RNA for assay validation or 5 µL eluate for sample testing in a 25 µL reaction.

Results: We first validated the RT-LAMP assay targeting the SARS-CoV-2 nucleocapsid (N) gene by testing serial dilutions of purified RNA. A logistic fit of hit ratios versus RNA concentration identified a limit of detection of approximately 65 copies per reaction at a 95% confidence level. A linear fit of threshold time as a function of RNA concentration produced an R^2 value of 0.86, indicating the feasibility of a semi-quantitative RT-LAMP assay. Testing deer nasal swabs (n=34) in triplicates with both the portable system and a lab-based RT-PCR assay as the gold standard yielded 80% sensitivity (95% CI: 56.34% to 94.27%) and 85.71% specificity (95% CI: 57.19% to 98.22%).

Conclusions: This portable system provides a rapid and cost-effective solution for detecting SARS-CoV-2 in deer nasal swab samples. Ongoing validation suggests it could be adapted for use in other animal species and diverse field environments. Its portability, ease of use, and point-of-care capabilities make it a valuable tool for surveillance in remote or farm settings.

Financial Support: Funding was provided by the American Rescue Plan Act through USDA NIFA APHIS (Collaborative award# 2023-70432-41395). The findings and conclusions are of the authors and should not be construed to represent any official USDA or U.S. Government determination or policy.

Notes:

P061 - Adapting a targeted NGS panel for carnivore pathogen detection, including SARS-CoV-2, to the Oxford Nanopore MinION

Nelly Elshafie[1], Jobin J. Kattoor[2], Rebecca P. Wilkes[1]

[1]Purdue University, [2]University of Pittsburgh. nelshafi@purdue.edu

Session: Diagnostic testing, 2025-01-20, 6:00 - 8:00

Objective: The critical need for cost-effective and versatile surveillance tests for SARS-CoV-2 and other pathogens in wildlife species drives the development of innovative diagnostic methods that laboratories can easily implement. Current nucleic acid amplification tests (NAATs), while reliable for SARS-CoV-2, are limited in scope. We propose adapting targeted next-generation sequencing (tNGS) panels to the Oxford Nanopore MinION platform with the use of a flongle to overcome these limitations by allowing the detection and sequencing of SARS-CoV-2 in the same assay but also allowing the detection of additional pathogens to expand wildlife surveillance. The use of this platform allows for a more rapid turnaround time for testing by reducing the need for sample batching while also keeping costs down.

Methods: We converted our existing tNGS panel for carnivores, previously validated on the Ion Torrent platform, to the MinION platform. We optimized the PCR and sequencing processes, including primer pool selection, cycle number adjustment, and barcoding strategy for multiple sample testing. We assessed relative analytical sensitivity using serial dilutions of positive SARS-CoV-2 samples spiked with nucleic acids from various wildlife species compared to a SARS-CoV-2 gold standard NAAT run in parallel. Wildlife samples negative for SARS-CoV-2 based on the NAAT were used for specificity testing, and results were compared with those obtained from testing the samples with the tNGS method on the Ion Torrent platform. We evaluated diagnostic sensitivity and specificity for the detection of SARS-CoV-2 compared to a gold standard NAAT.

Results: We processed 85 prospective wildlife samples to date, including bobcats, badgers, raccoons, opossums, and skunks. All samples tested negative for SARS-CoV-2 using NAAT. We validated the sensitivity of the ONT platform in comparison to the Ion Torrent system and confirmed SARS-CoV-2 detection with the ONT platform at a limit consistent with a Ct value of 34. Genome sequencing of positive samples produced ≥99% coverage for contrived positive samples with Ct values of 27-31, with accurate subvariant identification. Diagnostic sensitivity and specificity testing is ongoing but currently are both 100%, compared to the NAAT.

Conclusions: The adapted tNGS panel on the MinION platform demonstrated the ability to detect SARS-CoV-2 and other pathogens from wildlife samples with high specificity. Further optimization is required to enhance sensitivity. This project sets the foundation for low-cost, scalable pathogen surveillance in wildlife, with implications for public health and ecological conservation.

Financial Support: Funding for this project was provided by the American Rescue Plan Act through USDA APHIS.

Notes:

P062 - Development of modular multiplex RT-PCR assay to allow for a systems approach to poultry health

Matthew Koci[1], Natalie Roberts[1], Lauren Anderson[1], Tamara Pen[1]

[1]North Carolina State University. mdkoci@ncsu.edu

Session: Diagnostic testing, **2025-01-20, 6:00 - 8:00**

Objective: Animal health results from multiple biological systems functioning in equilibrium, yet it is often evaluated system by system. For instance, the gut hosts the greatest number of immune cells in the body, the highest concentration of neuro-endocrine cells outside the brain, and is among the most metabolically active organs. These activities involve constant cross-talk among systems, and any changes in diet, stress, or immune function inevitably affect others, impacting gut function, animal behavior, and overall performance. To understand the holistic effects of various therapies on animal health, we need tools to assess these systems collectively.

Methods: To address this need, we developed a modular, multiplex real-time RT-PCR assay system with 30 multiplex assays, each containing 3 target genes and one housekeeping gene, for a total of 90 different target genes. These genes span the major gut systems: immunity (39), stress (12), gut function (15), and metabolism (24). The multiplex assays can be used in different combinations to assess how treatments targeting one system influence gene expression both within that system and across others.

Results: We performed bioinformatic compatibility analyses for each multiplex assay, as well as initial validation studies.

Conclusions: This work presents the initial development of a modular, multiplex real-time RT-PCR assay designed to assess gene expression changes in poultry across multiple tissue and organ systems. This assay will enhance our understanding of how gene expression shifts within one system influence others, facilitating the development of new therapies and management strategies to optimize animal performance.

Financial Support: This work was sponsored by a grant from the North Carolina State University Animal Health and Nutrition Consortium.

Notes:

P063 - Bayesian latent class models for evaluating detection of macrolide-resistant *M. haemolytica*: Culture vs. sequencing

Jennifer Abi Younes[1], Lianne McLeod[1], Cheryl Waldner[1]

[1]Department of Large Animal Clinical Sciences, Western College of Veterinary Medicine. jabiyounes@gmail.com

Session: Diagnostic testing, 2025-01-20, 6:00 - 8:00

Objective: Bovine respiratory disease (BRD) is a persistent health challenge in feedlot cattle, heightened by growing threats of antimicrobial resistance (AMR) in key etiologic bacteria like *Mannheimia haemolytica*. Advances in metagenomic sequencing have the potential to provide timely and multifaceted results in disease diagnostics as compared to traditional culture-based techniques. To compare the accuracy of diagnostic tools in the absence of a gold standard test, Bayesian latent class models (BLCMs) can be used. The objective of this study was thus to use BLCMs to estimate and compare the sensitivity (Se) and specificity (Sp) of culture-based antimicrobial susceptibility testing and Nanopore metagenomic sequencing for detecting macrolide resistance in M. haemolytica in cattle.

Methods: Deep nasopharyngeal swab samples were collected from feedlot calves in the fall of 2020 and 2021 as part of a larger study. Samples were tested using culture-based antimicrobial susceptibility testing, with minimum inhibitory concentrations (MICs) determined using a commercial microdilution panel, as well as Nanopore metagenomic sequencing. BLCMs were developed to compare the detection of M. haemolytica with phenotypic resistance to specific macrolides to that of sequencing's detection of known antimicrobial resistance genes (ARGs) considered determinants of macrolide resistance found within *M. haemolytica* reads. The comparisons included phenotypic resistance to tulathromycin and the presence of mphE-msrE genes, tilmicosin resistance and the EstT gene, and tildipirosin resistance and the EstT gene. A two-test, three-population BLCM was used for the 2020 analysis, while a two-test, five-population model was applied for 2021.

Results: A total of 909 samples were analyzed in 2020 and 1,076 in 2021. In 2020, the BLCMs for detecting *M. haemolytica* with tulathromycin resistance showed a higher median sensitivity for culture-based detection [80% (95% CrI: 73-86%)] compared to metagenomic sequencing for identifying M. haemolytica with msrE and/or mphE genes [62% (95% CrI: 56-69%)]. Specificity was high for both methods: 99.8% (95% CrI: 99-99.99%) for culture-based detection and 98% (95% CrI: 96-99%) for metagenomic sequencing. For tilmicosin resistance, the culture-based method had a lower Se of 23% (95% CrI: 10-38%) compared to 43% (95% CrI: 22-65%) for detection of M. haemolytica with the EstT gene. Sensitivity for detecting tildipirosin resistance was low for culture-based method at 1.5% (95% CrI: 0.1-6%) but higher for sequencing detection of the EstT gene at 28% (95% CrI: 8-79%). In 2021, Se for detecting *M. haemolytica* with tilmicosin or tildipirosin resistance were similar for the culture-based method, at 38% (95% CrI: 25-51%) and 36% (95% CrI: 24-49%), respectively. In contrast, detection of the EstT gene had a higher Se of 75% (95% CrI: 58-90%) for tilmicosin and 74% (95% CrI: 57-89%) for tildipirosin. Specificity estimates remained high for both methods.

Conclusions: The use of BLCMs provided detailed insights into the diagnostic performance of traditional culture-based methods relative to metagenomic sequencing for identifying macrolide resistance in M. haemolytica recovered from feedlot calves, highlighting the potential for using metagenomic sequencing for detecting bacteria with ARGs conferring AMR to important antimicrobials.

Financial Support: This research was conducted as part of the "Antimicrobial Stewardship Systems from Evidence-based Treatment Strategies for Livestock" project funded by Genome Canada with support from Genome Prairie, Genome Alberta, the Saskatchewan Agriculture Development Fund and the University of Saskatchewan.

Notes:

P066 - Metabolomics analysis of the liver of pigs under metabolic and inflammatory distress

A.N. Gomez[1], B.R. Southey[1], S.L. Rodriguez-Zas[1]

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

Session: Disease pathogenesis, 2025-01-20, 6:00 - 8:00

Objective: Hepatic function can be influenced by environmental challenges, and conversely, the liver participates in the pig's response to insults and maintain homeostasis. Metabolomic analysis can assist in understanding the effects of environmental insults on the liver molecular pathways.

Methods: To advance the understanding of the liver metabolome, untargeted liquid chromatography mass spectrometry was used to evaluate the impact of inflammatory and fasting challenges in two-month-old female and male pigs. Metabolites were identified by matching the MS output against spectral libraries. Individual metabolomic analysis of thirty-six pigs evenly distributed across sex and distress factor levels was undertaken using a model of challenge (control, inflammatory, or fasting), sex (female or male), and the interaction between challenge and sex. Enrichment (over-representation) analysis of pathways among the significantly differentially abundant metabolites was conducted.

Results: More than 650 molecules, including over 120 metabolites with precise identification, showed significant interaction with sex and a main effect of challenge. Among the metabolites showing significant interaction effects (FDR-adjusted p-value < 0.1), the changes in hepatic levels elicited by fasting relative to control were comparable in magnitude and direction across sexes (average log10(fold change) =1.61). On the other hand, the changes elicited by inflammatory challenge relative to control were more extreme in males (average log10(fold change) =0.21, 16% higher in males). On average, the change in metabolite levels (log10(fold change)) elicited by fasting relative to control was 7.9-fold more extreme than that from the inflammatory challenge. The response to the inflammatory challenge was lower in overall magnitude and more variable between sexes than the response to fasting in relation to controls. For example, the level of spermine was higher in fasting than in control females and males (1.72 and 1.64, respectively). In contrast, the level was lower in immune-challenged females and higher in immune-challenged males relative to controls (-0.41, and 0.23, respectively). Conversely, the level of gluconic acid was lower in fasting than in control females and males (-1.46 and -1.36, respectively). In contrast, the level was higher in immune-challenged females and lower in immune-challenged males relative to controls (0.19, and -0.16, respectively). Metabolites in the G-protein coupled receptor process were less abundant in fasting than in control males (FDR-adjusted p-value < 0.1). On the other hand, metabolites in the urea cycle process were more abundant in fasting compared to control males.

Conclusions: These results indicate that fasting and inflammation can substantially impact liver metabolism. The detection of metabolome disruption that varies with sex suggests that practices to address liver dysfunction in response to distress must be tailored to the sex of the affected pigs.

Financial Support: This study is supported by USDA NIFA grant numbers 2022-38420-38610 and 2018-67015-27413 and NIH grant number P30 DA018310.

Notes:

P068 - Determining interactions between commensal *Corynebacterium* and pathogenic *Mannheimia*

S.M. Howe[1], J.G. Powell[1], E.B. Kegley[1], J. Zhao[1]

[1]Department of Animal Science, Division of Agriculture, Univeristy of Arkansas. smhowe@uark.edu

Session: Disease pathogenesis, 2025-01-20, 6:00 - 8:00

Objective: Bovine respiratory disease (BRD) is the leading cause of feedlot calf morbidity and mortality, and the most prevalent bacterial opportunistic pathogens, including Mannheimia haemolytica, have been identified as members of the healthy bovine upper respiratory tract microbiota. Commensal *Corynebacterium* have been identified as core members of the bovine respiratory microbiota, and a recent meta-analysis identified *Corynebacterium* as a health-associated member of the bovine respiratory tract. Additionally, in a mouse model, commensal *Corynebacterium* has been demonstrated to protect against Respiratory Syncytial Virus and secondary *Streptococcus pneumoniae* infection. As a result, the objective of this study is to investigate commensal Corynebacterium's potential health-promoting role within the bovine respiratory tract, specifically its interaction with pathogenic M. haemolytica.

Methods: Two previously isolated commensal *Corynebacterium* isolates (jzb023 and jzb029) underwent whole genome sequencing with an Oxford Nanopore MinIon sequencer. Biosynthetic gene clusters were detected using the antiSMASH algorithm. Growth curve analyses were conducted to assess interactions with M. haemolytica serotype 1. Briefly, an 18-24-hour culture of jzb023 and jzb029 was centrifuged at 4400 rpm for 10 minutes. The resulting supernatant was sterilized using a 0.2-micron syringe filter. M. haemolytica serotype 1 was inoculated into a 96-well plate containing the following culture media (0% supernatant (control), 50% jzb023 supernatant, 100% jzb023 supernatant) (n=4/group). The plate was incubated at 37ºC for 24 hours. Optical Density (OD600) readings were obtained at plate entry and every 15 minutes for the duration of the experimental period. This was repeated for jzb029 supernatant (n=4/group). The resulting growth curves were assessed using the QURVE R package.

Results: Whole genome sequencing indicates that both jzb023 and jzb029 possess multiple classes of biosynthetic gene clusters, indicating they likely produce secondary metabolites. The use of 100% jzb023 supernatant as the growth media completely inhibited the growth of *M. haemolytica* serotype 1. Additionally, growth curve analyses indicate that 50% supernatant of jzb023 significantly decreases *M. haemolytica* growth rate and maximum growth and increases *M. haemolytica* doubling time ($P < 0.05$). Similar results were observed for 50% jzb029 supernatant. Whether this anti-*M. haemolytica* effect is bacteriostatic or bactericidal is currently being assessed.

Conclusions: Our results indicate that jzb023 and jzb029 possess anti-BRD properties, potentially mediated by a secondary metabolite. Future plans involve metabolomics to investigate the mechanisms of jzb023 and jzb029 *M. haemolytica* growth inhibition and to assess both isolates' abilities to colonize the nasal cavity, modulate the bovine immune system, and provide colonization resistance against *M. haemolytica*.

Financial Support: This project was supported by Agriculture and Food Research Initiative Competitive Grant no. 2024-67012-42409 from the USDA National Institute of Food and Agriculture, as well as funds from the Arkansas Biosciences Institute.

Notes:

P069 - Gestational diabetes mellitus alters mammary and hepatic protein expression in lactating rats

Alexandra Pace[1], Denina Simmons[2], Chloe C Josefson[3], Patricia Villamediana[4], Amy L Skibiel[1]

[1]University of Idaho, [2]Ontario Tech University, [3]North Carolina Central University, [4]South Dakota State University. pace4745@vandals.uidaho.edu

Session: Disease pathogenesis, 2025-01-20, 6:00 - 8:00

Objective: Overnutrition is common in pets such as dogs and cats, resulting in conditions such as obesity, pancreatitis, and chronic inflammation. This can predispose individuals to diabetes mellitus, which has become a growing epidemic in pets. in conjunction with pregnancy, overnutrition can increase risk for gestational diabetes mellitus (GDM), a metabolic and endocrine disease, which can cause pregnancy complications and lactation impediments, including reduced milk yield and altered milk composition. Further, inadequacies in neonatal nutrition can program offspring metabolism, consequently increasing susceptibility to metabolic disorders later in life. However, the mechanisms underlying lactation insufficiencies after GDM are unresolved. We aimed to evaluate the hepatic and mammary proteome in a rodent GDM model during lactation to identify disparate expression of proteins in pathways related to metabolism and lactation in GDM-afflicted individuals.

Methods: Healthy primiparous female Sprague-Drawley rats were fed either a standard rodent chow (6% fat; CON: n=33) or a high-fat rodent chow (40% fat; GD: n=36). Females were mated and pregnancy confirmed by observation of a vaginal plug and semen presence in vaginal lavage. At 10 days of gestation, GD rats received intraperitoneal injections of streptozotocin (30mg/kg) to induce GDM, while CON rats received the vehicle (0.1M citrate buffer, 1mL/kg). GDM was confirmed if blood glucose concentrations surpassed 300 mg/dL. Blood glucose concentration was checked 2 times per day, and insulin was used to treat hyperglycemia as needed. After parturition, litter size was standardized to 4 female and 4 male pups. Dams were euthanized on day 11 of lactation, and hepatic and mammary tissue collected. Label-free shotgun proteomics were conducted on both tissues from a subset of dams (GD: n=8, CON: n=8) through nanoscale liquid chromatography coupled to mass spectrometry. Peptide analysis was performed using Spectrum Milli Software, and searches were conducted against the *Rattus norwegicus* UniProt Reference Proteome (ID no. UP000234681). Statistical analyses were performed using Metaboanalyst 5.0 to conduct principle component analyses and ANOVA with post-hoc Fisher's LSD tests. Proteins were considered differentially expressed at a fold change ± 1, and a *P*-value £ 0.05.

Results: A total of 439 proteins were identified in hepatic tissue, and 291 proteins in mammary tissue. A total of 11 proteins were differentially expressed in hepatic tissue, while 55 proteins were differentially expressed in mammary tissue. GD rats had more upregulated proteins in hepatic tissue, but more downregulated proteins in mammary tissue relative to CON. Differentially-expressed hepatic proteins are involved in pathways including mediator complex regulation (e.g, MED25), and multiple catabolic processes (e.g., Fah, Pecam1, Gls). Differentially expressed mammary proteins are involved in pathways including intraepithelial T-cell differentiation (e.g., pr19, Lipa), and protein localization (e.g., Kif3a).

Conclusions: Pathways affected by GDM in hepatic tissue are largely related to DNA-level alterations and repair mechanisms. Altered hepatic proteins are involved in gene expression regulation and catabolism, while mammary gland proteins are involved in immune processes and protein movement. These results provide a basis for future investigation into specific mechanisms underlying GDM-induced effects on hepatic and mammary tissue function and resulting deficits in lactation traits.

Financial Support: This research was supported by NIH Grant no. P20 GM103408 from the National Institute of General Medical Sciences.

Notes:

P070 - Acapsular or asialic mutants of *Mannheimia haemolytica* are attenuated in a calf lung challenge model

Harish Menghwar[1], Fred M. Tatum[2], Anna GoldKamp[2], Brad Chriswell[2], Robert E. Briggs[2], Carly Kanipe[3], 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, United States Department of Agriculture, [3]Bacterial Diseases of Livestock Research Unit, National Animal Disease Center. harish.menghwar@usda.gov

Session: Disease pathogenesis, 2025-01-20, 6:00 - 8:00

Objective: Bovine respiratory disease complex (BRDC) is a multifactorial syndrome that involves complex interactions between environmental, bacterial and viral pathogens, and host. It is generally recognized that Mannheimia haemolytica is the most significant bacterial pathogen associated with BRDC. We have previously shown removal of sialic acid from LPS (neuA, CMP-N-acetylneuraminic acid (Neu5Ac/sialic) synthetase) increases the susceptibility of M. haemolytica to phagocytic- and complement-mediated killing in-vitro. Although leukotoxin is known as the major virulence factor, roles of capsule and sialic acid (sialylated LPS) as putative virulence factors have not been examined in animals. Therefore, a study was undertaken to investigate the virulence of a M. haemolytica sialic acid mutant (ΔneuA) and a *M. haemolytica* capsular (Δcap) mutant in a calf lung challenge model.

Methods: *M. haemolytica* St1 strain D153 was used to generate in-frame capsular (glycosyltransferase ABCD) and sialic (neuA) deletion mutants (Fig. 1). Twelve seven-to-eight-week-old colostrum-deprived calves were divided into three groups (group 1: wildtype, group 2: capsular mutant, and group 3: sialic acid mutant) of four animals per each group and were housed in indoor isolation rooms. Animals were intratracheally administered with respective *M. haemolytica* inoculum (wildtype, Δcap or ΔneuA, 5×10^8 CFUs in 50 mL) followed by 50 mL Earle's balanced salt solution. Animals were observed three times per day for signs of pneumonia and were humanely euthanized two-to-three days post-bacterial challenge. Lungs were examined for gross pulmonary lesions, scored, and samples were collected for bacterial culture and histopathological analysis.

Results: Deletion of the capsule and sialic acid genes in the mutants was confirmed by PCR (Fig. 1). Animals in group 1 (wildtype) showed mild clinical signs of pneumonia, while animals in groups 2 and 3 (mutants) did not show any signs of pneumonia. Calves challenged with wildtype strain exhibited severe lung lesions characterized by extensive consolidation and hemorrhage, affecting ~10.8 % of total lung tissues. The difference in lung consolidation between wildtype and mutant groups was statistically significant ($P < 0.05$). The most severely affected lung lobes were the right cranial and right middle lobes, with ~50% consolidation. In contrast, calves challenged with ΔneuA and Δcap mutants compared to wildtype displayed mild lung lesions with ~2.9 % total lung consolidation ($P < 0.05$). The lesions were less severe in both mutant groups compared to the wildtype group, indicating a reduction in virulence was due to the loss of capsule or sialic acid (in LPS). Histopathological analysis showed animals challenged with wildtype strain had the most severe lesions ($P < 0.05$), such as necrotic and suppurative exudate with degenerate streaming nuclei (oat cells) as compared to animals challenged with the Δcap or the ΔneuA strains.

Conclusions: The lack of clinical signs and pneumonic lung lesions in calves receiving the M. haemolytica acapsular or asialic acid mutants indicate that both capsule and sialylated LPS contribute to virulence. Future studies are planned to determine whether capsular and sialic acid mutants could be used as potential vaccine candidates.

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:

P071 - Peromyscus deer mice as an acute rodent model of leptospirosis

Ellie Putz[1], Claire Andreasen[2], Paola Boggiatto[1], Mitchell Palmer[1], Luis Fernandes[1], Bienvenido Tibbs-Cortes[1], Judith Stasko[1], Camila Hamond[3], Steven Olsen[1], Jarlath Nally[1]

[1]National Animal Disease Center, [2]Iowa State University College of Veterinary Medicine, [3]National Veterinary Services Laboratory. luisgui530@gmail.com

Session: Disease pathogenesis, 2025-01-20, 6:00 - 8:00

Objective: Leptospirosis is a devastating global zoonotic disease affecting humans, companion animals, and domestic livestock. Wild rodents are the primary reservoir hosts of pathogen spread and similarly, laboratory rats and mice are largely asymptomatic. The Golden Syrian Hamster (Mesocricetus auratus) is the dominant model for acute disease; they are susceptible to most pathogenic serovars and are heavily utilized to maintain laboratory strain virulence and test bacterin vaccine efficacy. However, hamsters are primarily utilized in survival-based studies, and are limited by a lack of immunology characterizing reagents. Peromyscus leucopus (deer mice) are more closely related to hamsters than Mus musculus mice and historically have been alternative rodent models for other spirochete diseases such as Lyme disease. This work reports on deer mice as an alternative model of acute leptospirosis.

Methods: Male and female deer mice were challenged with strains of *Leptospira* (*L. interrogans*) serovar Canicola and L. borgpetersenii serovar Arborea) by intraperitoneal injection. Upon appearance of severe clinical signs of disease (weight loss, blood on nose/paws, bloody urine, lethargy, etc.) animals were humanely euthanized. Whole blood slides were Giemsa stained and manually evaluated for differential blood counts. Kidney and liver tissues were cultured and pathogen load determined by lipL32 qPCR. Statistical differences were determined using a linear regression model in R. Immunological tissues including kidney, spleen, liver, and lung were formalin fixed and sectioned for pathology analysis.

Results: Challenged deer mice developed severe clinical signs and were positive for Leptospira by culture and qPCR. Males were more susceptible than females to acute disease and contained higher bacterial burdens in tissues. Additionally, deer mice produced circulating foamy macrophages, a marker of disease severity characterized in hamsters, in response to Leptospira challenge. Pathology investigation revealed differentiating lesion severity between infecting Leptospira species.

Conclusions: This work describes for the first time that deer mice are susceptible to acute leptospirosis. Infected deer mice produce foamy macrophages, display susceptibility differences between sexes, and offer an alternative rodent model for acute leptospirosis.

Notes:

P072 - Porcine astrovirus 4 as a cause of tracheitis and bronchitis in pigs

Michael Rahe[1], Jazz Stephens[1], Rachel Derscheid[2], Calvin Ko[2], Andrew Noel[2], Phil Gauger[2], Panchan Sitthicharoenchai[1], Jennifer Groeltz-Thrush[2], Rebecca DuBois[3], Danielle Haley[3], Alexandra Buckley[4]

[1]North Carolina State University, [2]Iowa State University, [3]University of California Santa Cruz, [4]United States Department of Agriculture. mrahe@ncsu.edu

Session: Disease pathogenesis, 2025-01-20, 6:00 - 8:00

Objective: Astroviruses are single-stranded RNA viruses shown to be associated with gastroenteric and neurologic disease in several animal species, including humans. Porcine astrovirus 4 (PoAstV4) has previously been associated with clinical respiratory disease and lesions of tracheitis and bronchitis in pigs; however, experimental reproduction of respiratory disease has not been performed. The objective of this study was to fulfill Koch's postulates with PoAstV4 inoculum.

Methods: Four-week-old cesarean-derived colostrum-deprived piglets (n=18 challenged and n=11 negative controls) were inoculated with a PoAstV4 positive tissue homogenate or tissue culture media (negative controls) through both intratracheal and intranasal routes. Inoculum was previously screened with NGS for other primary pathogens of swine. Nasal swabs, fecal swabs, and serum were collected throughout the study. One third of the pigs were euthanized on day post-challenge 5 (DPC5) for trachea and lung tissue collection. Another one third of pigs were euthanized on DPC8 with the final pigs sacrificed on DPC 21. PoAstV4 PCRs were run on swabs, tissue, and serum. PoAstV4 RNAScope in situ hybridization and CD3 and CD20 immunohistochemistry were run on sections of trachea and bronchi with QuPath evaluation for scoring of direct detection signal.

Results: PoAstV4 PCR results on nasal swabs showed a robust infection with peak shedding detected at 6 days post-challenge (6DPC). Seroconversion against PoAstV4 was achieved, with IgG against the capsid spike protein detected at 14DPC and an IgM curve starting at DPC5, peaking at DPC14 and decreased by DPC21. At DPC8, there were significant differences ($p<0.05$) in the histologic scores of epithelial attenuation and mononuclear cell infiltration in the trachea and bronchi of challenged vs. negative control pigs. Infection of the epithelium of the nasal turbinates, tracheas, and bronchi of challenged pigs at 5DPC was confirmed with PoAstV4 in situ hybridization (ISH) with quantitative assessment pending. Additionally, the characterization of the lamina propria mononuclear infiltrate with CD3 and CD20 immunohistochemistry ongoing.

Conclusions: These preliminary findings show that PoAstV4 is a cause of tracheitis and bronchitis in pigs and subsequent analysis will further characterize both the infection and the host immune response.

Financial Support: This work was supported by Swine Health Information Center (SHIC) award #23-077.

Notes:

P073 - Oral florfenicol core-shell composite nanogels for efficient treatment of bacterial enteritis

Ali Dawood[1], Samah Algharib[1, 2], Luo Wanhe[3]

[1]College of Veterinary Medicine, Mississippi State University, [2]Benha University, Egypt, [3]College of Animal Science and Technology, Tarim University. asd324@msstate.edu

Session: Disease therapeutics, 2025-01-20, 2:45 - 3:00

Objective: To reduce the bitterness of florfenicol, avoid its degradation by gastric acid, and enhance its antibacterial activity against *Escherichia coli* (*E. coli*) by targeting and slowly releasing drugs at the site of intestinal infection, with pectin as an anion carrier and chitosan oligosaccharides (COS) as a cationic carrier, florfenicol-loaded COS@pectin core nanogels were self-assembled by electrostatic interaction and then encapsulated in sodium carboxymethylcellulose (CMCNa) shell nanogels through the complexation of CMCNa and Ca2+ to prepare florfenicol core-shell composite nanogels. In this study, the florfenicol core-shell composite nanogels were investigated for their formula choice, physicochemical characterization, pH-responsive performances, antibacterial activity, therapeutic efficacy, and in vitro and in vivo biosafety studies.

Methods: The florfenicol core-shell composite nanogels used in this investigation were made by electrostatic interactions and crosslinking. The loading capacity (LC) and encapsulation efficiency (EE) of CMCNa, CaCl2, COS, and pectin were used to determine the ideal concentrations. The physicochemical parameters of the formulated nanogels were investigated using several tools, including scanning electron microscopy (SEM), transmission electron microscopy (TEM), mean particle diameter, zeta potential (ZP), polydispersity index (PDI), fourier transform infrared (FTIR) spectrophotometer, X-ray diffraction (PXRD), stability studies, rheological analysis, and pH-responsiveness of the shell, core, and composite nanogels. Additionally, the antibacterial activity of the prepared nanogels was evaluated using inhibition zones, minimum inhibitory concentration (MIC), time-killing curves, live/dead bacterial staining, colony counting, and morphological analysis. In vitro and in vivo biosafety studies were performed using hemocompatibility and histopathological analysis. Moreover, the therapeutic effects of the shell, core, and composite nanogels were explored using *E. coli* mouse infection model. The SPSS 19.0 program was used to do one-way ANOVA on all experimental data.

Results: The results indicated that the optimized formula was 0.6 g florfenicol, 0.79 g CMCNa, 0.30 g CaCl2, 0.05 g COS, and 0.10 g pectin. In addition, the mean particle diameter, PDI, ZP, LC, and EE were 124.0 ± 7.2 nm, -22.9 ± 2.5 mV, 0.42 ± 0.03, 43.4 % ± 3.1 %, and 80.5 % ± 3.4 %, respectively. The appearance, lyophilized mass, resolvability, SEM, TEM, PXRD, and FTIR showed that the florfenicol core-shell composite nanogels were successfully prepared. Florfenicol core-shell composite nanogels had satisfactory stability, rheology, and pH-responsiveness, which were conducive to avoid degradation by gastric acid and achieve targeted and slow release at the intestinal infection sites. More importantly, florfenicol core-shell composite nanogels had excellent antibacterial activity against *E. coli*, satisfactory therapeutic effect, and good palatability.

Conclusions: Florfenicol core-shell composite nanogels demonstrated acceptable palatability, satisfactory therapeutic effect, and excellent bactericidal efficacy against *E. coli* . Florfenicol core-shell composite nanogels have shown great promise as biocompatible oral treatment for bacterial enteritis, according to studies on their biosafety conducted both in vivo and in vitro.

Financial Support: Second group of Tianshan Talent Training Program: Youth Support Talent Project (2023TSYCQNTJ0033), Natural Science Support Program of Xinjiang, Production and Construction Corps (2024DA029), the Fundamental Research Funds for the Central Universities (KYLH2023003), Engineering Laboratory for Tarim Animal Diseases Diagnosis (ELDC202402)

Notes:

P074 - Elucidating species-specific early adhesion mechanism of *Escherichia coli* O157:H7 in vitro

Caleb Skow[1, 2], Melha Mellata[1, 2]

[1]Department of Food Science and Human Nutrition, [2]Iowa State University. cskow02@iastate.edu

Session: Disease pathogenesis, 2025-01-20, 6:00 - 8:00

Objective: Gastrointestinal illness acquired from contaminated food products is a predominant cause of hospitalizations in vulnerable populations such as travelers, infants, and the elderly. Enterohemorrhagic *Escherichia coli* (EHEC) is an enteric pathogen of emergent concern in humans of particular concern as it is carried without disease in cattle. This allows for contamination of beef products in processing that is often undetected prior to harvest. While much of the pathogenesis dynamics are described for this microbe, initial adhesion dynamics are poorly understood. Our study aims to utilize in vitro cell culture models to elucidate the involvement of the host mechanistic target of rapamycin (mTOR) pathway in initial adhesion mechanisms. Cell growth processes potentially complicit in adhesion bacterial adhesion, such as protein and lipid formation, are tightly associated with active mTORC1 expression, whereas mTORC2 expression influences cytoskeletal rearrangement and cell proliferation. By utilizing select inhibitors of this pathway, we are able to observe how these bacteria adhere differently to both human and bovine intestinal epithelial cells (BIEC).

Methods: CACO-2 human colonic cells and BIEC isolated from an 18-week-old Angus steer were cultured for 4 days in 16-well glass chamber slides to reach semi-confluency. At 2 hours prior to inoculation, mTORC1 inhibitor rapamycin and mTORC1/2 inhibitor TORIN-1 were added to respective treated wells at a concentration of 100 nM. A log-phase inoculum of MG1655 (K12) (control) or 34(4) AKANF4 (O157:H7) at a multiplicity of infection of 5:1 was subsequently added to each well and incubated at 37°C 5% CO_2 rocking for 1.5 hours, rinsed thrice with warm PBS, replaced with clean media, and incubated for an additional 2 hours. Slides were rinsed, fixed with methanol, and stained with Giemsa's solution. Twenty high-power fields of view were captured from each well and enumerated for adherent bacterial cells. Statistical analysis was performed as t-tests between treated and untreated wells within each model and strain, as well as between strains and models for identical treatments.

Results: Data show both EHEC and K12 adhered to CACO-2 cells and BIEC at an average of 1.75 and 2 bacterial cells per host cell respectively, without significant difference between strains. Treatment of BIEC cells with rapamycin (mTORC1 inhibitor) significantly reduced the adhesion capacity of EHEC but not K12 to BIEC. TORIN-1 (mTORC1/2 inhibitor) did not affect bacterial adhesion to BIEC. Neither inhibitor significantly affected the adhesion of either strain in CACO-2 cells.

Conclusions: Understanding the adherence mechanisms of EHEC in cattle is important to design successful antibiotic-free strategies against bacterial persistence in carrier animals. We demonstrated adherence of EHEC to BIEC to be partially dependent on active mTORC1. Targeting mTORC1 in host cells to reduce adherent EHEC in cattle may inhibit shedding dynamics. This will improve food safety by preventing food-borne illnesses, such as hemolytic uremic syndrome and benefit the cattle industry by providing a novel mitigation method for carcass contamination.

Financial Support: United States Department of Agriculture (USDA)- Hatch project IOW05700-NC1202 to MM.

Notes:

P076 - Experimental challenge of lactating dairy cattle with highly pathogenic avian influenza virus A/bovine/Ohio/B24OSU-342/2024

Carolyn Lee[1, 2], Bryant Foreman[2], Hannah Cochran[2], Patricia Boley[3], Jennifer Schrock[3], Natalie Tarbuck[2], Olaitan Shekoni[3], Raksha Suresh[3], Sara Dolatyabi[3], Christina Sanders[4], Elizabeth Ohl[4], Kaitlynn Starr[5], Yehia Saif[3], Juliette Hanson[3, 5], Renukaradhya Gourapura[3], Cody Warren[4], Andrew Bowman[2], Scott Kenney[2, 3]

[1]Center for Food Animal Health, The Ohio State University, [2]Department of Veterinary Preventive Medicine, The Ohio State University, [3]Center for Food Animal Health, The Ohio State University; Department of Veterinary Preventive Medicine, The Ohio State University, [4]Department of Veterinary Biosciences, The Ohio State University, [5]The Ohio State University Plant and Animal Agricultural Research. lee.8757@osu.edu

Session: Disease pathogenesis, 2025-01-20, 6:00 - 8:00

Objective: In March 2024, highly pathogenic influenza (HPAI) virus (A/bovine/Ohio/B24OSU-342/2024 (H5N1)) was isolated from cow's milk on an Ohio dairy farm following the development of fever and an abrupt drop in milk production. The Ohio outbreak was traced to interstate transportation of animals from a Texas farm, and has since caused outbreaks in dairy cattle across 14 states, with zoonotic transmission to humans reported. Here, we sought to determine the viral pathogenesis and transmission dynamics of the Ohio strain of bovine H5N1 by intranasally (IN) or intramammary (IMM) inoculating peak lactation Holstein cattle under biosafety level 3 containment.

Methods: Cows were assessed for clinical signs of illness (e.g. fever, feed intake, milk production, and mastitis), and evidence of viral replication and dissemination using viral RNA and live virus detection methods. Further, we evaluated viral transmission to calves via bottle feeding of infected cow's milk, and transmission to commercial laying chickens co-housed with IN virus inoculated Holstein cattle.

Results: While IN inoculated cows did not display clinical signs of infection and viral RNA was not detected in the milk, lesions appeared in the upper respiratory tract by days post inoculation (DPI) 5 and persisted to the study endpoint (DPI 18). IMM inoculation of cows resulted in lethargy, high fever, and decreased feed intake and milk production from DPI 1. Both IMM inoculated cows met early endpoint criteria due to severe disease, with milk HPAI viral titers reaching 1 x 10^9 PFU/mL by DPI 2. Calves bottle fed infected milk for four feedings did not display apparent clinical signs associated with HPAI infection, and nasal and oral swabs collected from calves contained low HPAI viral RNA loads. HPAI viral RNA was not detected in chickens co-housed with IN inoculated cows.

Conclusions: Our data indicate that IN inoculation of the Ohio strain of bovine H5N1 causes mild respiratory disease, while direct virus introduction into the mammary gland is the primary cause of morbidity and mortality observed on affected dairy farms. While our data suggest that respiratory transmission is unlikely the primary route of transmission of HPAI H5N1 between cattle, it is probable that improper cleaning of milking equipment, combined with the potential of HPAI H5N1 to replicate in the mammary tissue of cows, contribute to viral spread.

Financial Support: This project has been funded in whole or in part with Federal funds from the National Institute of Allergy and Infectious Diseases, National Institutes of Health, under Contract No. 75N93021C00016.

Notes:

P077 - Pharmacokinetics of bumped-kinase inhibitor 1708 in horses for equine protozoal myeloencephalitis therapy

Izabela De Assis Rocha[1], Jenna McPeek[1], Steve Reed[2], Matthew Hulverson[3], Ryan Choi[3], Lynn Barrett[3], Samuel Arnold[3], Wesley Van Voorhis[3], Daniel Howe[1], Allen Page[1]

[1]University of Kentucky, [2]Rood and Riddle Equine Hospital, [3]University of Washington. izabela.idar@uky.edu

Session: Disease pathogenesis, 2025-01-20, 6:00 - 8:00

Objective: Bumped Kinase Inhibitors (BKI) are antiprotozoal drugs capable of halting apicomplexan growth at the nanomolar level. The current pre-clinical lead, BKI-1708, is now under investigation as a therapeutic candidate for equine protozoal myeloencephalitis (EPM) caused by Sarcocystis neurona. Here we report the oral pharmacokinetics (PK) of BKI-1708 in horses assessing systemic distribution and central nervous system (CNS) penetration.

Methods: Four horses were dosed orally with 15mg/kg of BKI-1708 once a day for 5 days. The drug was dissolved in a blank commercial paste and administered at a dose based on previous intravenous PK studies in horses. To assess systemic distribution, plasma was collected daily at 0h, 2h, 4h, 12h, and then 24/48/72h after the last dose. Additionally, indwelling subarachnoid catheters were installed in the lumbosacral space on day 3 to investigate CNS penetration by serial cerebrospinal fluid (CSF) collections at steady-state levels. Horses were monitored daily for adverse effects by physical exams, and drug quantitation was done by LCMS/MS with a lower limit of detection (LLOD) of 0.1µM. Data was analyzed by descriptive statistic methods (mean and standard deviation).

Results: Administration of BKI-1708 orally at 15mg/kg led to plasma levels of 5 ± 1.2 µM at steady-state, with an initial distribution phase followed by a terminal phase. A half-life of 25.25 ± 8 h was observed for BKI-1708. BKI-1862, a metabolite of BKI-1708, was also detected in the plasma samples, at levels close to the LLOD (0.13 ± 0.11 µM). The indwelling subarachnoid catheters were successfully placed in 3 out of 4 horses and remained patent for the entire study duration. The levels of BKI-1708 in CSF were only detectable at 52 h in 2 out of 3 horses (0.47 µM and 0.63 µM), and BKI-1862 was not identified in these samples. No adverse effects were observed.

Conclusions: The results of this study demonstrate that BKI-1708 is well-tolerated by horses and sustains plasma levels sufficient to halt parasite growth following a daily oral dosing regimen. The low drug levels in CSF could be due to i) the presence of efflux pumps in the blood-brain barrier controlling drug levels, or ii) drug concentration in CNS tissue rather than CSF, due to the lipophilicity of this drug class. Follow-up studies using drug escalation regimens and terminal experiments including postmortem CNS sampling are indicated to establish the neuropharmacokinetic profile of BKI-1708 in horses. If sufficient drug levels can be achieved in the CNS tissues, a therapeutic dosing regimen will be established, and horses enrolled in a non-inferiority clinical trial against the FDA-approved drug ponazuril.

Financial Support: United States Department of Agriculture - National Institute of Food and Agriculture (USDA-NIFA) AFRI grant # 2020-67015-30881.

Notes:

P078 - Preparedness and early detection of foot and mouth disease outbreaks in Armenia in 2022-2023

Liana S. Sargsyan[1], Tigran A. Andreasyan[1]

[1]Ministry of Economy of the Republic of Armenia. sahavovnaliana@gmail.com

Session: Epidemiology, 2025-01-20, 6:00 - 8:00

Objective: Foot and mouth disease (FMD) is a highly contagious zoonotic disease with multiple serotypes, and its presence can have significant economic impacts. In 2022, according to data from the World Organization for Animal Health, Iraq, Jordan, Israel, and Turkey experienced FMD outbreaks caused by a new FMD serotype. To address this health issue, well-organized action by the Veterinary Service and other competent authorities should be activated to prevent the spread of the FMD virus. To ensure the stable epizootic situation in Armenia, we performed a Strengths, Weaknesses, Opportunities, and Threats (SWOT) analysis to better understand Armenia's FMD preparedness and detection capabilities and address the systems' strengths and weaknesses.

Methods: We utilized the available data in Armenia from official documents, legal acts, and results of the coordinated actions taken by the Veterinary Service and other competent authorities in 2022-2023 to strengthen the control of the FMD in Armenia. The SWOT analysis was conducted by reviewing Armenia's regulatory framework, current strategy, management, laboratory capabilities, partnership, available human resources, financial strategy, infrastructure, procurement system, communications and information system related to FMD preparedness in Armenia to formulate our strengths, weaknesses, opportunities and threats using researchers, management, policy makers, and strategy specialists. In addition, interviews were conducted with all employees involved in the implementation of the FMD control strategy.

Results: The analysis of results from the Veterinary Service and other stakeholder activities included compiling the system SWOTs. Strengths included: 1) vigilance through operationally coordinated work of the Veterinary Service and other stakeholders; 2) well-trained laboratory staff; 3) procurement of vaccines; 4) capacity to vaccinate animals; 5) FMD surveillance; and 6) recently updated identification and registration of cattle in Armenia. Weaknesses included:1) lack of early warning legal systems and diagnostic materials; 2) incomplete international cooperation; and 3) lack of identification and registration of other sensitive animals. Opportunities included: 1) developing an early warning system; 2) developing and implementing an identification and registration program for all FMD sensitive animals; and 3) promoting international cooperation. Finally, threats included: 1) limited financial resources.

Conclusions: This analysis enables the development of a future strategy to maximize our strengths, reduce current vulnerabilities, capitalize on opportunities and, as a result, plan a more effective and coordinated national FMD outbreak response system.

Notes:

P079 - Epidemiology and AMR patterns of *Enterococcus* in the US: Phenotypic and genotypic insights

Muhammad Rashid Bajwa[1], Rebecca Lee Smith[1]

[1]University of Illinois Urbana Champaign. mkbajwa2@illinois.edu

Session: Epidemiology, 2025-01-20, 6:00 - 8:00

Objective: Enterococcus spp. are Gram-positive bacteria, part of the human gut microbiome. However, they can develop antibiotic resistance and transfer this to other species via "cross-resistance". Hence, studying the Enterococcal resistance is critical for monitoring the overall antibiotic resistance in the gut microbiota. Using the existing resistance data, the current study aims to: 1) Characterization of AMR prevalence and patterns in human and animal isolates 2) Assessment of the occurrence and distribution of multidrug resistance (MDR) 3) Comparison of AMR profiles between human and animal isolates 4) Investigation of associations between resistance phenotypes and genetic determinants.

Methods: Antimicrobial resistance data for Enterococcus isolates (n = 4,103), including human and animal samples, were obtained from the National Antimicrobial Resistance Monitoring System (NARMS) and the National Center for Biotechnology Information (NCBI) databases. Antibiotic resistance was determined based on predefined threshold levels for each antibiotic, while MDR was defined as resistance to ≥ 3 antibiotic classes. Chi-square tests were used to compare the prevalence of resistance genes between animal and human isolates.

Results: Antimicrobial resistance data revealed >90% of antibiotic resistance in Enterococcus isolates from both animals and humans. The highest resistance rates were observed for clindamycin (89.4%, 95% CI: 88.4-90.3%), ampicillin (84.5%, 95% CI: 83.3-85.6%), and ciprofloxacin (84.2%, 95% CI: 83.0-85.3%). The chi-square test between the top two antibiotics, ampicillin and clindamycin, revealed a significant association ($\chi^2 = 42.037$, $p < 0.001$), indicating a strong correlation in resistance patterns that warrants further investigation.

Conclusions: This comprehensive analysis of antimicrobial resistance data provides a solid foundation for understanding the patterns of antimicrobial resistance in Enterococcus. These results have significant implications for healthcare and agricultural practices, offering essential insights to shape antibiotic usage regulations, treatment strategies, and antimicrobial stewardship initiatives in medical and agricultural contexts. In the future, genetic data will be used to figure out how these resistance patterns work. This data will also be used to look into how resistance genes spread and to record possible transmission pathways through phylogenetic studies. This integrated approach will advance our understanding of antimicrobial resistance (AMR) in Enterococcus and guide specific measures to address this growing public health concern.

Notes:

P080 - Data-driven herd-level standards for diagnosing omphalitis in dairy calves based on umbilical stump diameter

Alexandre Rico[1], Alda F.A. Pires[1], Noelia Silva-del-Rio[1]

[1]University of California, Davis. aricofernandez@ucdavis.edu

Session: Epidemiology, 2025-01-20, 6:00 - 8:00

Objectives: Omphalitis prevalence on dairy farms is underdiagnosed, potentially due to the laborious nature of clinical examinations and the lack of a gold standard. In research settings, umbilical stump diameter has been used as a diagnostic criterion, but different thresholds based on arbitrary criteria have been used. Machine learning, through unsupervised clustering, offers a novel solution for characterizing pathological and non-pathological subpopulations. This study has two objectives, first to assess the reliability—intraobserver and interobserver agreements—of a technique for measuring the umbilical stump diameter, and second to propose data-driven thresholds for diagnosing omphalitis in Holstein (HO) and Jersey (JE) heifers aged 3 to 10 days.

Methods: The umbilical stump diameter was measured using a digital caliper positioned latero-laterally in relation to the calf as close as possible to the external umbilical ring. For our first objective, convenience samples of 4-day-old female calves were enrolled in the intraobserver (n = 157, 1 rater, 3 times) and interobserver (n = 44, 3 raters, 1 time) evaluations. For our second objective, we enrolled 667 female calves (HO, n = 437; JE, n = 230) aged 3 to 10 days from 17 source dairies. The statistical analysis was performed using the R language for statistical programming. The intraobserver agreement was assessed using the coefficient of variability (CV), while the interobserver agreement was assessed using the intraclass correlation coefficient (ICC). Thresholds were independently estimated for each breed using finite mixture models, Expectation-Maximization algorithm, parametric simulations, and ROC analysis. The main assumption was that the observed sample of umbilical stump diameters consists of a mixture of one non-pathological population (hypothetically non-diseased animals) and one pathological population (hypothetically diseased animals), both modeled as lognormal distributions.

Results: The intraobserver agreement [5.94% CV (95% CI: 5.31-6.57)] was classified as very good, and the interobserver agreement [ICC of 0.76 (95% CI: 0.65-0.85)] was fair to good. The ROC analysis showed AUCs of 0.62 (95% CI: 0.56-0.67) for HO and 0.74 (95% CI: 0.67-0.81) for JE. The optimal thresholds—the best trade-off between sensitivity (Sn) and specificity (Sp)—were identified at 16.6 mm for HO and 12.6 mm for JE. Both thresholds showed high Sp—around 0.95 in both breeds—but fair to poor Sn—0.38 in HO and 0.61 in JE. Achieving a Positive Predictive Value (PPV) of 0.99 is possible by setting the thresholds at 19.7 mm for HO and 14.6 mm for JE.

Conclusions: The umbilical stump diameter, measured at the external umbilical ring, is precise and can be used for discriminating between cases and non-cases of omphalitis in dairy heifers during the first days of life. The most convenient approach is to establish thresholds based on the estimated accuracy of predicting a case. Achieving a PPV of 0.99 is possible by setting the thresholds at 19.7 mm for HO and 14.6 mm for JE. The proposed thresholds should be considered as herd-level standards and applied for making decisions based on population-oriented approaches to provide reasonable targets for improving calf health.

Funding Support: This work was supported in part by Scholarship Program for Graduate Studies of the Fundación Caixa Rural Galega Tomás Notario Vacas (Lugo, Spain) and USDA National Institute of Food and Agriculture (Washington, DC; CFAH Animal Health under project #436).

Notes:

P083 - Brucellosis in livestock in Costa Rica: Outbreaks analysis based on retrospective data

D Areda[1], Ronald Castillo[2]

[1]Ottawa University, [2]World Organization for Animal Health. demelash.biffa@gmail.com

Session: Epidemiology, 2025-01-20, 6:00 - 8:00

Objective: Brucellosis is a significant disease affecting livestock productivity, especially in regions with limited animal health surveillance and services. Additionally, it is a recognized zoonotic disease, presenting a notable public health risk worldwide. This study aimed to analyze brucellosis outbreaks in livestock within Costa Rica, utilizing retrospective data from 2014 to 2017 to evaluate the disease's prevalence, distribution, and trends.

Methods: Data were obtained from the SIVE application (Integrated Epidemiological Surveillance System), developed by SENASA (Costa Rican Veterinary Services). SENASA investigates disease outbreaks as part of its surveillance system. Samples were submitted for laboratory analysis at various locations, including the central laboratory as well as its branches distributed across the country. Diagnostic testing included the Rose Bengal Test (RBT) for rapid screening of Brucella, followed by indirect ELISA (iELISA) or competitive ELISA (cELISA) for confirmation. Sample analysis was conducted by qualified laboratory technicians at each location. Data were analyzed using descriptive statistics, regression analysis, and time series analysis with R statistical software. An outbreak was defined as confirmed cases of brucellosis where samples from affected animals tested positive for the diagnostic tests used. Prevalence was defined as the proportion of animals that tested positive for brucellosis out of the total number of animals sampled during the study period.

Results: The analysis revealed a total of 4,643 confirmed brucellosis cases (positive for both RBT and iELISA) across 1,141 outbreaks, involving 107,614 animals at risk. Of the 63,555 animals sampled, prevalence of 12.37% and 7.31% were recorded based on RBT and iELISA, respectively. Time series analysis indicated a seasonality strength of 5.3%, suggesting that seasonality has a minimal impact on case variability, implying weak seasonal patterns in brucellosis cases. Monthly averages ranged from 3.04 cases in February to 4.87 cases in June, but these differences were not statistically significant (F-statistic=0.52, $p>0.05$).

Conclusions: The study highlights a modest overall prevalence of brucellosis in livestock in Costa Rica, with no significant monthly seasonal pattern observed in case numbers. These findings suggest that brucellosis outbreaks are less influenced by seasonal changes and may instead be driven by other risk factors. Further analysis will focus on identifying key risk factors associated with outbreak occurrences, including herd size, geographic location, and potential seasonal patterns through advanced regression, spatial, and time series analyses. These insights will be crucial for developing targeted strategies for brucellosis control and prevention in the region.

Notes:

P084 - Genomic characterization of equine rotavirus B circulating in central Kentucky

Chandika Gamage[1, 2], Amy Amy[3], Jennifer Morrow[3], Ganwu Li[4], Come J. Thieulent[1, 2], Udeni B. R. Balasuriya[1, 2], Mariano Carossino[1, 2]

[1]Dept. of Pathobiological Sciences, Louisiana State University, [2]Louisiana Animal Disease Diagnostic Laboratory, Louisiana State University, [3]Equine Diagnostic Solutions, [4]Dept. of Veterinary Diagnostic and Production Animal Medicine, Iowa State University. cgamage@lsu.edu

Session: Epidemiology, 2025-01-20, 6:00 - 8:00

Objective: In 2021, group B equine rotavirus (ERVB) was detected for the first time in diarrheic foals in Central Kentucky, USA. Since this first detection, ERVB has been detected in subsequent foaling seasons as a cause of foal diarrhea along with group A equine rotavirus (ERVA). Similar to ERVA, ERVB is a non-enveloped, double-stranded and segmented RNA virus in the family *Sedoreoviridae. Its genomic arrangement is similar to ERVA, with* 11 double-stranded RNA segments that encode six structural and five non-structural viral proteins. Based on phylogenetic analysis, it was determined that ERVB was related to RVB strains of ruminant origin. The aim of this study was to perform genomic characterization of ERVB strains currently circulating in Central Kentucky.

Methods: During the 2024 foaling season, fecal specimens from diarrheic foals were submitted to Equine Diagnostic Solutions (Lexington, KY) for ERVA and ERVB testing using a genotyping multiplex RT-qPCR assay developed by our laboratory. From a subset of 45 specimens that tested positive for ERVB we selected a total of 15 ERVB-positive samples with Ct values <22 for Illumina-based whole-genome sequencing. Coding-complete genome sequences were aligned using the strain RVB/Horse-wt/USA/KY/1518 as reference and phylogenetic analysis was performed for each genome segment using the maximum-likelihood method with 1,000 bootstrap replicates.

Results: Among the 45 samples that tested positive for ERVB, Ct values ranged from 16.87 to 35. We obtained coding-complete genome sequences for 15 samples with Ct values <22. Sequence analysis for the 11 genome segments demonstrated nucleotide sequence identities >99% compared to the RVB/Horse-wt/USA/KY/1518 reference strain. Phylogenetic analyses of genes encoding structural and non-structural proteins clustered closely with ruminants' origin RVB strains.

Conclusions: Our findings indicate that a single genomic constellation of ERVB is circulating in the equine population in Central Kentucky since its first identification in 2021, with no evidence of other ERVB genotypes circulating to date. Continued ERVB surveillance is crucial to monitor for potential antigenic drift or reassortment events and inform vaccine development.

Financial Support: This study was supported by a Grayson Jockey Club Research Foundation award (Award #863; GR-00011211), USDA 1433 Formula funds (GR-00010866; School of Veterinary Medicine, Louisiana State University) and start-up funds by the School of Veterinary Medicine to Mariano Carossino (PG009641).

Notes:

P085 - Bovine leukemia virus infection detection and dynamics in dairy youngstock

Madison E. Sokacz[1], Kelly R. B. Sporer[2], Bo Norby[3], Tasia M. Taxis[1]

[1]Michigan State University Department of Animal Science, [2]CentralStar Cooperative, Inc., [3]Michigan State University Large Animal Clinical Sciences. sokaczma@msu.edu

Session: Epidemiology, 2025-01-20, 6:00 - 8:00

Objective: Bovine leukemia virus (BLV) is the causative agent of enzootic bovine leukosis, a persistent infection with significant economic and animal health impacts in dairy herds. Although BLV transmission in adult cattle is well-documented, less is known about the infection dynamics in youngstock. This study aimed to longitudinally assess BLV infection dynamics in dairy youngstock across commercial dairy farms in Michigan.

Methods: A total of 254 heifer calves from five farms were enrolled and sampled at three early-life stages: neonates (4.9 ± 2.3 days of age), pre-breeding age (377.2 ± 31.8 days of age) and post- breeding (474.2 ± 37.1 days of age). Blood samples were analyzed using both enzyme-linked immunosorbent assay (ELISA) and quantitative polymerase chain reaction (qPCR) to assess BLV infection prevalence and incidence. Statistical analyses were conducted in R software using generalized linear models and pairwise comparisons to compare infection rates between time points and to assess the consistency between the two diagnostic assays. Additionally, Cohen's kappa was used to measure the agreement between the ELISA and qPCR assay results.

Results: At enrollment (neonates), apparent BLV prevalence was low by qPCR 1.2 % ± 1.25% but higher by ELISA 40.6 ±13.4%. By pre-breeding, the apparent prevalence by qPCR increased to 10.6 ± 24.1%, and further increased to 12.6 ± 28.1% by post-breeding. Apparent prevalence by ELISA decreased from the neonatal stage down to 13.4 ± 26.6% and 16.9 ± 24.1% at pre-breeding and post-breeding stages, respectively. The point incidence was highest between neonates and pre-breeding, with an average of 13.8 ± 3.3 % for qPCR and 17.2 ± 8.53% for ELISA. Analysis revealed a significant increase in infection rates by qPCR between the neonate stage and pre-breeding stage ($p<0.0001$), but no significant change between pre-breeding and post-breeding stages ($p=0.355$). Cohen's kappa was calculated to assess agreement between ELISA and qPCR, showing poor agreement at the neonatal stage and improving to moderate and strong agreement at future stages. Additionally, two subsets of youngstock were recognized. Fourteen youngstock were identified as persistently infected with four of those fourteen reaching high amounts of BLV by the post-breeding stage. Furthermore, a second subset including seven youngstock were characterized by transient infections, meaning the youngstock were exhibiting BLV positive results, either ELISA or qPCR, followed by subsequent negative results.

Conclusions: The study findings indicate that youngstock are particularly susceptible to BLV transmission during the first year of life. There is a possibility that sub-optimal management practices focused on youngstock are leading to previously overlooked transmission risks. Discrepancies between ELISA and qPCR at the neonatal stages suggest that ELISA is not ideal for testing at this age, due to potential interference from maternal antibodies. Given the identification of youngstock with high levels of BLV by post-breeding age, this study underscores the need for screening of BLV in order to identify and prevent those infections from entering the milking herd.

Financial Support: Thank you for the support of this project, provided by Agriculture and Food Research Initiative Competitive Grant;2020-67015-31562, and 2015-67028-23652 from the USDA National Institute of Food and Agriculture.

Notes:

P086 - Using time-dependent sensitivity analysis to help understand and manage dynamic ecological systems

Scott H. McArt[1], Wee Hao Ng[1], Stephen P. Ellner[1], Christopher R. Myers[1]

[1]Cornell University. shm33@cornell.edu

Session: Epidemiology, 2025-01-20, 6:00 - 8:00

Objective: Sensitivity analysis is often used to help understand and manage ecological systems by assessing how a constant change in vital rates or other model parameters might affect the management outcome. This allows the manager to identify the most favorable course of action. However, realistic changes are often localized in time - for example, a short period of culling leads to a temporary increase in the mortality rate over the period. Hence, knowing when to act may be just as important as knowing what to act on.

Methods: We introduce the method of time-dependent sensitivity analysis (TDSA) that simultaneously addresses both questions. We illustrate TDSA using three case studies: transient dynamics in static disease transmission networks, disease dynamics in a reservoir species with seasonal life history events, and endogenously driven population cycles in herbivorous invertebrate forest pests.

Results: We demonstrate how TDSA often provides useful biological insights, which are understandable in hindsight but would not have been easily discovered without the help of TDSA. However, as a caution, we also show how TDSA can produce results that mainly reflect uncertain modeling choices and are therefore potentially misleading. We provide guidelines to help users maximize the utility of TDSA while avoiding pitfalls.

Conclusions: TDSA can be viewed as a stripped-down version of optimal control theory, which brings both advantages and disadvantages. The disadvantage is that it provides less information because it is guaranteed to be accurate only for small perturbations. The main advantage is that it requires fewer assumptions and therefore imposes fewer constraints on the modeler. TDSA is also computationally much simpler and faster. By presenting a balanced view that highlights both the strengths of TDSA and the potential pitfalls, we hope that TDSA can become a useful addition to the toolkit of modelers and natural resource managers.

Financial Support: This work was funded by USDA-NIFA AFRI grant 2021-67015-35235 as part of the joint USDA-NSF-NIH-UKRI-BSF-NSFC Ecology and Evolution of Infectious Diseases program.

Notes:

P087 - Detection of influenza A virus antibodies in cattle in the US

Y. Lang[1, 2], L. Shi[1, 2], D. Gupta[1, 2], S. Roy[1, 2], C. Dai[1, 2], A. Khalid[1, 2], M. Zhang[1, 3], Shuping zhang[1, 3], R. Webby[4], W. Ma[1, 2]

[1]Dept. of Veterinary Pathobiology, University of Missouri, [2]Dept. of Molecular Microbiology & Immunology, University of Missouri, [3]Veterinary Medical Diagnostic Laboratory, University of Missouri, [4]St. Jude Children's Research Hospital. wma@missouri.edu

Session: Epidemiology, 2025-01-20, 6:00 - 8:00

Objective: Unprecedented outbreaks caused by the highly pathogenic avian influenza H5N1 virus among dairy cows in the US have raised significant concerns for public and animal health. To date, limited research regarding ruminants infected with influenza A viruses (IAVs) has been performed and the epidemiology of IAV infections in cattle in the US remains unknown.

Methods: We screened more than 1,700 serum samples collected from cattle in 15 states of the US since January 2023 using an Enzyme-linked immunosorbent assay (ELISA) to detect IAV infections and the positive samples were further tested by hemagglutination inhibition (HI) assay.

Results: Our results showed that more than 500 bovine serum samples collected in both 2023 and 2024 were ELISA positive, and some of them were positive to human H1N1 and H3N2 seasonal viruses as well as the H1N2 variant, the cluster I and IV H3N2 swine viruses by HI assay. None of the ELISA-positive samples were positive for the circulating H5N1 strain according to the HI assay results.

Conclusions: Our results indicate that IAVs can infect the bovine species, not limited to dairy cows. All results warrant the necessity to monitor bovine IAV epidemiology, as it might adapt to cattle and spread to humans, potentially causing future outbreaks.

Financial Support: This study is partially supported by the Centers of Excellence in Influenza Research and Response (CEIRR), contract number 75N93021C00016 and MU startup fund.

Notes:

P088 - Understanding strengths and challenges in the surplus calf industry

Samantha Locke[1], Alejandra Arevalo[1], Sara Sequeira[1], Devon Wilson[2], Andreia Arruda[1], Jessica Pempek[3], Greg Habing[1]

[1]The Ohio State University, [2]Dr. Devon Wilson, DVM, [3]USDA ARS - Livestock Behavior Research Unit. Locke.91@osu.edu

Session: Epidemiology, 2025-01-20, 6:00 - 8:00

Objective: Surplus calves are animals produced by the dairy industry but not retained on the farm as herd replacements, namely, male calves and excess females. These animals primarily enter dairy-beef or veal production systems. In recent years, surplus calf production has come under scrutiny due to welfare concerns, such as health outcomes and housing. To design and implement effective interventions, it is critical to understand the perspectives of industry stakeholders (i.e., calf marketers and calf raisers). However, little research has been conducted in surplus calf production systems in the United States. Therefore, the objective of this study was to understand surplus calf marketer and calf raiser perspectives of the strengths and challenges within the surplus calf system in the United States.

Methods: Twenty-two telephone interviews were conducted from June 2023 to January 2024. Participants included 7 dairy beef raisers, 6 veal industry stakeholders, 5 livestock market representatives, and 4 calf dealers. Individuals were located throughout the Northeast and Midwest US. The interview questionnaire was designed to take approximately 20 minutes to complete. Mean (range) interview duration was 31 minutes (11 to 69). Interviews were recorded, anonymized, and transcribed. Transcripts were then analyzed using inductive thematic analysis.

Results: Most participants expressed satisfaction with their day-to-day management strategies (e.g., health and nutrition programs, personnel). Varied opinions were expressed regarding the current market system for calves. When questioned about challenges or opportunities for improvement, participants discussed labor issues and lack of industry expertise in advisors. Participants also conveyed concerns regarding long-distance transport and the resultant stress on calves. Several individuals suggested simplifying calf marketing to reduce transport times. Focusing on improving conditions during calf transport may also result in improving other challenges expressed by participants, such as calf health when arriving at the calf raisers.

Concluisons: Future work to streamline calf marketing, as well as bolstering resources available to calf growers may be beneficial to the industry. Additionally, qualitative work to ensure a broad representation of stakeholder perspectives, particularly from other regions, may yield additional research avenues.

Financial Support: This research is funded by a grant from National Institute of Food and Agriculture No. 2022-68015-36628ca.

Notes:

P089 - Serengeti wildebeest exhibit lower fecal parasite burdens than two closely related resident ungulate species

Ricardo M Holdo[1], Jason E Donaldson[1], Basil Senso[1], T. Michael Anderson[2], Aidan Trentinus[3], Vanessa O Ezenwa[4]

[1]University of Georgia, [2]Wake Forest University, [3]Tanzania Centre for Research Cooperation, [4]Yale University. rholdo@uga.edu

Session: Epidemiology, 2025-01-20, 6:00 - 8:00

Objective: Migration has important consequences for infectious disease dynamics. Several mechanisms have been identified that could lead to lower parasite burdens in migratory than non-migratory (resident) animals, including migratory escape from habitats with high parasite loads and migratory culling of sick individuals. There is support for these mechanisms in some temperate migratory ungulates, but so far there has been little research on this topic in tropical ecosystems, where the drivers of migratory behavior are quite different. We used a multi-year dataset designed to investigate the effects of migration on resident-species parasite dynamics in the Serengeti ecosystem of East Africa to compare parasite burdens in closely related species with contrasting migratory behavior across the annual cycle.

Methods: Beginning in Oct 2021, we collected fecal samples from five ungulate species within a 1000-km^2 region of Serengeti National Park, Tanzania. Here we focus on three species: migratory wildebeest (Connochaetes taurinus) and two other closely-related species from the Alcelaphini tribe: topi (Damaliscus lunatus) and hartebeest (Alcelaphus bucelaphus). Wildebeest are migratory, and topi and hartebeest are year-round residents. Wildebeest show two peaks of high occupancy over the course of an annual cycle, during the southwards and northwards migration phases. We divided the focal area into seven regions, with a target sample collection of three fecal samples per species per region per month. We used vehicles to find individual animals or herds in each region, and immediately collected fresh fecal samples from observed defecations. We immediately transported samples in a cooler to the lab, where we processed them to quantify gastrointestinal nematode (GIN) egg counts, and lungworm larval counts. We tested for species differences in mass-adjusted fecal parasite burdens using mixed models with region as a random effect.

Results: Over three years of sampling, we collected >1000 fresh fecal samples each for wildebeest, topi, and hartebeest fecal samples. For both GIN and lungworms, we found consistently lower burdens in wildebeest than topi or hartebeest across seasons, suggesting lower parasite burdens in migratory than resident animals. The pattern was particularly striking for lungworm burdens: the two resident species had larval counts that were over an order of magnitude higher than those for wildebeest. Parasite burdens were comparable in the two-resident species.

Conclusions: Our results suggest that migratory escape and/or culling may be operating in this system, although our current data do not allow us to formally distinguish between these mechanisms, and further results are pending that compare parasite burdens in migratory and resident wildebeest. In terms of applied importance, wildebeest, as migrants, are the primary wild ungulate species coming into contact with livestock on the periphery of the ecosystem. Regardless of whether migration itself explains the lower parasite burdens in wildebeest, our results have implications for cross-species parasite transmission rates of nematodes from wildebeest to domestic species.

Financial Support: The work was supported by a USDA National Institute of Food and Agriculture Ecology and Evolution of Infectious Disease Grant (#2021-67015-33407).

Notes:

P090 - Prevalence and spread of antimicrobial resistant pathogens in backyard poultry farms

D. Reed Golden[1], Pankaj P. Gaonkar[1], Laura Huber[1], Brigid McCrea[2], Yagya Adhikari[3], Matthew Bailey[3], Kenneth Macklin[4]

[1]Pathobiology Department, College of Veterinary Medicine, Auburn University, [2]Alabama A&M and Auburn University Extension, [3]Department of Poultry Science, Auburn University, [4]Department of Poultry Science, Mississippi State University. drg0026@auburn.edu

Session: Epidemiology, 2025-01-20, 6:00 - 8:00

Objectives: Antimicrobial resistance (AMR) is a growing global health concern, with resistant pathogens found across various environmental reservoirs. While extensive research has shown that commercial poultry production exerts significant selective pressure that promotes the development and spread of AMR pathogens, little is known about the effects of small-scale backyard poultry farming on AMR prevalence and transmission. This study aims to address this gap by investigating the presence and spread of AMR pathogens in backyard poultry farms and assessing how this sector contributes to the overall risk of AMR in agricultural and community settings.

Methods: In this study, five sample types, including chicken fecal matter, litter, soil from inside (direct contact with chickens) and outside (no direct contact with chickens) the chicken coop, and a fecal sample from outside the chicken coop of unknown origin (wild or domestic animal) will be collected from each farm. Serial dilutions will be performed on the enriched samples to achieve three target dilution levels. Each dilution will be plated on CHROMagar, both with and without antibiotics for the selective culturing of susceptible and resistant-Escherichia coli and incubated at 37°C for colony enumeration. From each growth condition, three representative isolates will be selected. These isolates will then be used for disk diffusion tests and sequencing to further characterize their phenotypes and genotypes. In addition to screening for ESBL *E. coli*, 38 farms were screened for *Salmonella* spp. using MDS.

Results: Preliminary analysis indicates MDS-*Salmonella* detected in two bonus feces samples and one litter sample, though the organism could not be successfully cultured, while virulence genes associated with avian pathogenic Escherichia coli (APEC) were identified in at least one instance across all sample types. Additionally, three samples tested positive for extended-spectrum beta-lactamase (ESBL) producing *E. coli*. Complementary data from farm questionnaires on practices related to sanitation, species interactions, and antimicrobial usage (AMU) have been collected, will provide context for observed AMR patterns and informing potential risk factors associated with its transmission.

Conclusion: Although selective pressures associated with antimicrobial resistance are generally more pronounced in commercial poultry due to higher animal density and frequent AMU, the lack of biosecurity in backyard poultry farming presents unique risks. Common biosecurity gaps such as limited sanitation practices, open access to multiple species, and infrequent health monitoring create conditions conducive to AMR development and transmission. This study aims to reveal the prevalence and spread of AMR within backyard poultry environments, shedding light on the role these settings play in broader community AMR dynamics and informing targeted AMR mitigation strategies.

Financial Support: College of Veterinary Medicine, Auburn University

Notes:

P091 - Do swine farms have a mosquito problem? Assessing mosquito abundance and diversity in US commercial swine farms

Stephen Edache[1], David Edache[1], Lauryn Mauler[1], Lee Cohnstaedt[2], Ashley Thackrah[1], Vanessa Horton[1], Dana Mitzel[2], Andrea Dixon[1], Natalia Cernicchiaro[1]

[1]Kansas State University, [2]United States Department of Agriculture. edache@ksu.edu

Session: Epidemiology, 2025-01-20, 6:00 - 8:00

Objective: Pathogen transmission by mosquitoes is influenced by species abundance, diversity, and feeding behaviors. Certain animals, such as swine and birds, serve as reservoirs and amplifying hosts for certain mosquito-borne diseases, while others, like humans and horses, act as dead-end hosts. In commercial swine operations, the high density of potential hosts could lead to severe outbreaks if mosquito-borne pathogens are introduced. However, there is limited information on the presence of competent mosquito vectors around U.S. swine farms with varying biosecurity measures. Here, we investigated the abundance and species diversity of adult host-seeking and resting mosquitoes from commercial swine farms in southern Iowa between June and October 2024.

Methods: The study was conducted across four sow farms and four wean-to-market farms located in Southern Iowa. Three Biogents sentinel traps were set at each swine farm. Two traps, baited with dry ice, were set outdoors and positioned near mosquito-rich habitats such as sewage lagoons, ponds, and surrounding vegetation. The third trap was placed in the gestation barn of sow farms, and specific corners near visible cobwebs in the barns of wean-to-market farms. Adult resting mosquitoes were collected by aspirating selected outdoor habitats. Samples were collected biweekly on two consecutive days during the summer and early fall. The samples were shipped on dry ice to the entomology laboratory at Kansas State University for sorting, counting, sexing, and taxonomical identification. Mosquito species were initially identified using an artificial intelligence-based diagnostic system and later confirmed using morphological keys.

Results: Although mosquito numbers fluctuated over time, they were consistently higher on sow farms than on wean-to-market farms, with more found outdoors than indoors, and the majority captured in traps (for host-seeking mosquitoes) rather than in aspirators (for resting mosquitoes). The most abundant species belonged to the Culex genus, followed by mosquitoes from the Aedes and Anopheles genera, all of which are recognized as competent vectors of arboviruses in livestock settings.

Conclusions: The preliminary data presented here show the abundance and diversity of mosquito vectors on swine farms. This could be attributed to the presence of favorable habitats, such as lagoons, nearby vegetation, and farm management practices that promote water-breeding habitats. These findings highlight the critical need for mosquito surveillance in and around swine operations, offering valuable opportunities for developing effective vector control strategies, especially in the event of an arboviral outbreak.

Financial Support: Research funding is provided by USDA ARS NACA No. 58-3020-9-022, Center for Outcomes Research and Epidemiology (CORE), and College of Veterinary Medicine, Kansas State University (CVM-KSU).

Notes:

P092 - Seasonal white-tailed deer habitat use and implications for disease transmission

Marie L. J. Gilbertson[1], Alison C. Ketz[2], Matthew A. Hunsaker[1], Daniel P. Walsh[3], Daniel J. Storm[4], Wendy C. Turner[5]

[1]Wisconsin Cooperative Wildlife Research Unit, University of Wisconsin-Madison, [2]Funga, Austin, TX, [3]U.S. Geological Survey, University of Montana, [4]Wisconsin Department of Natural Resources, [5]U.S. Geological Survey, University of Wisconsin-Madison. mgilbertson5@wisc.edu

Session: Epidemiology, 2025-01-20, 6:00 - 8:00

Objective: Chronic wasting disease (CWD) is a fatal prion disease affecting cervid species, including white-tailed deer (*Odocoileus virginianus*; WTD), and has been increasing in prevalence and geographic range, despite decades of research, surveillance, and management efforts. CWD is transmitted both directly between deer and through the environment, with transmission even thought to be possible through deer consumption of infectious plant matter. As such, favored deer habitats may promote both direct interactions or indirect spatial overlap and subsequent environmental transmission. However, little is known about how individual animal space use translates to actual sites of overlap between individuals, leaving uncertainty in how habitat shapes the risk of direct or environmental CWD transmission. Our objectives were to determine how WTD space use varies by sex, age, and season, and how and when habitat may foster overlap and transmission potential between individuals.

Methods: We evaluated movement patterns for 596 WTD in southwest Wisconsin, a region with high CWD prevalence (>40% in some areas). Using integrated step-selection functions (iSSFs), we quantified deer seasonal habitat selection, including relative attraction or avoidance of between-group conspecifics. We also estimated seasonal encounter distributions—regions where a pair of deer were most likely to encounter each other—for all between-group pairs of WTD in our study. We quantified seasonal variation in the habitat composition of these areas using spatial generalized additive models (GAMs).

Results: We found that deer selection for agricultural or grass/pasture land use types was generally low, relative to forest, but was highest in the post-fawning (summer) and non-breeding (winter) seasons. We observed similar patterns for the composition of encounter distributions, suggesting that these habitat types may be attractive resources that facilitate between-group contact or overlap and subsequent transmission. Direct attraction to between-group individuals was low during the post-fawning season, but high during the breeding (fall) and non-breeding seasons. Across seasons, we observed that preferred habitat was not necessarily the habitat in which between-group individuals were most likely to interact.

Conclusions: Our results suggest that space use and habitat selection could increase the risk of environmental transmission in the fawning and post-fawning seasons, social selection could favor direct transmission risk in the breeding season, but combined social and habitat selection may shape risk of both direct and environmental transmission during the non-breeding season. By providing a detailed picture of deer movement and overlap through the year, our results can inform mechanistic models for CWD transmission, helping better target management and surveillance efforts to control this ecologically and economically significant disease.

Financial Support: The Federal Aid in Wildlife Restoration Act, administered through the Wisconsin Department of Natural Resources and the University of Wisconsin-Madison; the USDA, National Institute of Food and Agriculture (2022-05138) through the NSF-NIH-USDA Ecology and Evolution of Infectious Diseases program.

Notes:

P093 - Advancing animal disease research with FarmGTEx

George E. Liu[1]

[1]USDA ARS. george.liu@usda.gov

Session: General health and physiology, 2025-01-20, 6:00 - 8:00

Objectives: The Farm Animal Genotype-Tissue Expression (FarmGTEx) project, an ongoing international collaborative endeavor, aims to provide a comprehensive public resource for discovering tissue-specific genetic regulatory variants and predicting molecular phenotypes in farm animal species. The FarmGTEx project can significantly advance animal disease research by offering extensive gene expression data across various tissues in farm animals, facilitating an understanding of tissue-specific gene expression and regulation.

Methods: FarmGTEx collects and integrates gene expression profiles from farm animals. This resource aids researchers in identifying expression quantitative trait loci (eQTLs) and linking genetic variants to changes in gene expression. By comparing gene expression profiles between healthy and diseased tissues, FarmGTEx enables the identification of candidate genes and genetic variations involved in disease development, supporting pathway analysis and the study of complex diseases.

Results: Through cross-tissue analyses, FarmGTEx reveals interactions between genes and their expression in different tissues, aiding in biomarker discovery and the identification of therapeutic targets. This resource has uncovered eQTLs related to disease susceptibility in livestock, helping to identify genetic variations affecting tissue expression in diseases such as mastitis in dairy cattle. It also uncovers genetic factors influencing disease resistance, promoting translational research and the development of precision livestock farming.

Conclusions: FarmGTEx provides extensive insights into the genetic basis of diseases in farm animals, supporting the development of targeted interventions and breeding strategies for improved animal health. It enables the study of shared genetic mechanisms and tissue-specific vulnerabilities, enhancing the understanding of disease biology and aiding in the discovery of therapeutic targets and biomarkers for disease resistance.

Financial Support: This work was supported by AFRI grant numbers 2019-67015-29321 and 2021-67015-33409 from the USDA National Institute of Food and Agriculture (NIFA) Animal Genome Programs.

Notes:

P094 - Effect of stressors on amygdala gene profiles in the pig

S. Bhamidi[1], B.R. Southey[1], S.L. Rodriguez-Zas[1]

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

Session: General health and physiology, 2025-01-20, 6:00 - 8:00

Objective: The amygdala regulates many behavioral processes, including feeding, movement, and socialization, which can impact the performance and health of the pig. Management practices and environmental challenges can influence the molecular plasticity of the pig's amygdala, hindering growth, reproduction, and well-being. The objective of this study was to characterize the changes in the molecular mechanisms and gene profiles in the amygdala.

Methods: An RNA-sequencing analysis of the amygdala from two-month-old male and female pigs exposed to inflammatory or fasting challenges and controls was undertaken. Differential expression of genes across conditions and enrichment analysis were used to characterize the profiles.

Results: Over 1,450 genes were differentially expressed (FDR-adjusted p-value < 0.05) between pigs exposed to different challenges within each sex or between sexes within a treatment. The effect of fasting in the amygdala of males was detected in genes annotated to the endocrine process and characterized by the under-expression of the genes CGA, OXTR, and POMC. In females, the effect of fasting was on genes annotated to the stem cell proliferation process and characterized by under-expression of the genes IRF6, SHOX2, and WNT11. The inflammatory challenge had a similar effect across sexes, eliciting over-expression genes in cytokine-related processes, including CXCL10, CCL2, and NFKBIA. Differences between sexes in gene expression under inflammatory conditions were detected in genes pertaining to the neuropeptide signaling pathway, including over-expression of the genes CARTPT, GALR1, and NPY.

Conclusions: Our results indicate that fasting can disrupt the amygdala mechanisms and affect growth or behavior in males and that an inflammatory challenge can elicit generalized responses from many immune-related genes in both sexes. These findings can assist in the identification of practices to ameliorate the effects of environmental challenges on amygdala-associated behavior and physiological processes.

Financial Support: This study is supported by USDA NIFA grant numbers 2022-38420-38610 and 2018-67015-27413 and NIH grant number P30 DA018310.

Notes:

P095 - Validation of a food frequency questionnaire (FFQ) for use in dog nutrition research

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

[1]Virginia-Maryland College of Veterinary Medicine. janiceobrien@vt.edu

Session: General health and physiology, 2025-01-20, 6:00 - 8:00

Objective: To validate the Semi-Quantitative Food Frequency Questionnaire used by the Dog Aging Project to collect dietary information for the prospective cohort study so that diet researchers who use the diet data to correlate with future health outcomes can transparently report the validity of their findings.

Methods: A stratified random sample of 3043 Dog Aging Project participants consuming eight (8) different commercially prepared diets was taken. These were participants who had already completed the comprehensive dietary questionnaire (FFQ) at least once and were invited to retake the questionnaire (FFQ retest), followed by a 2-week time period in which the participants were invited to complete a 3-day food diary (FD) of all food items consumed by their pet. The food diaries were reviewed and the food items reported were categorized and their daily equivalent frequency (DEF) is being calculated. Statistical analysis will be conducted in R studio. The Wilcoxon signed-rank test for two dependent samples will be used to compare the mean values of intakes of dietary components. The relative difference between mean values of intakes of dietary components will be calculated with FFQ compared to the reference method (FD or FFQ retest) and expressed as a percentage, with Spearman's correlation coefficient, and by calculating the intraclass correlation coefficient (ICC) to assess the test-retest reproducibility. Since the use of correlation could be misleading, the Bland-Altman method will be employed to assess agreement between two methods (FFQ retest and FD) and between both administrations of the FFQ. The Bland-Altman method will be interpretable at both group and individual levels. Using the Bland-Altman method, mean intake from both methods, the mean difference in intake between both methods, 95% limits of agreement (LOA) as 1.96 standard deviations of the mean difference, and the Bland-Altman index defined as a percentage of respondents beyond LOA will be calculated. The strength of correlation will be interpreted as follows: 0.01-0.09 negligible, 0.10-0.29 weak, 0.30-0.49 moderate, 0.50-0.69 strong, 0.70-0.89 very strong, 0.90-1.00 perfect. The ICC will be interpreted as follows: <0.50 poor reliability, 0.50-0.74 moderate, 0.75-0.90 good, >0.90 excellent.

Results: Of the 3043 participants invited, 1236 completed both the retake questionnaire and the 3-day food diary. The Daily Equivalent Frequencies are still being calculated and recorded for the 1236 respondents, however this work will be completed within 1 month, and the results will be calculated.

Conclusions: This is the first time a FFQ has been validated using the same methods and standards applied in human nutrition epidemiology. This work will be instrumental for any future researchers using DAP data, and this FFQ will have applications beyond research: it will be able to be used by veterinary practitioners in the clinic to calculate both the macro- and micronutrients the pet is consuming.

Notes:

P096 - Immune and pulmonary responses to wildfire-PM2.5 exposure in dairy heifer calves

Delaney Sarantopulos[1], Jenifer Cruickshank[2], Sierra Lepiane[2], Mostafa Kamyabi[1], Alexandra Pace[1], Juliana Ranches[2], Denise Konetchy[1], Pedram Rezamand[3], Amy Skibiel[1]

[1]University of Idaho, [2]Oregon State University, [3]University of Connecticut. askibiel@uidaho.edu

Session: General health and physiology, 2025-01-20, 6:00 - 8:00

Objective: Wildfires have detrimental impacts on the environment and animal health. They release pollutants into the atmosphere, especially fine particulate matter ($PM_{2.5}$), one of the most hazardous components of wildfire smoke. Numerous negative health outcomes and premature mortality in humans have been associated with exposure to wildfire-$PM_{2.5}$. Wildfire-$PM_{2.5}$ inhalation is also linked to increased mortality risk in dairy calves along with coughing, ocular discharge, and lung consolidation. However, the pathogenesis of wildfire-$PM_{2.5}$ -induced health effects is not well understood. Our objective was to investigate effects of wildfire-$PM_{2.5}$ inhalation on pulmonary and systemic immune cell populations, immune cell function, and pulmonary function.

Methods: Holstein heifer calves were housed at Oregon State University (CON, n = 18) or University of Idaho (WF, n = 20) dairy centers. WF calves were exposed to wildfire-$PM_{2.5}$ (17-33 mg/m^3) for six consecutive days when calves were approximately two weeks old, whereas age-matched CON calves were not exposed to wildfire smoke ($PM_{2.5}$ < 3.8 mg/m^3). All calves were born healthy; nutrition and management protocols were the same between groups. Health scores, rectal temperature, respiration rates, and heart rates were recorded, blood was collected, tidal volume and minute ventilation were determined by pneumotachography, and transtracheal washes (TTW) were performed on WF calves 2 d after the last day of elevated $PM_{2.5}$ exposure and on CON calves at the same age. Immunophenotyping (percent lymphocytes, monocytes and macrophages, and granulocytes) was performed on TTW fluid via flow cytometry. These data are presented as percent of live cells. Differential cell counts were performed on whole blood, and respiratory burst of phagocytes in whole blood was determined by conversion of dihydrorhodamine-123 to fluorescent rhodamine-123 by reactive oxygen species produced by activated phagocytes. Data were analyzed with T-tests or Mann-Whitney U tests.

Results: Health scores were not different between groups. Rectal temperature and heart rate were higher in WF compared to CON calves ($P < 0.0001$). Compared to CON, WF had lower white blood cell count, lymphocyte, monocyte, eosinophil and basophil concentrations in whole blood ($P < 0.04$). Respiratory burst of neutrophils and monocytes in whole blood was lower in WF compared to CON (42.96 ± 2.94 vs. 65.62 ± 5.87% rhodamine 123-positive cells; $P < 0.01$). TTW fluid from WF had fewer B lymphocytes, but more granulocytes compared to CON (B lymphocytes: 2.43 ± 0.63 vs. 13.25 ± 4.73%; granulocytes: 63.83 ± 3.82 vs. 48.95 ± 4.88%; $P < 0.05$). Tidal volume did not differ between groups, but minute ventilation was greater in WF compared to CON because of higher respiration rates (minute ventilation: 14.61 ± 1.32 vs. 8.69 ± 0.73 L/min; $P < 0.001$).

Conclusions: Our results indicate both a local and systemic immune response to wildfire smoke exposure. Pulmonary function, as measured by tidal volume, did not appear to be affected by wildfire-$PM_{2.5}$ exposure, but minute ventilation was increased, potentially increasing deposition of pollutants into the calf's lungs. Understanding calf responses to smoke inhalation can aid in developing strategies to mitigate negative health outcomes associated with poor air quality.

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:

P098 - The connection between rumination and lactose concentration in early-lactation dairy cows

Samanta Arlauskaitė[1], Ramūnas Antanaitis[1], Akvilė Girdauskaitė[1], Karina Džermeikaitė[1], Justina Krištolaitytė[1], Mindaugas Televičius[1], Dovilė Malašauskienė[1], Mingaudas Urbutis[1], Kotryna Tolkačiovaitė[1], Walter Baumgartner[2]

[1]Lithuanian University of Health Sciences, [2]University of Veterinary Medicine, Vienna. samanta.arlauskaite@lsmu.lt

Session: General health and physiology, 2025-01-20, 6:00 - 8:00

Objective: Monitoring milk lactose concentration and rumination time is beneficial for managing cattle health and can lead to timely treatment, reducing veterinary costs and preventing decreases in milk yield. It is known that reduced lactose levels may be associated with diseases such as mastitis and acidosis. A decrease in rumination time can also indicate the onset or presence of illness. Today, innovative technologies provide veterinarians with the ability to track lactose levels and rumination in real-time without causing additional stress or discomfort to the cow, enabling faster monitoring of disease progression. The aim of the current study was to investigate the connection between rumination and lactose concentration in early-lactation dairy cows.

Methods: The research was conducted at a free-stall farm in Lithuania in 2023. Out of 1,160 cows, 502 were selected and divided into two groups: the first group had a lactose concentration lower than 4.7%, and the second group had a concentration of 4.7% or higher. The average milk yield per lactation was 12,500 kg. Milk lactose concentration was regularly monitored using the laser-based BROLIS HERDLINE (Vilnius, Lithuania) in-line milk analyzer, which records the composition of individual cow's milk every 5 seconds. Rumination time was assessed using the RumiWatch sensor (RWS**;** Itin and Hoch GmbH, Liestal, Switzerland), a halter equipped with pressure sensors connected wirelessly, allowing for continuous, real time data transmission.

Results: Rumination: Our study revealed a significant increase ($p < 0.01$) of 13.84% in rumination chews (RCs) second group (ML ≥ 4.70%) compared to first group (ML < 4.70%). The average RCs value in second group was 1,280.80 (n/h) (±52.28), while in first group, the average was 1,103.58 (n/h) (±23.37). Other Chews: The findings showed a significant increase ($p < 0.01$) of 14.09% in other chews in second (ML ≥ 4.70%) compared to first group (ML < 4.70%). The average value of other chews in first group was 163.24 (n/h) (±3.46), while in second group it was 190.02 (n/h) (±8.08).

Conclusions: Our study indicates that evaluating the connection between milk lactose concentrations and rumination time may help predict cow illness and determine the success of treatment. From a practical standpoint, we recommend monitoring milk lactose levels as indicators of both behavioral and physiological changes. For cows with lactose levels of ≥4.70%, dietary adjustments should be made to maintain milk protein quality while supporting higher milk production. Additionally, steps should be taken to encourage physical activity in cows with reduced movement to enhance overall well-being and performance.

Notes:

P099 - Monitoring the in-line milk fat-to-protein ratio in dairy cows during early lactation for assessing metabolic health and inflammatory status

Karina Džermeikaitė[1], Justina Krištolaitytė[1], Ramūnas Antanaitis[1], Samanta Arlauskaitė[1], Akvilė Girdauskaitė[1]

[1]Large Animal Clinic, Veterinary Academy, Lithuania University of Health Sciences. karina.dzermeikaite@lsmu.lt

Session: General health and physiology, 2025-01-20, 6:00 - 8:00

Objective: The objective of our investigation, as per our hypothesis, is to evaluate the kinetics of changes in serum amyloid A (SAA) levels, the metabolic profile, the quality of milk, and the behaviour of dairy cows during the early lactation period in healthy cows. Our hypothesis is that cows with metabolic abnormalities at the start of lactation are more likely to acquire SAA during this period.

Methods: The study was carried out at a dairy cattle farm in Lithuania in 2024. The milk composition of each milk sample (milk yield, milk fat, and protein) was determined using the BROLIS HerdLine in-line milk analyser, which was developed by Brolis Sensor Technology in Vilnius, Lithuania. Using specialised boluses, we assessed the cows' behaviour parameters, including rumination time, body temperature, and cows' activity. The quantities of non-esterified fatty acids (NEFA) and SAA were evaluated in blood samples collected from 71 cows at various intervals during the early lactation period. All blood samples were selected and categorised into two groups according to their NEFA concentration: group I-NEFA (n=43) had a NEFA concentration of 0.24 ± 0.12 mmol/l, while group II-NEFA (n=28) had a concentration of 0.87 ± 0.23 mmol/l.

Results: The study revealed a strong positive association between the milk fat-to-protein (F/P) ratio ($r = 0.516$, $p < 0.001$) and NEFA. Compared to cows with reduced NEFA levels, those with elevated NEFA levels exhibited a significantly higher milk F/P ratio. The findings suggested a significant negative correlation between NEFA and SAA ($r = -0.441$, $p < 0.001$). A substantial negative correlation was observed between SAA and both milk fat ($r = -0.426$, $p < 0.001$) and the milk F/P ratio ($r = -0.535$, $p < 0.001$). Enhanced cow activity was correlated with elevated SAA levels ($r = 0.382$, $p < 0.001$), suggesting that inflammation may induce behavioural changes as a result of physical discomfort.

Conclusions: This study investigated blood biomarkers, milk composition, and behaviour parameters from dairy cows. Blood SAA levels decrease as NEFA levels increase. In the class II-NEFA, the F/P ratio in milk was 13.67 % higher than in the I-NEFA class. This suggests that elevated NEFA levels may serve as a sign of energy mobilisation from body reserves, a phenomenon that is frequently observed during metabolic stress or energy deficits. The milk F/P ratio exhibits a significant inverse relationship with SAA. This suggests that inflammation may obstruct the synthesis of milk lipids. An increase in SAA levels was linked to an increase in cow activity, suggesting that the behavioural alterations were caused by inflammation-induced pain. The metabolic status of dairy cows is indicated by fluctuations in the ratio of milk F/P. The observations indicate that the metabolic and inflammatory conditions in dairy cows are mirrored in the composition of milk, which could serve as an alternative to blood samples for the assessment of energy balance and health.

Notes:

P100 - Hematological and biochemical analytes in free-ranging Marsican brown bears in the Central Apennines, Italy

Michela Toro[1], Leonardo Getuli[2], Giammarco Quaglia[3], Nausica D'Aurelio[1], Sara Serrani[1], Alessandra Sferrella[1], Daniele Giansante[1], Vincenza Di Pirro[2], Valentina Zenobio[1], Daria Di Sabatino[1], Leonardo Gentile[2], Fabrizio De Massis[1]

[1]Istituto Zooprofilattico dell'Abruzzo e del Molise "G. Caporale", [2]Ente Parco Nazionale d'Abruzzo Lazio e Molise, [3]Italian National Institute of Statistics. f.demassis@izs.it

Session: General health and physiology, 2025-01-20, 6:00 - 8:00

Objective: The Marsican brown bear (MBB, Ursus arctos marsicanus) is an endemic brown bear subspecies of Central Apennine in Italy, of about 50-60 bears. To the best of our knowledge, normal hematology and biochemistry values in this animal species have never been reported in the International peer-reviewed literature. This study aims to investigate hematological and biochemical values in this animal population.

Methods: Sixty-two samples from 39 free-ranging MBBs (23 females and 16 males) were evaluated for hematological analyses and 71 samples from 44 free-ranging MBBs were evaluated for biochemical analyses. All MBBs were between one and 15 years old and were caught between January 1991 and May 2023. Bears were captured using a baited trap with Aldrich's snare during the hunting season. The bears were anesthetized with 0.08 mg/kg medetomidine (Zalopine, Orion-yhtymä Oy, Espoo, Finland) and 4 mg/kg ketamine (Ketavet 100, Intervet Productions, Aprilia, Italy) intramuscularly using a CO_2-powered immobilizing gun (CO_2 PI, Dan-Inject Aps, Børkop, Denmark). Physical examinations and serological screening for infectious disease were done on all bears and only the healthy ones were included in this study. Hematological analyses were performed using an Advia 120 hematology system (Siemens, Bayer Diagnostics, NY, USA) equipped with specific software for veterinary use. No hemolysis and lipemia were documented. Biochemical analyses were performed using an Ilab 650 automated system (Instrumentation Laboratory, MA, USA).

Results: Hematological analyses were performed for the following: red blood cell count (RBC), hematocrit (HCT), hemoglobin (HGB), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), hemoglobin distribution width (HDW), red cell distribution width (RDW), mean corpuscular volume (MCV), platelet count (PLT), white blood cells count (WBC), basophils, eosinophils, lymphocytes, monocytes, neutrophils. Biochemical analyses were determined for the following: uric acid, albumin, amylase, blood urea nitrogen (BUN), total bilirubin, calcium, cholesterol, creatinine, alkaline phosphatase (ALP), gamma-glutamyl-transferase (GGT), glucose, aspartate-transaminase (AST), alanine-aminotransferase (ALT), total protein and triglycerides. All results are discussed.

Conclusions: This study is an important step ahead in the understanding of the physiology of MBB. Hematological and biochemical analyte values were similar to those found in free-ranging European Brown Bear. The only variation recorded has been the higher range of WBC and a higher mean in glucose in MBBs. The capture and immobilization procedures (snare's catching versus darting from a helicopter), may have affected these values. The stress of being trapped for 20-30 minutes with a leg-hold snare against the brief chase with the helicopter could be the cause of the rise in blood glucose and the WBC cells for the release of glucocorticoid hormones. Also, medetomidine may increase the concentration of glucose in different animal species. Since samples sizes in this study were small, and some individuals were sampled multiple times, further studies are required to better understand the biological value of hematological and biochemical analytes found in this study.

Notes:

P101 - Pain control for elastration of newborn bull calves

Mariah M Crevier[1], Derek B Haley[1], Jeffrey A Rau[2], Jessica L Gordon[3]

[1]Dept. of Population Medicine, Ontario Veterinary College, [2]Dept. of Health Sciences, Ontario Veterinary College, [3]Dept. of Large Animal Clinical Sciences, Michigan State University. mcrevier@uoguelph.ca

Session: General health and physiology, 2025-01-20, 6:00 - 8:00

Objective: Castration is a common routine husbandry practice and is known to cause pain. There are many studies on pain control for the castration of bull calves older than 1 week of age, however, there is little research on pain control for castration when performed at or near the time of birth. Therefore, the purpose of this study was to investigate the effects of different pain control regimens after newborn bull calves were castrated by elastration.

Methods: Eighty-five, newborn bull calves born at the Ontario Beef Research Centre were enrolled and randomly assigned 1 of 5 treatment groups: (1) 1 dose of oral meloxicam at castration (1 oral), (2) 1 dose of oral meloxicam at castration and 1 week later (2 oral), (3) 1 dose of injectable meloxicam at castration (1 injectable), (4) sham castration (sham), or (5) castration without pain control (control). Meloxicam was dosed based on calf weight, following label instructions for mg/kg body weight (1.0mg/ml for oral dose, 0.5mg/ml for injectable dose). Calves were treated within 24 hours of birth and followed for 14 days. Calves were observed for pain-associated behaviour such as tail flicks, and licking of the castration site for 2 hours at the following times: day of castration (day 0), day 1, 2, 7, 8, and 9. Behavioural observations were recorded as count data; marked as an event each time a behaviour occurred. Behavioural data were analyzed in SAS using a mixed model. To record general activity, HOBO Pendant G data loggers were placed on the calves' leg on day -1 and remained there until day 14. General activity data was analyzed in SAS using a mixed repeated measures model. Calves were weighed weekly until sent out to pasture and continued to be weighed bi-weekly until weaning. Weight data was analyzed in SAS using a mixed model.

Results: Out of thirteen behaviours recorded, five were statistically significant between treatment groups. Control calves had approximately three times the rate of licking compared to meloxicam-treated groups, and four times the rate of licking compared to the sham group. Additionally, control calves had two times the rate of tail flicks compared to meloxicam-treated groups, and five times the rate of tail flicks compared to the sham group. A treatment effect ($P=0.0267$) was observed for standing time with the control group spending the least amount of time standing in a 24 h period. However, no statistical time differences were seen between treatment groups. A tendency was seen for treatment effect on lying time ($P=0.0581$). No treatment effect ($P>0.1$) was seen for average daily gain when comparing weights during data collection, up to week 8, and weaning weights. Weight at time of castration was significant ($P=0.0023$), however, calves were not blocked by weight when assigned treatment.

Conclusions: Behavioural responses recorded after castration suggested pain control was able to alleviate pain. Differences were found between control calves and meloxicam-treated calves. There was no impact on average daily gain. It was concluded that meloxicam has the potential to alleviate pain post-castration.

Financial Support: OMFRA Agri-Food Alliance, Beef Farmers of Ontario, Solvet.

Notes:

P102 - An avian-specific bacterium for gut immune maturation of newly hatched conventional layer chickens

Jonathan Rodriguez-Gallegos[1], Jared Meinen-Jochum[1], Melha Mellata[1]

[1]Iowa State University. jrod11@iastate.edu

Session: General health and physiology, 2025-01-20, 6:00 - 8:00

Objectives: Conventional Lohman Select Layer (LSL) chickens are dominantly used in U.S. commercial farms. However, chicks are hatched away from their progenitors, thus mainly exposed to environmental microbes lacking host-specific microbes that help gut maturation in early life. We have previously shown that early introduction of key host-specific gut bacteria, like Segmented Filamentous Bacteria (SFB), increase the maturation of the gut immune system in specific-pathogen free (SPF) layers. However, SPF chickens, mainly maintained in tightly controlled environments, do not mimic the poultry farm environment. Thus, this study aimed to study the SFB colonization and its impact on conventional LSL layers acquired from commercial poultry facilities. The objectives of this study were to demonstrate the introduction of SFB at day-of-hatch will 1) increase its ileal colonization; 2) colonize other sections of the intestine; and 3) impact the size of intestinal organs and overall homeostasis through the increase of anti-inflammatory cytokines in conventional layers.

Methods: One-day-old conventional LSL chickens (n=12 per group) were treated with PBS (CON) or Layer-derived SFB (L-SFB) orally. At 4, 7, 15, and 29 days post-inoculation (dpi), distal ileum scrapings and ceca content were collected post euthanasia. SFB were enumerated via qPCR. Visualization of the filamentous form of SFB was confirmed via fluorescent in situ hybridization (FISH) and Gram-stain microscopy. Reverse transcription qPCR (RT-qPCR) was utilized to assess the expression of homeostatic cytokines in the distal ileum. Finally, the lengths of the ileum (measured from the ileo-ceca junction to Meckel's diverticulum) and ceca were measured at all times tested.

Results: In CON birds, the level of SFB was below the limit of detection as assessed by qPCR in the distal ileum, the primary colonization site of SFB, at all times tested. However, at 7 and 15 dpi, L-SFB birds demonstrated significantly higher levels of SFB compared to the CON birds in the distal ileum ($P < 0.0001$). CON birds demonstrated sporadic detection of filamentous SFB morphologies compared to L-SFB birds that showed high levels of filamentous SFB at all times tested in the distal ileum. In the ceca content, SFB was readily detected by conventional PCR in both groups. Compared to CON birds, there was a significant increase in the expression of the homeostatic cytokine IL-10 in L-SFB birds at 15 dpi ($P < 0.0001$). Finally, ceca and ileum lengths were significantly increased in L-SFB compared to CON birds at 7 and 15 dpi, respectively ($P < 0.01$ and $P < 0.05$).

Conclusions: Introducing key early gut colonizers like SFB on the day of the hatch is essential for the development of the gut immune system in chickens, allowing for increased resistance to pathogens in lieu of antibiotics. Our team has previously demonstrated these interactions in SPF layers. This study shows that our strategy for promoting early gut colonization of SFB in SPF layers is repeatable in commercial LSL chickens, but differences exist in the conventional chickens' response to the treatment, indicating the importance of understanding host-bacteria interactions occurring in conventional birds that used in commercial farms.

Financial Support: This research was supported by the United States Department of Agriculture (USDA) National Institute of Food and Agriculture (NIFA) project #20236701539078 and USDA- Hatch projects (IOW05700-NC1202) to MM.

Notes:

P103 - Single-nucleus transcriptomics delineates distinct cell types in porcine cecum and colon

Crystal L. Loving[1], Kristen A. Byrne[1], Sharu Paul Sharma[2]

[1]USDA-ARS-NADC, [2]ISU-Genome Informatics Facility. crystal.loving@usda.gov

Session: General health and physiology, 2025-01-20, 6:00 - 8:00

Objective: Detecting and differentiating cell types in complex tissues is central to understanding cell functions, interactions, and changes during different animal states (eg, weaning and infection). The gastrointestinal tract is critical for food digestion, nutrient absorption, and water reabsorption, as well as microbial defense. The large intestine of the pig is comprised of the cecum and colon, both microbial rich sites. Single-cell RNA-sequencing has revolutionized characterizing cell populations and related gene expression, but requires creating a single-cell suspension from complex tissues for immediate partitioning for labeling individual cells. Instead, isolation of nuclei from snap frozen tissues allows for later isolation and partitioning for single-nucleus RNA-sequencing (snRNAseq). Thus, to establish basic cell identity and gene expression profiles, snRNAseq of porcine cecum and colon was performed.

Methods: Small sections of cecum and colon were collected from 70-day-old pigs (n=2) and snap frozen in small plastic bags. Smaller sections (0.1 g) were later recovered and placed into a GentleMACS C-tube with lysis buffer for mechanical and enzymatic digestion. Isolated nuclei were partitioned to a single nucleus for tagging during first-strand cDNA synthesis, and subsequently Illumina libraries constructed and sequenced. Gene count matrices were generated by mapping to the porcine genome and data were filtered, normalized, and used to cluster the cell types (based on nucleus RNA sequencing) with the Seurat package. Colon and cecum datasets were respectively integrated and clustered. Major cell types, including T and B lymphocytes, myeloid cells, and various epithelial cells, were assigned to the initial clusters using canonical marker gene expression. Reference based mapping was used to compare similar cell types between the cecum and colon.

Results: Gene expression was assayed within nearly 31,000 nuclei from porcine cecum and colon, with approximately 15,000 nuclei recovered from each tissue type. Initial clustering revealed 16 clusters of cell types within cecum and 17 clusters in colon. The most abundant predicted cell type was epithelial cells, with at least four distinct types in both tissues. Endothelial cells and fibroblasts were also detected. Several clusters of leukocytes were noted, but included T and B cells, antibody-secreting cells (ASC), and macrophages. Comparable cell types were identified between cecum and colon, with noted differences in some gene expression profiles. Immunohistochemistry and in situ hybridization are being used to confirm location of epithelial and leukocyte populations.

Conclusions: Single-nucleus transcriptomics of porcine cecum and colon identified key cell populations, and subtype-specific gene markers. Use of snap frozen tissues for subsequent nuclei isolation eliminates to the need to recover live cells from tissues for immediate partitioning, which increases feasibility of experimental design and sample processing. Overall, the snRNAseq dataset expanded our understanding of key cell populations in both cecum and colon of domestic swine.

Financial Support: USDA ARS CRIS 5030-32000-225.

Notes:

P104 - Characterization of maternal and fetal vitamin D metabolism during pregnancy in Holstein cows

Madilyn Martin[1], Ahmad Fraz[1], Firmansyah Saputra[1], Jeniffer Laguna[1], Milerky Perdomo[1]

[1]University of Florida. cdnelson@ufl.edu

Session: General health and physiology, 2025-01-20, 6:00 - 8:00

Objective: Compelling evidence suggests maternal vitamin D shapes fetal immune development. Objecives were to test effects of the amount and source of maternal vitamin D on maternal and fetal vitamin D metabolism and fetal immune development.

Methods: Twenty-four Holstein cows between 42- and 96-days gestation at the University of Florida Dairy Research Unit were blocked by conception date and randomly assigned to receive one of four treatments: 0.2 mg cholecalciferol (LCHOL), 1.0 mg cholecalciferol (HCHOL), 0.2 mg calcidiol (LCAL), and 1.0 mg calcidiol (HCAL). The treatments were combined with 100 g of cornmeal and delivered as a daily topdressing to the TMR. Body weights, body condition scores, and blood sampled from the coccygeal vein were collected at 0, 7, 14, 21, 28, and 35 days relative to treatment. Cows were slaughtered between 88- and 138-days gestation, and fetal and maternal tissues were collected for analysis of vitamin D metabolism and fetal immune development. Data were analyzed using mixed models with fixed effect of treatment and random effect of block. Interactions ($P < 0.05$) between source and amount of vitamin D were observed for maternal and fetal plasma 25-hydroxyvitamin D concentrations.

Results: Plasma 25-hydroxyvitamin D concentrations of cows receiving HCAL was greater ($P < 0.001$) than cows receiving LCAL, HCHOL, or LCHOL (LCHOL = 50.2, HCHOL = 49.1, LCAL = 53.7, and HCAL = 81.4 ± 4.9 ng/mL). Plasma 25-hydroxyvitamin D concentrations of fetuses from cows receiving HCAL was greater ($P < 0.05$) than fetal 25-hydroxyvitamin D from cows receiving LCAL or HCHOL (LCHOL = 3.9, HCHOL = 3.0, LCAL = 2.7, and HCAL = 6.9 ± 1.2 ng/mL). Analysis of immune development was not completed at time of abstract submission but will include analysis of fetal blood leukocytes by flow cytometry, and analysis of fetal bone marrow, spleen and thymus development.

Conclusions: Maternal vitamin D source and amount affects fetal plasma 25-hydroxyvitamin D concentrations.

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:

P105 - Extracellular vesicles characterization from bovine colostrum

R. Santos[1], L. Kluppel[1], K. Holzapfel[1], S. Gade[1], U. Bickel[2], F. Rosa[1]

[1]School of Veterinary Medicine, Texas Tech University, [2]Jerry H. Hodge School of Pharmacy, Texas Tech Health Sciences Center. rafsanto@ttu.edu

Session: General health and physiology, 2025-01-20, 6:00 - 8:00

Objective: Extracellular vesicles (EVs) quantification and characterization from bovine colostrum can potentially enhance the role of EVs and their cargo in animal health. However, colostrum heterogeneity presents a challenge for EVs isolation. Therefore, this study aimed to identify and quantify EVs isolated from bovine colostrum using different approaches including a novel single-particle interferometric reflectance imaging sensing (SP-IRIS).

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. Colostral-EVs were isolated by ultracentrifugation at 340,000 × g for 60 min and EVs size distribution was measured using dynamic light scattering (Zetasizer Nano ZS90, Malvern). Colostral-EVs aliquots were subjected to Transmission Electron Microscopy (TEM) with negative staining (1% uranyl acetate) and to Cryo-Electron Microscopy (Cryo-EM). Following, bovine EV samples were analyzed using the SP-IRIS imaging detection on a Leprechaun analyzer (Unchained Labs, Pleasonton, CA). Colostral EVs were placed onto the microchip (Luni Flex chip, Unchained Labs) and incubated with antibodies markers of bovine EVs CD-9 and CD-63 (CC25 and IVA50, Thermofisher).

Results: The TEM analysis identified EVs isolated from bovine colostrum in the range of 40-150 nm. The Cryo-EM analysis showed nanoparticles with clear lipid bilayer boundaries, and the classical donut shape for the bovine colostral-EVs. The bovine colostral-EVs had an average diameter of 45 nm detected using single particle interferometric reflectance imaging. The bovine CD9 and CD63-bound EVs subpopulations detected were 40% and 50%, respectively. In contrast, human specific CD9 and CD63 antibodies were unable to detect the bovine colostral-EVs.

Conclusions: Although bovine colostrum heterogeneity presents a challenge for EVs isolation, our findings suggest that bovine colostral-EVs characterization using interferometry and fluorescence analysis validated our species-specific EV detection. Studies evaluating bovine colostrum derived extracellular vesicle cargo transfer from the mother to the offspring 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:

P106 - Investigation of fetal liver, heart, and thymus transcriptomes for prediction of reproductive failure

Kristen Walker[1], Alex Pasternak[2], Milan Royer[2], Angelica Van Goor[3], J.C.S. Harding[4], Joan Lunney[1]

[1]USDA, ARS, BARC, APDL, [2]Purdue University, [3]USDA, NIFA, Division of Animal Systems, [4]University of Saskatchewan. joan.lunney@usda.gov

Session: Immunology, 2025-01-20, 6:00 - 8:00

Objective: Porcine reproductive and respiratory syndrome (PRRS) is one of the costliest diseases to pork producers worldwide. The pregnant gilt model (PGM) of PRRS virus (PRRSV) infection has facilitated research into vertical viral transmission and evaluation of the fetal response to infection. The goal of this study is to identify critical tissues and genes that forecast fetal resistance (a fetus with no viral load despite maternal infection), resilience (a fetus which survives despite PRRSV RNA in fetal thymus and serum following maternal infection), or susceptibility (>5 log PRRSV RNA and/or meconium staining) to congenital PRRSV infection. We hypothesize that specific genes associated with innate immunity may provide some protection to foster the resilience observed in fetuses which contract no or maintain low levels of virus.

Methods: Pregnant gilts (N=30) were infected with PRRSV at day 85 of gestation. At 21 days post infection, the gilts and fetuses were euthanized, and fetal tissues collected for further investigation. The fetuses in this study were selected based on PRRS viral load (VL) in fetal serum (SER), placenta (PLC), and thymus (THY). We compared six distinct fetal groups; control (CTRL) (mock infected/no viral load), uninfected (UNIF) (<1 log/ml SER or per mg PLC or THY), PLC-Only VL (>0.5 log virus in PLC; <1 log in SER and THY), PLC+SER VL (>0.5 log in PLC, 0.5-2.0 log in SER; <1 log THY), THY LOW VL (>0.5 log in PLC, 0.5-2.0 log in SER and THY), and THY MED VL (>0.5 log in PLC, >5.0 log in SER and 2.5-4.99 log THY). The first 3 groups had no VL in fetal serum or thymus. Total tissue RNA was extracted and purified from fetal liver, heart, and thymus. These samples were analyzed using Agilent Bioanalyzer for RNA concentration and overall quality (RIN #). Expression of 7 housekeeping genes and 179 gene targets was analyzed using NanoString transcriptomics. The codeset included genes based on 12 pathways hypothesized to be involved in fetal resilience or susceptibility including innate immune, and Nidovirus associated response pathways.

Results: Analyses revealed that in the THY differential gene expression was limited to only two interferon inducible genes. Expression results in the HRT revealed more substantial impacts on immune genes including cytokines, NOD-like receptors, and other Nidovirus response genes. Liver data is pending.

Conclusions: Results will provide insight into mechanisms of PRRSV resistance and susceptibility, focusing on differential gene expression between fetuses with low or medium levels of PRRSV infection versus those which successfully prevent infection (UNIF). They will provide information on targeted tissue responses and clues to their mechanism of fetal protection following maternal infection, to inform treatment options and swine facility management strategies.

Financial Support: Lab work supported by USDA ARS Project: 8042-32000-117-00D. Sample collection funding from Genome Canada (Project 2014LSARP_8202) and Genome Prairie (Saskatchewan Ministry of Agriculture, Project #346143) with administrative support provided by Genome Alberta.

Notes:

P107 - Validation of a rapid test to evaluate humoral immunity in Labrador retrievers

Fiona B. Mccracken[1], Sarah M. Dickerson[1], Jason W. Fowler[1], Patrick M. Skaggs[1], Claire L. Timlin[1], Craig N. Coon[1]

[1]Four Rivers Kennel. burmeisteri.fiona@gmail.com

Session: Immunology, 2025-01-20, 6:00 - 8:00

Objective: This study sought to validate the D2Dx rapid immunity test in canines and investigate the age-related changes in the humoral immune system of healthy large breed dogs. Currently used as a research tool in livestock species such as cattle and swine, and tested in primates, humans, and mice, the D2Dx test has not been previously validated in any companion animal species.

Methods: Blood plasma with EDTA anticoagulant was collected from 80 healthy Labrador Retrievers housed at Four Rivers Kennel under IACUC protocol FRK-56. Age groups were defined as follows: puppy 0-1.5 years, adults 1.5-7 years, seniors 7-10, and geriatric 10-14. Immunity biomarkers including c-reactive protein (CRP), interleukin 4 (IL-4), interferon gamma (IFN-γ), complement protein 3 (C3), and immunoglobulin G (IgG) were determined through canine specific enzyme-linked immunosorbent assay (ELISA) kits. The D2Dx test which evaluates the cumulative humoral immune response by measuring the aggregation between pseudo pathogen nanoparticles and circulating immune molecules including antibodies and complement proteins, was also performed. Data was analyzed using a one-way ANOVA between age groups, with a Tukey's post hoc, and correlations between variables were evaluated through multivariate analysis.

Results: The D2Dx score was correlated with weight, age, IgG, and C3 validating that the test was able to adequately measure the humoral immunity in canines ($r \geq 0.49$). D2Dx score was significantly different between age groups ($p < 0.01$) with puppies having significantly reduced scores compared to all other groups ($p < 0.01$). C3 was significantly different between age groups ($p < 0.01$), with puppies significantly lower than other life stages ($p \leq 0.03$). Adults' C3 averaged significantly higher than seniors and trended higher than geriatric subjects ($p \leq 0.01, 0.07$). IgG between ages was also significantly different, with puppies significantly lower than other age groups ($p < 0.01$). There was no significant difference between age groups for IFN-γ: Il-4 ratio, IFN-γ, Il-4, or CRP ($p \geq 0.18$).

Conclusions: This study showed that the D2Dx Rapid Immunity Test is able to detect changes to humoral immunity in canines. The test shows promise as a clinical test for multiple conditions including infection or immunodeficiency, and as a research tool to understand the humoral immune response to various stimuli.

Financial Support: This study was funded by Four Rivers Kennel.

Notes:

P108 - Enteral immunization with virulent and avirulent *R. equi* in newborn foals

Rafaela Klein[1], Bibiana da Silveirea[1], Jocelyne Bray[1], Garrett Wehmeyer[1], Joanne Hardy[1], Noah Cohen[1], John C. Blazier[1], Angela Bordin[1]

[1]Texas A&M University. abordin@tamu.edu

Session: Immunology, 2025-01-20, 6:00 - 8:00

Objectives: Evidence exists that host-pathogen interactions in the gut can modulate immune development in the neonatal period. Using a foal model of early enteral immunization and subsequent intrabronchial infection with virulent *Rhodococcus equi* (VRE), we previously demonstrated that enteral immunization with VRE at age 2 and 4 days induces epigenetic changes in circulating monocytes, differentially-expressed genes in neutrophils, and protection against intrabronchial infection at age 28 days. Here, our goals were to determine whether enteral immunization with either VRE or avirulent *R. equi* (AvRE): 1) induced transcriptomic changes in myeloid progenitor cells using single-cell RNA-Seq; 2) increased *in vitro* neutrophil opsonophagocytic killing assay (OPK) of *R. equi* or heterologous infection with *Staphylococcus aureus;* and 3) protected against *in vivo* intrabronchial infection with *R. equi.*

Methods: Foals (n = 24) were immunized with either saline (control), VRE (10^{10} CFU), or AvRE (10^{10} CFU) at ages 2 and 4 days. At age 28 days, foals were infected intrabronchially with 2×10^6 CFU of VRE and clinically monitored until either age 64 days or recovered from pneumonia. Bone marrow aspirates were collected from foals at age 14 days. Neutrophils were collected at ages 2 (before immunization), 14, 28 (before challenge), and 56 days (after challenge). Single-cell 3´ library analysis was performed using Cell Ranger (v3.0.2), in which RNA reads were aligned to the equine (EquCab3.0) transcriptome and a gene expression matrix generated. Using the R (v3.6.2) package Seurat (v3.1.2), the gene expression matrix was used to cluster individual cells based on the expression of conserved marker genes in each group (VRE, AvRE, and control), and their proportions compared between the 3 groups using chi-squared testing. For OPK data, effects of group, sample time, and their interaction will be analyzed using linear mixed-effects regression with the nlme package in R to account for repeated measures in foals. Post hoc comparisons of pairwise differences between groups will be made using the method of Tukey with the multcomp package in R. Significance will be defined as $P < 0.05$.

Results: No foals gavaged with VRE developed pneumonia (8/8) whereas 63% of foals gavaged with AvRE (3/8) and 75% of control foals (2/8) developed pneumonia. Bone marrow scRNA-Seq and OPK data analysis are pending but will be presented at the conference.

Conclusions: Enteral VRE, but not AvRE, protected foals against *R. equi* pneumonia, indicating the gut-lung axis is functional a few days after birth and that virulent, but not avirulent, bacteria induce cross-talk between gut and lungs generating protective innate and adaptive immune responses. Our pending results will clarify the role of the bone marrow in the gut-lung axis-mediated protection against *R. equi* and whether enteral immunization with *R. equi* induces heterologous immune responses in foals.

Financial Support: USDA-NIFA (2023-67015-39097), Grayson-Jockey Club Research Foundation, and Link Equine Research Endowment.

Notes:

P109 - Assessing the impact of a liposome-TLR agonist (LTC) on the mammary gland immune response in non-lactating Holstein dairy cows

Emily Leonard[1], Brian Crooker[2], Steve Dow[3], Luciano Caixeta[1]

[1]Department of Veterinary Population Medicine, University of Minnesota - Twin Cities, [2]Department of Animal Science, University of Minnesota - Twin Cities, [3]Department of Clinical Sciences, Colorado State University. leona454@umn.edu

Session: Immunology, 2025-01-20, 6:00 - 8:00

Objective: Mastitis on dairy farms is commonly controlled using antibiotics, but concerns for antimicrobial stewardship are rising. Another possible 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 was to determine whether administration of LTC activated the immune response in the mammary gland of non-lactating dairy cows.

Methods: Four non-pregnant, non-lactating Holstein cows were enrolled in this pilot study. Undiluted LTC was administered intramammarily into the left hind quarter of each cow in the treatment group (TRT; n=4), while the left front quarter received the LTC diluent (Tris-buffered with 10% sucrose, pH 7.2) for the control group (CON; n=3). After treatment administration, teat cistern cells were collected using a cytobrush (Medscand® Cytobrush Plus®). Samples were collected at 12h, 24h, and 48h after treatment. A 0h, pre-treatment sample was not collected to ensure that any immune response measured at 12h was due to treatment and not the sampling method. The effect of LTC on somatic cell count (SCC) and cytokine expression via qRT-PCR 24h after LTC administration was assessed with two sample T-tests.

Results: The SCC of TRT and CON at 12h was 9,005 cells/mL*1000 and 2,298 cells/mL*1000, respectively (P=0.22). The SCC of TRT and CON at 24h was 3,323 cells/mL*1000 and 5,814 cells/mL*1000, respectively (P=0.11). The SCC of TRT and CON at 48h was 4,554 cells/mL*1000 and 2,915 cells/mL*1000, respectively (P=0.53). The qRT-PCR analysis revealed significant upregulation of MCP-1 at 24h ($P<0.05$) and no significant change in expression of CXCL8, IFN-α, or IFN-γ.

Conclusions: No meaningful difference in SCC was observed when comparing samples collected from quarters receiving or not receiving LTC. This result is likely due to the small sample size and large amount of variation between cows. However, upregulation of MCP-1 is an important finding as MCP-1 is the key cytokine for recruiting monocytes to the mammary gland. Such findings support the premise that administration of LTC activates the immune system 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:

P110 - Females with obesity suffer from more severe disease following influenza A virus infection

Saurav Pantha[1], Saranya Vijayakumar[1], Shristy Budha Magar[1], Brian Wolfe[1], Tawfik Aboellail[2], Santosh Dhakal[1]

[1]Department of Diagnostic Medicine and Pathobiology, Kansas State University, [2]Kansas State Veterinary Diagnostic Laboratory, Kansas State University. sauravvet@vet.k-state.edu

Session: Immunology, 2025-01-20, 6:00 - 8:00

Objective: Influenza A viruses (IAVs) cause seasonal influenza outbreaks and have the potential to cause pandemics. Available evidence suggests that biological sex (i.e., being male or female), as well as obesity, independently play important roles in IAV pathogenesis. However, their interaction during IAV pathogenesis has not been pursued yet. This study aims to explore the effects of biological sex and obesity during IAV pathogenesis in a diet-induced obesity (DIO) mouse model.

Methods: Four- to five-week-old male and female C57BL/6 mice were fed with either a high-fat diet (HFD, 60kcal% fat) or a control diet (10kcal% fat) for 13-14 weeks, and body mass was recorded weekly. Obesity was defined as having ≥20% body mass compared to the average body mass of age- and sex-matched mice on the control diet. There were four experimental groups: non-obese males, non-obese females, obese males, and obese females. Mice were inoculated intranasally either with a lethal/high dose (10^3 $TCID_{50}$) or a sublethal/low dose ($10^{1.5}$ $TCID_{50}$) of mouse-adapted A/California/04/2009 H1N1 IAV. Change in body mass was recorded daily up to 21 days post-infection (dpi), and mice that lost ≥25% of body mass were humanely euthanized. Subsets of mice were euthanized on different dpi to collect tissue samples for virus titration, histopathology, flow cytometry, and cytokines/chemokines measurement.

Results: After 14 weeks of HFD treatment, 100% of the male and 70% of the female mice became obese, indicating the sex difference in the progression of obesity. However, the male and female mice with obesity similarly had significantly greater body mass, adipose tissue deposition, plasma leptin and total cholesterol concentrations, and glucose intolerance compared to their non-obese controls. After a lethal dose infection, the median survival time was shorter for female non-obese and obese mice compared with their male counterparts. A subset of mice, infected with the lethal dose and euthanized at 3 dpi, showed similar virus replication in the lungs. However, hematoxylin and eosin (H&E) staining showed significantly higher inflammation of lung parenchyma in female mice with obesity. Obese female mice also had higher fold changes in cytokines associated with the Th17 pathway, including IL-17A, IL-23, and IL-22, and chemokine MIP1α responsible for immune cell infiltration. After the sublethal dose infection, mice from all groups became sick, and obese mice had greater body mass loss. Among the four groups, the percentage change in body mass from the baseline was highest in females with obesity. Importantly, 25% (i.e. 2/8) of the females with obesity reached the humane endpoint and required euthanasia, indicating that they suffer from more severe IAV pathogenesis than other groups. Flow cytometry analysis of the lungs at 3 dpi, after sublethal dose infection, showed comparable neutrophil and alveolar macrophages but reduced interstitial and inflammatory monocytes and macrophages in obese males and females.

Conclusions: Our study shows that females with obesity experience greater severity of IAV pathogenesis in a mouse model. This is likely to be mediated by dysregulated inflammatory responses, including Th17-mediated immunopathology, cytokines/chemokines alteration, and disproportionate immune cell infiltration.

Financial Support: The research was supported by NIH through the Center on Emerging and Zoonotic Infectious Diseases (CEZID) at the Kansas State University (award number P20GM130448) (S.D.) and the start-up funds provided to S.D. by the College of Veterinary Medicine at KSU.

Notes:

P111 - Sex differences in influenza vaccine-induced immunity and protection in mice with obesity

Saurav Pantha[1], Saranya Vijayakumar[1], Shristy Budha Magar[1], Brian Wolfe[1], Tawfik Aboellail[2], Santosh Dhakal[1]

[1]Department of Diagnostic Medicine and Pathobiology, Kansas State University, [2]Kansas State Veterinary Diagnostic Laboratory, Kansas State University. sauravvet@vet.k-state.edu

Session: Immunology, 2025-01-20, 6:00 - 8:00

Objective: Host-associated factors, including biological sex (i.e., male or female as determined by chromosomes, gonads, and sex steroids) and obesity, impact immune responses to influenza vaccines. Whether or not obesity impacts influenza virus vaccine responses in a sex-specific manner is not known. Our objectives were to investigate sex differences in influenza vaccine-induced immunity and protection in a mouse model of diet-induced obesity (DIO).

Methods: Five- to six-week-old male and female C57BL/6 mice were randomly assigned to either a high-fat diet (HFD, 60kcal% fat) or a control diet (10kcal% fat), and body mass was recorded weekly. Mice on HFD with body mass ≥20% than the average body mass of age- and sex-matched mice on the control diet were considered obese. Obese and non-obese male and female mice, obtained after a diet treatment for 12 weeks, were vaccinated with an inactivated A/California/04/2009 H1N1 vaccine (20µg in 40µL phosphate buffered saline) and boosted after 21 days post-vaccination (dpv). At 35 dpv, plasma samples were collected to measure antibody responses by enzyme-linked immunosorbent assay (ELISA) and virus-neutralizing assays. At 42 dpv, vaccinated mice were inoculated intranasally with a drift variant of the H1N1 virus (10^5 $TCID_{50}$ in 30µL of Dulbecco's Modified Eagle Medium). Body mass was recorded daily for 21 days post-challenge (dpc) to determine disease severity and protection. Subsets of mice were euthanized at 35 dpv to quantify B cells in bone marrow by flow cytometry and at 3 dpc to collect lung samples to measure replicating virus titers.

Results: After 12 weeks of diet treatment, 100% of the male and 67% of female mice became obese. Males and females with obesity had significantly greater body mass, glucose intolerance, and body mass index than the non-obese controls ($p<0.05$ in each case). At 35 dpv, IgG, IgG2c, and virus-neutralizing antibody (nAb) titers were higher in females than in male mice, irrespective of obesity. Importantly, males with obesity had the lowest levels of IgG, IgG2c, and nAb titers than the other groups. After the virus challenge, change in body mass was compared for 21 dpc. In agreement with the lowest antibody levels, vaccinated obese males were least protected as evidenced by higher absolute (i.e., in gm) and relative (i.e., percentage change from the baseline) body mass loss. Similarly, both obese and non-obese females with higher antibody titers cleared replicating viruses from the lungs within 3 dpc. While 75% (i.e., 3/4) of non-obese males cleared, vaccinated obese males (i.e., 4/4) couldn't clear replicating viruses from the lungs. Flow cytometry analysis of bone marrow-derived cells showed a similar frequency of memory B cells among the four groups. However, the frequency of plasma cells was significantly higher in female non-obese and obese mice compared with their male counterparts.

Conclusion: Our data illustrate that antibody production after influenza vaccination is inferior in males with obesity and they are less protected from subsequent influenza virus challenge. This is likely mediated by the inefficiency of B cells to produce antibodies in obese males, which warrants further investigation.

Financial Support: The research was supported by NIH through the Center on Emerging and Zoonotic Infectious Diseases (CEZID) at the Kansas State University (award number P20GM130448) (S.D.) and the start-up funds provided to S.D. by the College of Veterinary Medicine at KSU.

Notes:

P112 - Effect of isoprostane class on bovine monocyte-derived macrophage function under in vitro inflammatory challenge

Eric Owczarzak[1], Angel Abuelo[1]

[1]Michigan State University. owczar12@msu.edu

Session: Immunology, 2025-01-20, 6:00 - 8:00

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 isoPs have been extensively studied as a biomarker of oxidative stress, there is still a gap in knowledge regarding the biological activity of these molecules. One possible function of isoPs could be activity on monocyte-derived macrophages (MDMs), MDMs are essential in the initiation, maintenance, and resolution of inflammation during the periparturient period, however, MDMs are also impaired by oxidative stress (OS) during this period. Thus, the objective of this study was to compare in vitro effects of F2- and F3-isoP on macrophage function under in vitro inflammatory challenge.

Methods: Monocytes were isolated via magnetic-activated cell sorting separation and differentiated into macrophages from 6 healthy mid-lactation dairy cows and cultured for 18 h with different biologically relevant concentrations (0, 25, 250, and 500 nM + vehicle control) of F2- and F3-isoP. Lipopolysaccharides (LPS) and 2,2'azobis dihydrochloride (AAPH) were used as the in vitro inflammatory challenge as these are effective in mimicking in vivo OS conditions in cattle. Following incubation, the following MDM functions were evaluated: (1) Cell adhesion and spreading via inverted fluorescence microscopy, (2) motility using a Boyden chamber assay, (3) phagocytosis capacity via flow cytometry, (4) differentiation of MDMs into a particular phenotype by measuring surface markers via flow cytometry, and (5) expression of cytokines and enzymes relevant to the inflammatory response of MDMs via RT-qPCR and wester blotting. Mixed models were built for all outcome variables. Treatment was the fixed effect and animal was the random effect to account for individual variability. Tukey's honest significance test was used for post hoc pairwise comparisons.

Results: The data analysis is being completed, and the results will be finalized for the conference.

Conclusions: The analysis of the results will let us assess whether favoring F3- or F2-isoP production during periods of oxidative stress could improve the impaired function of MDMs observed in periparturient cattle.

Financial Support: USDA-NIFA 2022-67015-36350.

Notes:

P113 - Single-cell RNA sequencing analysis of influenza virus-infected CD1D-deficient pigs

Weihong Gu[1], Darling Melany de Carvahlo madrid[1], Sadie Clements[1], Laurie Touchard[1], Nathan Bivens[1], Grant Zane[1], Mingyi Zhou[1], Kiho Lee[1], Joh Driver[1]

[1]University of Missouri. driverjp@missouri.edu

Session: Immunology, 2025-01-20, 6:00 - 8:00

Objective: We developed pig-specific T cell receptor (TCR) and B cell receptor (BCR) primers compatible with the droplet-based protocols of the 10x Genomics Next GEM Single Cell 5' RNA sequencing (scRNAseq) protocol. These assays were used to compare cryopreserved lung cells from influenza-infected pigs genetically engineered to lack CD1D. This is a gene required for the development of natural killer T (NKT) cells, a subset of innate-like T cell that accumulates in barrier organs such as the lungs. Our objective was to determine if NKT cell-derived immune signals influence lymphocyte receptor diversity after one or two influenza infections.

Methods: scRNAseq was coupled with enrichment of TCR and BCR repertoires using primers that target the C regions in mRNA transcripts of TCR and BCR chains and isotypes. This allowed construction of expressed TCRs and BCRs of individual cells including genomic rearrangements between the variable (V), diversity (D), and joining (J) regions of the TCR and BCR intervals responsible for creating diversity in receptor binding surfaces. These assays were used to compare cryopreserved lung cells from NKT cell-intact and -deficient swine. CD1D-intact and -deficient pigs were analyzed after one or two infections with influenza (n=3 per group) to interrogate the evolution of the TCR and BCR repertoires after primary or secondary infection and to determine if NKT cells exert T helper cell functions that influence receptor diversity. For this, one cohort of pigs was necropsied five days after a single infection with H1N1 A/Missouri/CS20N08/2020 while a second cohort of pigs was infected with H1N1 A/Missouri/CS20N08/2020 virus two weeks after an initial infection with H1N1 A/California/04/2009 and necropsied 5 days later.

Results: Among the 12 samples in this dataset, we detected almost all V(D)J gene segments annotated for TCR alpha and beta chains, including some that were annotated as pseudogenes. Examination of Vb/Jb combinations confirmed previous studies showing that certain rearrangements are favored in pigs. In several pigs, VDJ rearrangements clustered by major histocompatibility complex inheritance patterns. The most expanded clones were among $CD4^+$ and $CD8^+$ tissue resident memory T cells, cytotoxic $CD8^+$ T cells, and proliferating T cells, consistent with reports that these populations harbor antigen experienced T cells that are poised for rapid responses during influenza infection. As expected, twice-infected pigs had more expanded clones than once-infected pigs. This was less apparent in NKT cell-deficient compared to NKT cell-intact pigs, which might suggest that induction of influenza-specific lung T cells is reduced in the absence of NKT cell helper functions.

Conclusions: The assays presented in this study can easily be applied to 5' 10x Genomics protocols for use in swine. Our protocols can be employed to profile TCRs and BCRs in the same sample which enhances the utility of the method as most adaptive immune responses involve both cellular and humoral responses. Accordingly, the combined protocol could shed light on acquired immunity that develops in response to vaccination and infection in production pigs, as well as in the growing number of immune-related pig models being developed for biomedical use.

Finanical Support: This research was funded jointly by the U.S. Department of Agriculture grant 2021-67015 and the National Institutes of Health grants HD092286 and AI158477.

Notes:

P114 - Reduction of reactive oxygen species production in bovine monocytes and neutrophils from cows with subclinical Johne's disease

Maria A. Colombatti Olivieri[1], John P. Bannantine[1]

[1]USDA-Agricultural Research Service, National Animal Disease Center, Ames, IA, USA. alejandra.colombatti@usda.gov

Session: Immunology, 2025-01-20, 6:00 - 8:00

Objective: Johne's Disease (JD) is caused by Mycobacterium avium subsp. paratuberculosis (MAP) and poses a significant economic challenge in the dairy industry worldwide. There is still limited understanding of the innate immune response in naturally infected animals, particularly concerning the role of neutrophils in this disease. This study involved infecting bovine whole blood with MAP to assess the production of reactive oxygen species (ROS) in bovine monocytes and neutrophils from healthy cows and those with subclinical JD.

Methods: MAP strains were cultured in Middlebrook 7H9 media. Heparinized whole blood was collected from healthy control cows and cows infected with Johne's Disease (JD), with eight cows in each group. The blood samples were stimulated with lipopolysaccharide (LPS) and either MAP sonicate (MPS) or were infected with three different MAP strains, which varied in virulence based on their survival in bovine macrophages. After three hours of incubation, CellROX Deep Red was added to measure ROS production. Red blood cells (RBCs) were then lysed and fixed using BD FACS Lysis Solution. Cell labeling was carried out with an anti-neutrophil antibody (MM20A) and an anti-CD14 antibody for monocyte labeling. The samples were analyzed using flow cytometry. Additionally, purified neutrophils from the peripheral blood of three healthy cows were infected with the same MAP strains to assess the neutrophils' ability to kill the bacteria at both 3- and 24-hours post-infection. All tests were performed in triplicate.

Results: In the neutrophil killing assay, all strains exhibited a reduction in survival at both evaluated time points. However, this reduction was only significant at 24 hours post-infection (hpi) for Map strain 285 compared to Map K10 and Kay strains, which had demonstrated greater survival in bovine macrophages. Additionally, flow cytometry results indicated that monocytes and neutrophils from cows with JD had significantly lower reactive oxygen species (ROS) production when infected with Map or stimulated with lipopolysaccharide (LPS) or modified polysaccharide (MPS), compared to healthy animals.

Conclusions: These results suggest that neutrophils do kill Map, but not initially. Furthermore, a reduced innate immune response, as measured by ROS production, occurs in JD-infected animals.

Financial Support: Dr Colombatti Olivieri is supported by the Oak Ridge Institute for Science and Education (ORISE), ARS Participation Program, Oak Ridge, TN, United States.

Notes:

P115 - Diversity and T-cell antigenic potentials of *Mycoplasma mycoides* subsp. mycoides vaccine candidates

Emily Wynn[1], Rohana Dassanayake[2], Daniel Nielsen[2], Eduardo Casas[2], Michael Clawson[1]

[1]USDA - ARS, US Meat Animal Research Center, [2]USDA - ARS, National Animal Disease Center. emily.wynn@usda.gov

Session: Immunology, 2025-01-20, 6:00 - 8:00

Objective: Mycoplasma mycoides subsp mycoides (Mmm) is the causative agent of contagious bovine pleuropneumonia (CBPP). CBPP is a severe respiratory disease of cattle in sub-Saharan African countries where it causes significant economic losses and there is a need for efficacious vaccines to help bring it under control. To that end, we used publicly available sequences of Mmm strains isolated from cattle to identify the core genome of Mmm, compare the sequence diversity of proteins known or predicted to be outer membrane or extracellular, and to predict epitope binding sites for bovine major histocompatibility complex (MHC) proteins.

Methods: All publicly available, non-redundant genomes of Mmm in GenBank were downloaded for this study (n = 15). The genomes represent strains isolated from Europe, Africa, Asia, and Australia within the 20th or 21st centuries that all likely emerged from a European ancestor within the last 300 years. They were annotated with DFAST, and core and pan genome analyses were performed using the bioinformatic software package EDGAR. Two hundred and eight known or predicted outer membrane or extracellular proteins of Mmm were identified from the literature and their sequences were compared at the community level. Regional epitope binding efficiencies of these proteins to bovine MHC Class I and II were predicted with NetMHCpan and NetMHCIIpan.

Results: The 15 Mmm genomes have a 94% core genome of 793 genes. Eighty-six of the 208 genes encoding outer membrane and extracellular proteins identified from the literature are present in the core genome. A majority of those genes and their encoded proteins products are highly conserved at the sequence level, including at the sites of predicted epitope binding with bovine MHC Class I and II. Despite the high sequence conservation, multiple proteins have large differences in the numbers of MHC Class I and II epitopes and their predicted binding strengths.

Conclusions: This study underscores the importance of core genome analyses when characterizing targets for vaccine studies. The high conservation of protein sequences across the 15 Mmm genomes and their predicted epitope sequences provide opportunities for the development of new diagnostic and preventive mitigation measures, including new vaccines. Additionally, the genes of several vaccine protein candidates identified in previous studies were not part of the core genome and may not provide efficient protection across diverse Mmm populations. However, despite the geographical diversity of the strains represented in this study, their genomes are unlikely to represent the complete diversity of extant Mmm in Africa. Consequently, sequencing more contemporary strains could better direct future vaccine work.

Financial Support: This work was funded by USDA - ARS project numbers 3040-32000-036-000D and 5030-32000-236-000-D. Dr Wynn is supported by ORISE contract number DE-SC0014664.

Notes:

P116 - C3b and C1q complement in foals: Serum concentration and effects on killing of intracellular *Rhodococcus equi*

R. L. Klein[1], J. McCaskill[1], N. D. Cohen[1], B. P. da Silveira[1], A. I. Bordin[1]

[1]Department of Large Animal Clinical Sciences, Texas A&M University. kleinrafaela@tamu.edu

Session: Immunology, 2025-01-20, 6:00 - 8:00

Objective: *Rhodococcus equi* (*R. equi*) is an intracellular bacterium that causes severe pneumonia in foals worldwide. Immunization with enteral R. equi induces both innate and adaptive immune responses and protects foals against pneumonia. Moreover, enteral immunization induces expression of complement system proteins which play a vital role in induction of inflammatory responses and uptake and killing of pathogens by phagocytic cells. Our objectives were: 1) to determine whether serum concentrations of C3b receptor (C3bR), C3b fragment (C3bF) and C1q were affected by enteral immunization, age, and pneumonia status in foals; and 2) to determine the effect of opsonization with different concentrations of C1q and C3b on R. equi phagocytosis and intracellular replication in J774A.1 macrophages.

Methods: Healthy Quarter Horse foals (n=24) were randomly assigned to receive enteral administration of saline (100 ml of NaCl 0.9%; n=8), live virulent R. equi (VRE; 10^{10} R. equi in 100 ml of NaCl 0.9%; n=8), or live avirulent R. equi (AvRE; 10^{10} R. equi in 100 ml of NaCl 0.9%; n=8) at ages 2 and 4 days. Foals were challenged intrabronchially with 2×10^{6} R. equi at age28 days and were monitored for the development of clinical pneumonia until age 64 days or when deemed healthy. Serum samples were collected at ages 2, 14, 28, and 56 days and tested by ELISA for C3bF, C3bR, and C1q concentrations using commercial kits (MyBioSource). The association between serum concentrations of C1q, C3bF or C3Br, and age, study group, and the interaction of age and study group, or age and pneumonia status will be analyzed using linear mixed-effects modeling ($P<0.05$). For the R. equi intracellular killing assay, approximately 1×10^{4} cells/ml of J774A.1 murine macrophages were infected with R. equi opsonized with either 0, 1, 10, or 100 µg/ml of purified C1q and C3b. Cells were lysed immediately or after 48 hours post-infection. Quantitative cultures were conducted to determine the colony-forming units (CFU) for each complement concentration.

Results: Preliminary analysis comparing ages 2 and 28 days indicate age and pneumonia status, but not immunization, were associated with serum concentrations of C1q and C3b receptor in foals. Final data analysis of serum complement concentrations for all ages and effects of complement components on intracellular killing are pending but will be presented at the conference.

Conclusions: Preliminary results suggest that complement plays an important role in susceptibility and protection against R. equi pneumonia in foals.

Financial Suport: Research grant: Equine Institute Research Grant; Agriculture and Food Research Initiative Competitive Grant no. 2023-67015-39097 from the USDA-NIFA; Grayson-Jockey Club Research Foundation; Link Equine Research Endowment. Student support: Boehringer Ingelheim VSP; Texas A&M University School of Veterinary Medicine & Biomedical Sciences.

Notes:

P117 - Anti-rotaviral antibody activity in mares and their foals after maternal vaccination with VP8 mRNA

K.E.R. Borba[1], N.M. Canaday[2], R.M. Legere[1], J.W. Skrobarczyk[3], S.S. Campbell[2], Z.W.T. Arnold[2], E. Cotton-Betteridge[1], C. Poveda[4, 5], C. Mancino[6, 7], F. Taraballi[6, 7], M.F. Criscitiello[8], A.I. Bordin[1], L.R. Berghman[3, 8], J.B.K. Pollet[4, 5], N.D. Cohen[1]

[1]Departament of Large Animal Clinical Sciences, Texas A&M University, [2]6666 Ranch, Gutherie, Texas, [3]Departament of Poultry Scince, Texas A&M University, [4]Texas Children's Hospital Center for Vaccine Development, Baylor College of Medicine, [5]National School of Tropical Medicine, Baylor College of Medicine, [6]Orthopedics and Sports Medicine & Biomedical Sciences, [7]Center for Musculoskeletal Regeneration, Houston Methodist Research Institute, [8]Departament of Veterinary Pathobiology, Texas A&M University. karinborba@tamu.edu

Session: Immunology, 2025-01-20, 6:00 - 8:00

Objectives: Diarrhea caused by equine group A rotavirus (ERVA) is common in foals worldwide. The vaccine available in the United States (US) is a conditionally licensed, killed whole virus (KWV) product composed of a single ERVA genotype administered to pregnant mares to protect their foals against ERVA infection during the neonatal period. Given the limitations of KWV vaccines and epidemiological evidence indicating that at least 2 genotypes of ERVA infect foals in the US, we developed novel ERVA vaccines targeting the highly conserved VP8 surface protein using either synthetic VP8 peptides or mRNA encoding ERVA VP8. Our objective was to compare the immunogenicity of the VP8 vaccines to the KWV vaccine.

Methods: Mares (12 per group) were randomly assigned to be immunized intramuscularly (IM) at 8 and 10 months of pregnancy with either synthetic peptides of VP8 (400 µg) with an oil-in-water adjuvant or VP8 mRNA (400 µg) encapsulated in lipid nanoparticles (LNPs); the KWV was administered IM at 8, 9, and 10 months of pregnancy according to the manufacturer's recommendations; 12 unvaccinated mares were included as negative controls. Serum samples were collected from mares before vaccination and from mares and their foals when foals were 1, 35, and 49 days of age. We assessed immunogenicity by measuring serum antibody activity levels against VP8 in mares and their foals using an indirect ELISA. An in vitro virus neutralization assay was performed to evaluate neutralization titers of anti-ERV in serum samples of mares and foals. Data were analyzed using linear mixed-effects regression to compare effects of sample time, vaccine group, and their interaction with post hoc comparisons using the Turkey method and significance at $P<0.05$.

Results: At foaling, mares in all vaccine groups had anti-VP8 activity levels significantly ($P<0.001$) higher than controls, but mares immunized with mRNA had significantly ($P<0.001$) higher antibody activity levels than mares in the KWV or peptide groups. At ages 1, 35, and 49 days, foals born to vaccinated mares had significantly ($P<0.001$) higher anti-VP8 activity levels than control foals, but values in the mRNA group were significantly ($P<0.001$) higher than those for mares in the KWV or peptide groups; values for foals in KWV and peptide groups did not differ significantly ($P=0.935$). Virus neutralization titers in mares on the day of foaling were significantly ($P < 0.05$) higher in the mRNA mares (median, 1:108) compared to control mares (median, 1:14). At age 1-day, median neutralizing titers were significantly ($P<0.05$) higher for foals in the mRNA group (median, 1:26) than control foals (median, 1:7).

Conclusions: Antibody activity levels against ERVA VP8 remained elevated longer in foals from mares vaccinated with a VP8 mRNA vaccine than the KWV or peptide vaccines. Moreover, mRNA vaccination resulted in serum with greater viral neutralizing titers against ERVA than in control foals. Our results suggest that mRNA vaccines offer superior antibody responses to a neutralizing epitope (viz., VP8) and higher viral neutralization titers compared with traditional vaccines. Our promising results support further investigation to assess efficacy of this mRNA vaccine.

Financial Support: Texas A&M AgriLife Research Formula Animal Health Funds; Link Equine Research Endowment, Texas A&M University; Glenn Blodgett Chair in Equine Studies; 6666 Ranch; Baylor College of Medicine; Houston Methodist

Notes:

P118 - Dynamics of maternal systemic and mucosal influenza A antibody in swine

Meghan Wymore Brand[1], Bailey L. Arruda[1], Giovana Zanella[1, 2], Amy L. Baker[1]

[1]National Animal Disease Center, USDA-ARS, [2]Iowa State University. meghan.brand@usda.gov

Session: Immunology, 2025-01-20, 6:00 - 8:00

Objective: Maternal antibody transfer via colostrum and milk is critical for protection against pathogens in neonatal pigs, but the dynamics of maternal antibodies present at mucosal surfaces in the respiratory tract is not well understood. The objective of this study was to assess maternal influenza A virus (IAV)-specific antibodies in pig serum and at the respiratory mucosa, and to assess the impact of waning maternal antibody on infection with IAV.

Methods: Pregnant sows were naturally exposed to a human H3N2 IAV and developed antibody titers prior to farrowing. Piglets were weaned at three weeks of age, and serum was collected throughout the study to assess HI antibody and IgG and IgA IAV-specific antibodies by ELISA. HI titers and litter were used to allocate piglets into groups. A subset of piglets was necropsied at weaning to collect nasal wash fluid (NW), tracheal wash fluid (TW), and bronchoalveolar lavage fluid (BALF). Remaining piglets were split into 4 groups, naïve and homologous challenge at 3.5 or 8.5 weeks post weaning (WPW), and a group of contacts for each challenge. Nasal swabs (NS) were collected daily after challenge, and pigs necropsied at 4 days post infection (DPI) at 4 and 9 WPW. Contact pigs were introduced at two DPI and maintained for five weeks. At all necropsies, macroscopic lung lesions were scored, and NW, TW, and BALF were collected to assess levels of IAV-specific maternal IgG and IgA, and IAV levels by qPCR titer equivalence.

Results: HI antibody titers waned 2-8-fold from weaning to the end of the study 9 weeks later. Serum IAV-specific IgG waned throughout the study, and was minimally detected by 9 WPW. Serum IAV-specific IgA was mainly detected at and prior to weaning, and no IAV-specific IgA was detected in BALF, TW, or NW. IAV-specific IgG was detected in BALF and TW at weaning and 4 WPW, but only detected at low levels in a small number of animals by 9 WPW. In NW, IAV-specific IgG was detected at weaning but was only detected in a few animals at 4 WPW. All contact pigs developed serum, BALF, and TW IAV-specific IgG and IgA but only a subset had detectable antibody in NW. The 3.5 WPW challenge had reduced viral titers in 2 and 3 DPI NS, while 1 and 4 DPI NS, 4 DPI BALF, and 4DPI NW viral titers were equivalent between challenge timepoints. Minimal macroscopic lung lesions were observed at either challenge timepoint.

Conclusions: Low levels of serum IAV-specific IgA were detected in piglets at weaning and quickly waned, and no mucosal IgA was detected. Serum IgG and mucosal IgG were detected at weaning and 4 weeks post weaning, with minimal levels detected by 9 WPW. The only difference in viral titers was a decrease in early infection (2 and 3 DPI NS) when maternal antibody was present. This may be due to minimal protection of maternal antibody under these study conditions or other factors including age.

Financial Support: U.S. Department of Agriculture (USDA) Agricultural Research Service (ARS project number 5030-32000-231-000-D).

Notes:

P119 - PEDV infection alters gene expression in all cell types of porcine jejunal Peyer's patches: A single-cell study

Jayne Wiarda[1], Eraldo Zanella[1], Jianqiang Zhang[2], Samantha Hau[1], Alexandra Buckley[1]

[1]National Animal Disease Center, ARS-USDA, [2]Iowa State University. jayne.wiarda@usda.gov

Session: Immunology, 2025-01-20, 6:00 - 8:00

Objective: Porcine epidemic diarrhea virus (PEDV) is an enteric pathogen infecting pig intestinal epithelial and immune cells, causing morbidity and mortality associated with gastrointestinal immunopathology and diarrhea. However, the effect of PEDV infection on Peyer's patches (PPs), sites of organized lymphoid tissue found throughout the small intestine, is relatively unexplored. PPs are leukocyte-rich lymphoid areas with unique immune cell compositions compared to other intestinal locations, lending to their unique role as organized sites of intestinal immune induction. Therefore, understanding how PEDV affects the PP cellular landscape could be critical in determining how effective versus ineffective immune responses to PEDV infection are induced in the intestine.

Methods: Single-cell RNA sequencing (scRNA-seq) was utilized to assess the cellular landscape of PPs from mock-challenged (n=3) or PEDV-challenged pigs (n=5). Pigs (~4.5 weeks old) were orally inoculated with ~$1x10^5$ TCID50/mL non-S-INDEL PEDV USA/NC49469/2013 or mock inoculum. Rectal swabs were collected daily and tested via RT-qPCR for the presence of PEDV RNA. Clinical scores were also collected daily. At 5 days post-inoculation, pigs were humanely euthanized, and the most proximally-located jejunal PP was grossly identified and excised for cell isolations. Mechanical and enzyme dissociation was performed to obtain single-cell suspensions submitted for scRNA-seq partitioning, library preparation, and sequencing. Sequenced reads were processed following standard procedures of the Seurat analysis package. Differential abundance testing was performed with miloR to assess potential differences in cell type proportions occurring between PEDV- and mock-inoculated samples. Differential gene expression analysis was performed with Seurat between PEDV- and mock-inoculated cells of each annotated cell type with >50 total cells recovered.

Results: While mock-inoculated animals exhibited no clinical signs and had PEDV-negative rectal swabs throughout the study, PEDV-inoculated pigs exhibited diarrhea as early as 1-day post-inoculation, and all had PEDV-positive rectal swabs at euthanasia. scRNA-seq resulted in a dataset of 44,661 cells annotated into 31 cell types. Differential abundance was not observed for any cell types between challenge groups; however, differentially expressed genes (DEGs) were observed between challenge groups for all cell types tested, with the most DEGs being recovered from epithelial cell types followed by B cells.

Conclusions: PEDV infection caused no perturbation to cell type abundances but did cause global disruption of transcriptional profiles for all cell types in jejunal PPs. Epithelial cells had the most altered gene expression profiles upon infection, suggesting PEDV causes greatest functional disruption to host cells at the epithelial barrier. B cells, known to comprise the majority of PP-associated cells, had the next largest amounts of DEGs, and various T cells, innate lymphoid cells, and myeloid cells also had gene expression profiles altered by PEDV infection, suggesting functional shifts to the underlying immune network. B cells and several other affected cell types play pivotal roles in PP immune induction, indicating such biological processes may be affected by PEDV infection. Further research is being conducted to identify which cells are actively infected with PEDV (i.e. contain viral RNA) and how this contributes to transcriptional alterations, including in the context of PP immune induction.

Financial Support: This work was funded by USDA-ARS CRIS project 5030-32000-230-000-D. Mention of trade names or products is for information purposes only and does not imply endorsement by the USDA. USDA is an equal opportunity employer and provider.

Notes:

P120 - Unveiling novel mechanisms of immune dysfunction in Bovine Leukemia Virus-infected dairy cows

Oscar Benitez[1, 2], Clarissa Strieder Barboza[1, 2]

[1]Department of Veterinary Sciences, Davis College of Agricultural Sciences and Natural Resources, Texas Tech University, [2]School of Veterinary Medicine Texas Tech University. obenitez@ttu.edu

Session: Immunology, 2025-01-20, 6:00 - 8:00

Objective: Bovine Leukemia Virus (BLV) is a delta-retrovirus that infects nearly 84% of the dairy herds in the U.S., costing up to $2.7 billion in annual milk production losses. BLV infects the immune system of cows and causes immune dysfunction. Elucidating the mechanisms of BLV pathogenesis will aid in developing strategies to control disease spread and improve dairy health. Single-cell RNA sequencing of peripheral blood mononuclear cells from infected and uninfected animals will provide a comprehensive understanding of the mechanisms of BLV pathogenesis at the molecular and cellular levels by revealing transcriptional changes at the cellular level.

Methods: Clinically healthy, mid-lactation Holstein dairy cows will be screened for BLV antibodies, proviral load (PVL), and lymphocyte count (LC). BLV-negative cows (n=5) and BLV-positive cows (n=15) will be categorized into low, moderate, and high PVL groups. Peripheral blood mononuclear cells (PBMCs) will be isolated using a Ficoll-Paque density gradient and prepared for single-cell RNA sequencing to analyze transcriptional changes in lymphocyte and monocyte populations (Aim 1). B-cells from BLV+ cows will be isolated and co-cultured with CD4+ T-cells to assess how BLV infection affects B-cell function and B-T cell interactions during antigen presentation.

Results: Data generated from this work will include gene expression profiles of thousands of cells in the form of gene expression matrices, principal component analysis, cell clustering, and pseudotime data. This data will reveal results such as PBMC subtypes that change in proportion and transcriptional profile with BLV and PVL levels; unique B- and T-cell states of dysfunctional transformation and activation as BLV PVL increases; cell level disease progression; uniquely expressed genes in BLV-infected B-cells; defective gene pathways of dysfunctional B-cells infected by BLV. Results from B-T cell co-culture will include T-cell proliferation rates assessed by flow cytometry, cytokine secretion profiling (ELISA), antibody profiling (ELISA), and RT-qPCR gene expression data for T-cell anergy genes.

Conclusions: The analysis of this data will yield insights into the molecular mechanisms by which BLV regulates gene expression in B-cells, highlighting altered signal cascades that impact immune function. It will identify dysfunctional cell states or activation patterns linked to disease progression. Results from B-T cell co-culture experiments will allow conclusions regarding T-cell activation and proliferation dynamics when in contact with BLV-infected B-cells and changes in cytokine secretion profiles leading to the expression of anergy-associated genes in T-cells. Antibody profiling will give further insight into the effect of BLV on humoral responses. This data will further clarify the interactions between B- and T-cells during BLV infection. These results will also inform strategies to combat immune dysfunction by targeting key cell receptors and transcription factors the virus affects. Overall, the findings will contribute to understanding how BLV drives immune disruption and will aid in developing targeted therapeutic interventions to restore immune balance and mitigate disease impact.

Financial Support: USDA NIFA 2024-67016-42408.

Notes:

P121 - Single-cell transcriptomics reveals marker genes for immune cell subtypes in turkey peripheral blood

Melissa S. Monson[1], Sharu Paul Sharma[2], Kristen A. Byrne[1], Crystal L. Loving[1]

[1]National Animal Disease Center, USDA-ARS, [2]Office of Biotechnology, Iowa State University. melissa.monson@usda.gov

Session: Immunology, 2025-01-20, 6:00 - 8:00

Objective: Detecting and differentiating immune cell types is central to understanding the role each specific cell subtype plays in immune recognition and clearance or tolerance of different pathogens. However, distinguishing cell subtype-specific responses in turkeys is challenging due to limitations in species-compatible antibodies and other immune reagents. Single-cell RNA-sequencing (scRNA-seq) can capture the transcriptional profiles of individual cells and use them to separate cells into distinct populations without the need for turkey specific reagents. Therefore, this study used scRNA-seq to characterize the expression patterns of turkey leukocytes from peripheral blood.

Methods: Using two turkeys to gain initial insight into expression patterns within turkey immune cells, peripheral blood samples were collected from two healthy male commercial turkeys at approximately 17 weeks of age from the flock at the Iowa State University Stanley L. Balloun Turkey Teaching and Research Facility. Blood samples were divided and used to isolate peripheral blood mononuclear cells (PBMCs; n = 2) or all white blood cells (WBCs; n = 2); isolated samples were partitioned to single cells before library construction and sequencing. Gene count matrices were generated by mapping to the turkey genome and data were filtered, normalized, and used to cluster the cells (0.3 cluster resolution) with the Seurat package. PBMC and WBC datasets were clustered separately due to expected differences in cell type compositions. Major cell types were assigned to the initial clusters using canonical marker genes, then lymphocytes, monocytes, and heterophils were independently subclustered to refine predictions of specific cell subtypes and identify novel markers within their gene expression profiles.

Results: Gene expression was assayed within nearly 80,000 individual cells from turkey peripheral blood, with approximately 22,000 cells per PBMC library and 18,000 cells per WBC library. Initial clustering revealed 14 clusters of cells within PBMCs and 18 clusters in WBCs. The most abundant predicted cell type was thrombocytes, followed by heterophils (only present in WBCs) and T lymphocytes. Only a few subclusters of monocytes and B lymphocytes were identified in PBMCs and WBCs, with clear indications of plasma B cell and SOX5 expressing B cell populations. Heterophils and T lymphocytes were resolved into a larger number of subclusters with variable expression in both canonical and novel marker genes. For example, different granzymes, NK-lysin, and other effector genes were variably expressed across 4 subclusters of cytotoxic T cells in both PBMCs and WBCs, likely reflecting differences in their functional states.

Conclusions: Single-cell transcriptomics in turkey peripheral blood identified cell subtype-specific gene markers and extended our knowledge of the expression patterns of turkey leukocyte subtypes. Future research could employ these markers and scRNA-seq to help disentangle subtype-specific responses to and control of the diverse pathogens seen in the turkey.

Financial Support: Research was supported through U.S. Department of Agriculture appropriated funds.

Notes:

P122 - Commercially available anti-human/-mouse monoclonal antibodies cross-reactive to agriculture and wildlife species

Kristen Byrne[1], Crystal Loving[1]

[1]USDA-ARS-NADC. kristen.byrne@usda.gov

Session: Immunology, 2025-01-20, 6:00 - 8:00

Objective: Animal health is a key component of "One Health", reducing the spread of zoonotic diseases and ensuring a safe food supply. However, reagents required to monitor immune status in agricultural and wildlife species are lacking compared to available human and rodent reagents. Therefore, our objective was to increase access to immune specific reagents by identifying commercially available monoclonal antibodies (mAbs) that cross-reactive with agricultural and wildlife species.

Methods: Commercially available flow cytometric panels of over 400 anti-human and anti-mouse mAbs were selected for cross-reactivity testing against seven agriculture and wildlife species. Species include bison, chicken, dairy cows, pigs, sheep, turkeys, or white tail deer and utilized either single cell suspensions from immune tissues (spleen, lymph node, thymus, or bursa of Fabricius) or peripheral blood mononuclear cells (PBMCs). Tissue cell were stained either directly after isolation, while PBMCs were cultured for 16h with or without various stimulants (ConA, ionomycin, LPS, and/or PMA) to increase expression of activation markers. Cells were stained with a fixable viability dye and then manufacturer's protocol was followed for staining of approximately 5e5 cells per mAb. Data were acquired on a BD FACSymphony, and percent positive determined using a fluorescent minus one gating strategy after exclusion of doublets and dead cells.

Results: Of the 417 monoclonal antibodies tested, 230 were punitive cross-reactive (at least 2-fold greater reactivity than the matched isotype control) to at least one species and sample type (PBMC or tissue) combination. Turkeys and chickens had the lowest punitive cross-reactivity with 47 and 79 mAbs identified respectively, while pig PBMCs had the highest reactivity with 115 mAbs identified. Putative cross-reactive mAbs included rare markers such as CD180, a B cell subset marker, with 0.9% positive events on pig PBMCs, to very abundant markers such as MHC Class I with 97% of bovine PBMCs positive. We identified 32 mAbs as cross-reactive with at least 3 species and at least 5% positive events and plan to further validate specificity and cross-reactivity. To date, three of those 32 mAbs, B-ly4 (CD21), 3A6 (CD166), and Tu39 (MHC Class II), have been validated via flow cytometry with directly conjugated mAbs and confirmed as cross reactive in bison, sheep, cattle, and pigs.

Conclusions: Identification of commercially available, fluorescently labeled mAbs cross-reactive to livestock and wildlife immune cells will enhance relevant research programs across the globe. While further research is needed to confirm the punitive cross-reactive clones identified herein, this research may be useful to other agriculture and wildlife researchers as a framework to quickly identify commercially available cross-reactive mAbs and expand research capabilities.

Financial Support: USDA-ARS CRIS project 5030-32000-225-000D.

Notes:

P123 - Gene expression profile of CD14+ monocytes and alveolar macrophages showing a trained immune phenotype upon BCG vaccination in weaned heifers

Beulah Esther Rani Samuel[1], Pengxin Yang[1], Christopher K Tuggle[1], Jodi L McGill[1]

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

Session: Immunology, 2025-01-20, 6:00 - 8:00

Objective: The BCG vaccine induces non-specific protection effects in immunized individuals, partly attributed to trained immunity. Our previous works showed that BCG induces a trained immune phenotype in bovine PBMCs, monocytes, and γδ T cells in calves. While we have evidence of enhanced cytokine production by bovine monocytes, the signaling pathways associated with trained immunity in cattle remain poorly defined. There is also limited evidence of innate training in lung-resident alveolar macrophages from any species. Therefore, our current study aimed to immunize calves with BCG, assess trained immunity through cytokine production, and explore transcriptional changes in bovine monocytes and alveolar macrophages.

Methods: Weaned Holstein x Angus heifers were assigned to control (n=10) and BCG (n=10) groups. 6 x 10^6 CFU BCG Danish was administered subcutaneously to BCG heifers on days 0 and 14. The control heifers received saline. Blood was drawn at 4- and 5-week post-BCG administration and CD14+ monocytes were isolated by MACS separation. At 6- and 7-week post-BCG, bronchoalveolar lavage was performed to collect alveolar macrophages. Both cell types were stimulated in vitro with cRPMI, *E. coli* LPS, PAM3CSK4, or PolyI:C/Imiquimod for 4 hours (mRNA expression) or 48 hours (cytokine production). IL-1β and IL-6 production were measured by ELISA of culture supernatants. Total RNA was isolated from LPS-stimulated or unstimulated monocytes and alveolar macrophages, and QuantSeq 3'mRNA sequencing was performed. Differentially expressed genes (DEG) were analyzed using a linear model for limma, and significance was determined by adjusted p-value (BH method).

Results: CD14+ monocytes from BCG heifers produced more IL-1β (p=0.022, p=<0.0001) and IL-6 (p=0.015, p=<0.0001) than monocytes from control heifers in response to in vitro stimulation with LPS and Poly(I:C)/Imiquimod. Alveolar macrophages from BCG heifers produced more IL-1β (p = 0.041) than those from control heifers upon in vitro stimulation with *E. coli* LPS. Transcriptional analysis revealed a 1 to 2 log2FC increase in DEGs such as IL-1β, IL-6, and TNF-α due to LPS stimulation in monocytes from BCG-immunized heifers compared to control. The BCG group also showed upregulation in DEGs related to cell adhesion and recruitment, innate signaling pathways, and defense responses. LPS stimulation of alveolar macrophages from the control group resulted in higher log2FC in DEGs related to chemokines and immune cell recruitment compared to the BCG-treated heifers. Alveolar macrophages from the BCG group showed DEGs upregulated in antiviral functions and alternative activation.

Conclusions: Our results showed that bovine monocytes adopt a memory-like phenotype following BCG immunization, comparable to the trained immune phenotype in humans and mice. Despite the non-inflammatory nature of alveolar macrophages in the lung environment, subcutaneous BCG administration can also induce innate training in alveolar macrophages. Investigation of transcriptional changes associated with BCG-induced trained immunity in bovine monocytes revealed additional genes and pathways linked to trained immunity. Gene expression in alveolar macrophages indicates a shift away from the typical proinflammatory response of trained immunity, aiming to protect against infection and prevent hyperinflammation. Our future work will focus on the molecular mechanisms involved in BCG-induced trained immunity.

Financial Support: U.S. Department of Agriculture, National Institute for Food and Agriculture. (R01 HD099104-01).

Notes:

P124 - Dynamics of the CD25 marker in activated T-cell in calves and cows following *Salmonella* Dublin stimulation

Rafael Castro-Vargas[1], Angel Abuelo[1, 2]

[1]College of Veterinary Medicine, Michigan State University, [2]Michigan State University Extension, Michigan State University. castrov8@msu.edu

Session: Immunology, 2025-01-20, 6:00 - 8:00

Objective: *Salmonella* Dublin (S. Dublin) represents a high risk of infection for young calves in the first two months of life due to potential intrauterine transmission and bacterial shedding through feces and colostrum. Despite the availability of vaccines to prevent the disease, there is a lack of foundational knowledge related to the age-specific T-cell activation response after S. Dublin stimulation in young cattle. This gap is critical based on the intrinsic differences of the non-fully functional immune calves' system that could result in a weaker response than adult cows. For this reason, critical immunomarkers for monitoring T-cell activation, such as CD25, could grant us insights into the immune readiness of calves following exposure to S. Dublin. Thus, the objective was to compare the dynamics of the CD25 marker on activated T-cells from calves and cows in response to heat-inactivated S. Dublin on day 3 post-stimulation. We hypothesized that CD25 expression in calves would be significantly lower than in adult cows after in vitro stimulation with S. Dublin, reflecting their immature immune status.

Methods: Blood samples were collected from one-week-old calves (n = 4) and adult mid-lactation cows (n = 4). Peripheral blood mononuclear cells were isolated using a density gradient centrifugation method. The isolated PBMCs were exposed to heat-inactivated wild-type S. Dublin, pokeweed mitogen as a positive control, or media alone as a negative control, with each condition set up in duplicate. After 3 days of incubation, the cultured cells were labeled with antibodies against CD3 and CD25, along with the Zombie Green viability dye, and subsequently analyzed via flow cytometry. During flow cytometric analysis, singlet T-cells were gated based on size and granularity, viability, and CD3 and CD25 expression. For each sample, 10,000 events were recorded within the CD3+ gate. Statistical comparisons between groups will be performed using a one-way ANOVA with multiple comparisons, with significance set at $P < 0.05$.

Results: Data from these experiments are currently being processed and analyzed.

Conclusions: By examining T-cell activation dynamics using CD25 immunomarker, we expect to establish the groundwork for assays that assess vaccination responsiveness in calves after S. Dublin stimulation.

Notes:

P125 - Binding of WC1 SRCR domains from multiple species to *Mycobacterium* spp and *Leptospira* spp.

Janice C. Telfer[1], Oriana Attridge[1], Lauren Poirier[1], Owen Fanning[1]

[1]University of Massachusetts Amherst. telfer@vasci.umass.edu

Session: Immunology, 2025-01-20, 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 the WC1 genes across species have co-evolved with pathogens and thus sought to characterize their diversity and ligand-binding potential.

Methods: Genes from cattle, sheep, goats, swine, dogs, and cats were mapped. Sequences encoding SRCR domains from cattle, swine, dogs and cats were cloned into a mammalian expression vector. After transfection of the SRCR domain vector into suspension 293F, recombinant SRCR domain proteins were purified and quantified. ELISA and bacteria binding pull-down assays were carried out with recombinant WC1 SRCR protein and fractionated or fixed whole Mycobacteria spp and Leptospira spp.

Results: Unlike the WC1 multigenic arrays in cattle, sheep, goats and swine, there is a single WC1 6-SRCR domain gene in dogs and cats. We confirmed that selected WC1 SRCR domains from cattle, swine, cats and dogs are capable of directly binding with high affinity to fractionated and whole fixed Leptospira spp or Mycobacterium spp.

Conclusions: Selected WC1 SRCR domains from cattle, swine, dogs and cats bind selectively and with high affinity to Leptospira spp and Mycobacterium spp, supporting the hypothesis that they have co-evolved with important pathogens as a hybrid co-receptor and pattern recognition receptor on gamma delta T cells.

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 and from Massachusetts Agricultural Experimental Station grant funds.

Notes:

P126 - Effects of cytochrome P450 pathway manipulation on inflammation in an in-vitro bovine mastitis model

Joe D. Wilson[1], Vengai Mavangira[2]

[1]Veterinary Microbiology and Preventive Medicine, Iowa State University, [2]Veterinary Microbiology and Preventive Medicine, Veterinary Diagnostic and Production Animal Medicine, Iowa State University. joew@iastate.edu

Session: Immunology, 2025-01-20, 6:00 - 8:00

Objective: Oxygenated lipid mediators derived from the cytochrome P450 (CYP450) pathway were recently described during bovine clinical mastitis. However, their role in the disease pathophysiology is unknown. We previously demonstrated early increases in direct CYP450 enzyme activities and significant alterations of their lipid oxygenated metabolite (epoxylipids) profiles preceding clinical signs during experimental coliform mastitis in lactating dairy cows. Epoxylipid profiles have demonstrated therapeutic potential in comparable disease models in other species. We hypothesized that limiting the production of CYP450-derived inflammatory mediators through the inhibition of the hydration pathway, soluble epoxide hydrolase (sEH), would limit the degree of inflammation during an in-vitro coliform mastitis model.

Methods: Using a previously utilized in-vitro approach of whole blood stimulation and bovine peripheral blood mononuclear cells (PBMC) cultures, we investigated different approaches to enhancing the initial CYP450-derived epoxy lipids inflammation. Whole blood or PBMCs isolated from healthy lactating Holstein dairy cows were stimulated with endotoxin and treated with compounds targeting the CYP450 pathway. The incubation times targeted peak epoxylipid production (6 hr.) we previously identified in milk and plasma during in-vivo intramammary *E. coli* infusion, time at development of clinical signs (12 hr.), and a later time point (24 hr.). Treatments included endotoxin (lipopolysaccharide, LPS) to mimic coliform mastitis, soluble epoxide hydrolase inhibitor (sEHI) alone, preincubation with sEHI followed by endotoxin (sEHI+LPS), preincubation with CYP450-derived metabolites known as epoxyfatty acids (EpoxyFA) with or without sEHI, and various controls including non-treatment referent (control) and compound vehicles. The inflammatory response was based on cytokine analyses using ELISAs for IL-1β, IL-6, and TNF-α. Data were analyzed using nonparametric analyses in Prism software 8.4.

Results: In both whole blood and PBMC stimulation approaches, LPS induced significant IL-1β production relevant to untreated controls. However, sEHI treatment decreased IL-1β and TNF-α production in PBMCs only. No treatment effects were observed for IL-6 production in either experimental approach. No differences were noted among treatments in whole blood stimulations. Exogenous EpoxyFA supplementation had no effect on inflammation in either experimental approach at any time point.

Conclusions: Targeting CYP450 and sEH pathways reduces pro-inflammatory cytokine production in PBMCs; however, whole blood stimulation showed no differences between treatments, suggesting that pure isolation of immune cells, such as neutrophils and monocytes, may be necessary to better evaluate inflammatory response in coliform mastitis models. Better experimental models that have expression of the CYP450 and sEH pathways are needed to better understand their roles during inflammation.

Financial Support: This project was supported by Agriculture and Food Research Initiative Competitive Grant no. 2020-67015-31541 from the USDA National Institute of Food and Agriculture.

Notes:

P127 - Improving the ruminant immune response to bovine neonatal gastroenteritis through commensal bacterial modulation

R.B. Dauwen-Lile[1], T.C. Berry[2], J.L.C. Borgogna[1], C.J. Yeoman[1]

[1]Dept. of Animal & Range Sciences, Montana State University, [2]Dept. of Microbiology & Cell Biology, Montana State University. river.dauwen@student.montana.edu

Session: Immunology, 2025-01-20, 6:00 - 8:00

Objective: Bovine neonatal gastroenteritis, commonly known as scours, is a diarrheal infection that, if untreated, can lead to dehydration, electrolyte loss, acidosis, and even death in scouring calves. Scours also causes more financial loss than any other disease to cow-calf producers. Vaccinations and antibiotics cannot adequately address this poly-microbial disease. Beneficial commensal gut bacteria directly interact with the gut-associated lymphoid tissue (GALT) to support barrier function and the rapid development of a mature immune system. This study aims to use bacterial immunomodulation to reduce severity of illness and mortality in young ruminants and reduce the impact on producers.

Methods: 24 colostrum-deprived calves are being direct fed 20mL of either *Akkermansia muciniphila*, *Aristaella hokkaidonensis R7*, or *Rikenellaceae* RC9 at $1x10^6$ cells/mL in milk replacer twice daily for 14 days. An untreated group of 6 calves will be cared for on the same protocol without one of the bacterial strains. A baseline control group of three animals will be harvested at birth as well. Blood and serum samples were collected weekly throughout the experiment. Fecal DNA extraction was used to test for the presence of the direct fed microbial by absolute qPCR. Intestinal tissue of the jejunum, ileum, and cecum will be surgically removed from harvested animals and examined for number of Peyer's patches (primary gut immune tissue) and prevalence of associated follicles. Biopsies will be submitted for histochemical analysis and microbiome analysis. Remaining tissue will be examined for barrier function by transepithelial electric resistance.

Results: Microbial populations were analyzed in young ruminants from birth until a stable microbial community was formed. These bacteria were correlated with the following immunological benefits with p values < 0.05. *A. muciniphila* is associated with increased IgG (most common antibody in blood and tissue); r^2=0.66. *A. hokkaidonensis* is associated with increased IgA (found in mucus) and IgM (first antibody produced in immune response); r^2=0.66 and 0.63. *Rikenellaceae* RC9 is associated with increased IgG (most common antibody in blood and tissue); r^2=0.71. Further results of the animal trial will be shared.

Conclusions: Our preliminary results suggest that specific microbes are responsible for much of the foundational immune development in ruminant animals rather than the whole microbiome. The current study will further elucidate whether beneficial commensal bacteria can be used to stimulate and shape immunological development through barrier function, circulating immunoglobulins, and GALT morphology.

Financial Support: This work was supported by USDA-AFRI grant 2020-67016-31676, the Bair Ranch Foundation, and the Montana Agricultural Experiment Station. All findings presented are those of the authors and should not be construed to represent those of the USDA or U.S. Government.

Notes:

P128 - IgY-based immunomodulatory combo succeeds at tissular cure of *Staphylococcus aureus* mastitis in a mouse model

Jatna I. Rivas Zarete[1, 2], Benjamin Adu-Addai[2]

[1]Tuskegee University, Integrative Biosciences Program, [2]Tuskegee University, Biomedical Department. jrivas4108@tuskegee.edu

Session: Mastitis, 2025-01-20, 6:00 - 8:00

Objective: *Staphylococcus aureus (S. aureus)* is a bacterial agent responsible for about 10% of clinical mastitis cases in dairy cows in the United States. *S. aureus* causes chronic mastitis with abscesses and persistent infections. The infections persist due to the survival of *S. aureus* inside the mammary epithelial cells (MECs), avoiding destruction by humoral immunity and antibiotics. *S. aureus* doesn't actively enter the cells: instead, MECs themselves are non-professional phagocytes that actively capture and consume pathogens near them. The "ingestion" is called internalization, and should destroy the pathogen, but *S. aureus* survives. Vitamin D3 and IgY have been previously found to, together or independently, prevent internalization of *S. aureus* by mammary epithelial cells. Moreover, previous studies show that IgY disrupts *S. aureus'* capacity for biofilm formation, inhibits its growth, targets germs for destruction by professional immune cells only, and can inhibit the effect of toxins. The peptide RP185 polarizes M2 macrophages (vulnerable to *S. aureus*) to M1 (less vulnerable), which should in turn destroy infected cells and clear *S. aureus* present in the lumen of the gland. Our study combines these elements to allow the animal's body to eradicate *S. aureus*: preventing internalization of *S. aureus* by MECs by reducing MEC non-professional phagocytosis directly (vitamin D3 acts on the cells) and indirectly (IgY binds to *S. aureus)*, and promoting the killing of already infected cells, and biofilm and bacterial clearance by M1 macrophages. Together, these tools are meant to enable the host's immunity to clear the infection.

Methods: Lactating mouse dams (n=49) were inoculated with *S. aureus* (100 CFU) through their teats, causing mastitis, one week after giving birth. They were then assigned one of four treatments: the control treatment (+CTRL, n=12); 15ul of anti-SpA IgY 5ug/ml (IgY, n=12); anti-SpA IgY 5ug/ml+80nM of Vitamin D3 (VitD, n=12); 5ug/ml anti-SpA IgY+80nM of Vitamin D3+10ug/ml of RP185 (RP, n=13). The treatments were administered every 12h for 6 days. Thirteen lactating dams were used as negative control (-CTRL) (no infection, no treatment). Results were infection/cure, weekly survival/death, through Kaplan-Meier survival analysis.

Results: Survival rates were: 46% (+CTRL), 80% (IgY), 80% (VitD), 91.7% (RP) and 92.3% (-CTRL), with +CTRL having significantly higher mortality than -CTRL and RP. Two weeks after the treatment (dry period) the animals were sacrificed and their mammary glands were cultured. Tissue-level cure rates were 30% (+CTRL), 70% (IgY), 90% (VitD), 54% (RP), and N/A (-CTR), correspondingly, with VitD having the best chances of cure. This suggests that tissue-level clearance of *S. aureus* infections of the murine mammary gland can be achieved by immunomodulatory treatments alone.

Conclusions: Intramammary combination treatments of anti-SpA IgY+Vitamin D3 may be a viable solution for the prevention of chronic *S. aureus* mastitis and *S. aureus* mastitis relapses derived from incomplete tissular cure. Treatment combinations including RP can be considered for animals of very high genetic value to reduce disease severity. The significance of the tissular cure observed in VitD groups and the reduction in mortality observed in RP groups, is worth exploring in large animals.

Financial Support: We are grateful to the USDA/NIFA (Award #2022-38821-37363) for making this project possible!

Notes:

P129 - Induced *Staphylococcus* chromogenes teat end colonization in dairy heifers

Pamela R. F. Adkins[1], Ben R. Byrd[1]

[1]University of Missouri. adkinsp@missouri.edu

Session: Mastitis, 2025-01-20, 6:00 - 8:00

Objectives: The objective of this study was to inoculate heifer teat ends with a specific strain of *Staphylococcus chromogenes* and determine if the inoculated strain could be identified on the teats over time.

Methods: This study was conducted by using a teat dip challenge model. The trial was approved by IACUC (Protocol #28281). The challenge isolate was *Staphylococcus chromogenes* #304 (SC304), which was selected because it originated from a teat end swab and has known ability to inhibit the growth of *Staphylococcus aureus in vitro.* To allow for strain identification, the selected isolate was added to the matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF) library following the manufactures instructions. The challenge dip was prepared by growing SC304 in 250 mL of Tryptic Soy Broth to a concentration of 1 X 10^5 CFU/mL. Breeding age heifers from the University of Missouri Foremost Dairy farm were enrolled in the study if they were Holstein breed and had an apparently healthy mammary gland based on visual inspection. Two randomly selected quarters of each enrolled heifer were dipped once daily for 2 days by inserting the teat into the challenge broth for 15 seconds. Teats were allowed to dry for 5 minutes prior to releasing the heifer from the chute. Teat ends of enrolled quarters were sampled on day 1, 3, 7, 14, and 28 post challenge. Teat end swabs were collected by brushing a dry swab across the selected teat end. Swabs were then placed in sterile tubes and plated on Columbia Blood Agar. For each sample, 4 colonies with phenotypic characteristics similar to SC304 were selected for characterization. All selected isolates were speciated using MALDI-TOF plate extraction method, following the manufacturer's instructions. Next, all isolates identified as *S. chromogenes* were evaluated using the MALDI-TOF tube extraction method to allow for identification of isolates that matched the SC304 MALDI-TOF library entry. Isolates that matched SC304 as the top match with difference between the top and second match were classified as a SC304 isolate.

Results: A total of 20 quarters (10 heifers) were enrolled in the study. All enrolled quarters were apparently healthy at the time of enrollment and remained clinically normal throughout the study. Overall, SC304 was identified on 75% (15/20), 60% (12/20), and 35% (7/20) of quarters on days 1, 3, and 7, respectively.

Conclusion: Induced teat end colonization with *S. chromogenes* using a teat dip challenge technique was successful. Future studies will use this technique to determine if induce colonization with potentially protective strains of *S. chromogenes* can provide quarter level protection from major mastitis pathogens.

Financial Support: USDA-NIFA Award #2022-67015-37123

Notes:

P130 - Intramammary infection induces mammary epithelial cell death in heifer mammary glands during late gestation

B.D. Enger[1], M. X. S. Oliveira[1], P. H. Baker[1], K. M. Enger[1]

[1]The Ohio State University, Department of Animal Sciences. enger.5@osu.edu

Session: Mastitis, 2025-01-20, 6:00 - 8:00

Objective: Intramammary infections commonly occur in pregnant dairy heifers. Dairy heifers that begin their lactation with intramammary infections have reduced milk yields, and it is suspected that the prepartum infections are disrupting mammary gland growth and microstructural development. The objective of this study was to assess how *Staphylococcus aureus* intramammary infections affect mammary glands of pregnant heifers during progressing stages in late gestation.

Methods: Twenty-one pregnant Holstien dairy heifers, equally divided across 3 gestational stages (5.75, 6.75, and 7.75 months pregnant) were enrolled. One randomly selected mammary gland of each heifer was infused with *Staphylococcus aureus* (STAPH) while another was designated a control and infused with saline (SAL). Mammary secretions were sequentially collected after challenge and mammary tissues were collected 21 d post challenge from mammary parenchyma located at the center of the mammary parenchymal mass and near the distal edge of the mammary parenchyma, near the abdominal wall. Mammary tissues were fixed and subject to histological analysis.

Results: All STAPH quarters remained infected throughout the trial while SAL quarters stayed culture negative. Quarter treatment somatic cell counts (SCC) were similar at challenge but SCC of STAPH quarters markedly increased, and remained greater than that of SAL quarters, for the duration of the study. Tissue area responses were less pronounced and were inconsistent compared to our previous studies by our group. For instance, STAPH quarters of heifers that were 7.5 months pregnant displayed less epithelial area than SAL quarters, but CHALL quarters of heifers that were 6.5 months pregnant displayed more epithelial area than SAL quarters. Interestingly, no differences were detected between SAL and CHALL quarters for epithelium tissue areas at 8.5 months pregnant. Complementing the epithelial area changes resulting from infection was that centrally located epithelium had a greater proportion of epithelial cells undergoing apoptosis in STAPH mammary glands than in SAL mammary glands across all gestational ages (0.63% vs 0.51% ± 0.1%, P = 0.15). This pattern was not present in epithelium located at the distal edge of the mammary tissues.

Conclusions: In conclusion, our findings indicate that intramammary infections during pregnancy increases epithelial cell apoptosis but that spatial location of the mammary tissues in the mammary gland influences the degree of tissue damage that results from intramammary infection. The increased death of epithelial cells in STAPH mammary glands suggest that IMI disrupts normal mammary development, which may compromise future milk production. These results highlight the need to reduce IMI occurrence in non-lactating, pregnant heifers to ensure maximal mammary gland productivity in the ensuing lactation.

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:

P131 - Intramammary cholera toxin-based *Staphylococcus aureus* vaccine with subcutaneous boost shows safety, immunogenicity

Elise Overgaard[1, 2], Haley A. Bridgewater[2, 3], Nicholas K. Van Engen[4], Juliette K. Tinker[2, 3]

[1]Pentamer Biologics, LLC, [2]Biological Sciences, Boise State University, [3]Biomolecular Sciences, Boise State University, [4]Johnson Research, LLC. eliseovergaard@boisestate.edu

Session: Mastitis, 2025-01-20, 6:00 - 8:00

Objective: Staphylococcus aureus is one of the most common agents of bovine mastitis, a costly disease that affects dairy cows worldwide. Cholera toxin (CT), a bacterial enterotoxin, binds to receptors on epithelial membranes and can be conjugated to other bacterial antigens, supporting mucosal vaccination via a Trojan horse-style antigen delivery system. In this study, we aimed to assess the safety and immunogenicity of a non-toxic CT-based S. aureus vaccine (IsdA+ClfA-CTA2/B) to prevent bovine mastitis. The vaccine contains two highly conserved S. aureus antigens derived from strain Newbould 305: clumping factor A (ClfA) and iron-regulated surface determinant protein A (IsdA).

Methods: On Day 0, six Holstein cows with no clinical or systemic evidence of mastitis received intramammary IsdA+ClfA-CTA2/B or PBS control. On Day 14, vaccinated cows received subcutaneous boost and unvaccinated cows received subcutaneous PBS control. On Day 20, all cows received intramammary challenge in opposite quarters with S. aureus Newbould 305. Safety was monitored throughout the study. Milk and blood samples were collected on Days 0, 7, 14, 17, 20, 23, 27, and 30, and additional milk samples were collected each day of the challenge period. Bacterial shedding and somatic cell count (SCCs) in milk samples were quantified. Bacterial counts were log transformed prior to analysis. Immunogenicity was assessed using ELISA to detect antibodies against vaccine components. ELISA data were reported as the natural log of the ratio of absorbance on Day X compared to absorbance on Day 0 (LN (Day X/Day 0)). Bacterial counts, SCCs, and ELISA data were analyzed using two-way repeated measures ANOVA followed by Dunnett's test to evaluate multiple comparisons among treatment means.

Results: No systemic or local adverse events (AEs) for either administration route were observed. One control cow developed moderate mastitis in one quarter due to an *E. coli* infection shortly after Day 0, as well as a subclinical Staphylococcal infection in another quarter. The Staph infection progressed into clinical mastitis during the challenge period. Both infections were possibly related to intramammary administration. No other vaccine-related AEs were observed. Vaccinated cows appeared to have lower amounts of bacterial shedding and SCCs compared to control animals for the first several days of the challenge period, although these differences were not statistically significant. Vaccinated animals produced immunogenic responses in milk and serum with significant whey IgG responses against IsdA ($p<0.05$ on Day20-Day27), ClfA ($p<0.05$ on Day20-Day30), and CTA2/B ($p<0.05$ on Day20-Day30), serum IgG1 responses against IsdA ($p<0.05$ on Day17-Day27), ClfA ($p<0.05$ on Day17-Day30) and CTA2/B ($p<0.05$ on Day17-Day30), and serum IgG2 responses against IsdA ($p<0.05$ on Day20-Day30) and CTA2/B ($p<0.05$ on Day27-Day30).

Conclusions: The intramammary route requires extra attention to sterile technique during administration, but these findings reveal a good safety profile for the IsdA+ClfA-CTA2/B vaccine and provide evidence that it can induce antigen-specific immunity when administered via the intramammary route with a subcutaneous boost. Further trials with larger sample sizes are required to better assess efficacy in bacterial shedding and SCC reduction and to determine whether the vaccine provides protection against clinical mastitis.

Financial Support: This research was funded by a 2023 USDA/NIFA SBIR Phase I Grant (Contract Number 2023-00817, PD Tinker, Co-PD Overgaard).

Notes:

P132 - Changes in the components of milk in dairy cattle with subclinical mastitis

Ramūnas Antanaitis[1], Samanta Arlauskaitė[1], Mindaugas Televičius[1], Dovilė Malašauskienė[1], Mingaudas Urbutis[1], Karina Džermeikaitė[1], Justina Krištolaitytė[1], Akvilė Girdauskaitė[1]

[1]Lithuanian University of Health Sciences. akvile.girdauskaite@lsmu.lt

Session: Mastitis, 2025-01-20, 6:00 - 8:00

Objective: Subclinical mastitis is significantly more prevalent than clinical mastitis in dairy cattle herds. With subclinical mastitis, there are no visible changes in the milk, which often leads to farms being unaware that some of their milking cows are affected by this form of mastitis. If subclinical mastitis is not diagnosed in time, milk production decreases, treatment costs increase, and milk quality deteriorates. There is also a risk that the subclinical form of the disease may develop into the clinical form of mastitis. The aim of this study was to determine how the components of milk change when a cow develops subclinical mastitis.

Methods: The study was conducted on a dairy cattle farm in Lithuania. 550 lactating cows are kept in the farm. 20 fresh lactating cows were selected for the study. 10 of them were diagnosed with subclinical mastitis, and 10 were clinically healthy. Subclinical mastitis was diagnosed by identifying increased electrical conductivity of milk, decreased milk production, an increased number of somatic cells in the milk, and through a general clinical examination along with a positive result from the California Mastitis Test. The milk biomarkers were recorded by the DeLaval (Sweden) herd management software, combined with the Herd Navigator system. Milk samples were taken to determine milk components on the day of subclinical mastitis diagnosis. Milk samples were also taken from the cows in the control group. Once the results were obtained, the milk components of the cows with subclinical mastitis were compared to those of the healthy cows.

Results: When evaluating changes in milk composition, it was found that the somatic cell count in cows with subclinical mastitis is 160% higher than in clinically healthy cows. The milk of cows with subclinical mastitis had 5.5% less fat, 1.6% less protein, and 2.4% less lactose compared to the milk of clinically healthy cows. The urea concentration in the milk of affected cows was 9.9% higher compared to the milk of the control group of cows.

Conclusions: The obtained data were not statistically reliable due to the small number of animals involved in the study. For future similar studies, it would be useful to increase the number of animal groups being studied. However, this study emphasizes how subclinical mastitis significantly affects dairy cow's milk composition. Significantly elevated somatic cell counts and altered milk component concentrations, such as lower levels of fat, protein, and lactose, as well as higher levels of urea, are observed in cows with subclinical mastitis. These modifications impact milk quality and also indicate higher health concerns, which eventually harm the profitability of dairy farms. Early detection and management of subclinical mastitis are crucial to minimizing its negative effects on milk production, quality and animal welfare.

Notes:

P133 - Antimicrobial susceptibility of mastitis bacterial pathogens isolated from North American dairy cattle, 2011-2022

Bryce L. Lunt[1], Michael T. Sweeney[1], Lacie Gunnett[1], Dipu Mohan Kumar[1], Abhijit Gurjar[1], Véronique Moulin[1]

[1]Zoetis. bryce.lunt@zoetis.com

Session: Mastitis, 2025-01-20, 6:00 - 8:00

Objectives: To determine the in vitro activity of ceftiofur, pirlimycin, penicillin-novobiocin, cefoperazone, ampicillin, erythromycin, oxacillin, and cephalothin against bacterial pathogens from naturally occurring cases of bovine mastitis in the United States and Canada.

Methods: A total of 10,890 bacterial isolates of Streptococcus dysgalactiae, Streptococcus uberis, Staphylococcus aureus, and Escherichia coli isolated as etiological agents from dairy cows with mastitis by 29 veterinary laboratories across North America between 2011 and 2022 were tested for in vitro antimicrobial susceptibility by broth microdilution according to Clinical and Laboratory Standards Institute (CLSI) standards.

Results: Using available clinical breakpoints, antimicrobial resistance among S. dysgalactiae (n=2406) was low for penicillin-novobiocin (0% resistance), ceftiofur (0.1%), erythromycin (3.2%), and pirlimycin (4.6%). Among S. uberis (n=2398), resistance was low for ampicillin (0%) and ceftiofur (0.2%) and moderate for erythromycin (11.9%) and pirlimycin (18.4%). For S. aureus (n=3194), resistance was low for penicillin-novobiocin (0%), ceftiofur (0.1%), oxacillin (0.2%), erythromycin (0.7%), cefoperazone (1.2%), and pirlimycin (2.8%). For *E. coli* (n=2892), resistance was low for ceftiofur (2.8%) and cefoperazone (3.4%) and moderate for ampicillin (9.2%).

Conclusions: Mastitis pathogens in the United States and Canada have not shown any substantial changes in the in vitro susceptibility to antimicrobial drugs used for mastitis management over the 12 years of the study, or among that of a previous survey from 2002-2010. The data supports the conclusion that bacterial resistance to common antimicrobial drugs among mastitis pathogens, even to drugs that have been used on dairies for mastitis management for many years, continues to remain low.

Notes:

P135 - MinION library preparation using recombinase polymerase amplification (RPA) for avian reovirus characterization

Vicente Avila-Reyes[1], Morgan L. Cunningham[2], Kelsey T. Young[3], Holly S. Sellers[4], James B. Stanton[1]

[1]Pathology Department, University of Georgia, [2]College of Veterinary Medicine, University of Georgia, [3]Commonwealth of Pennsylvania, [4]Poultry Diagnostic and Research Center, University of Georgia. jbs@uga.edu

Session: Microbiome, 2025-01-20, 6:00 - 8:00

Objective: Avian orthoreoviruses (ARV) are worldwide pathogens of numerous avian species, though, poultry are the most economically importantly population. ARV's ubiquity throughout poultry facilities, their segmented RNA genome that makes them prone to mutate rapidly and reassort, and the presence of mixed strains in one sample are factors that complicate control measures. Current ARV genetic typing is valuable for characterization; however, it is labor intensive and time consuming. Additionally, focusing on a single gene fails to address the role of reassortment and the role of the other segments in pathogenicity. Recombinase polymerase amplification (RPA) is an emerging technology that amplifies a targeted nucleic acid at a constant temperature in a short period of time compared with conventional nucleic acid amplification technologies. The objectives of this study were to test if using RPA for MinION library preparation reduces the time required for library preparation and its effectiveness in genomic sequencing of ARV.

Methods: RNA was extracted from cultured avian reovirus samples using Trizol. Reverse transcription, strandswitching, and PCR were performed as previously described with random hexamers as the reverse primers. RPA reactions for barcoding were run utilizing TwistAmp Basic Kit (TwistDx Inc) following manufacturer recommendations and using PCR primers from ONT's PCR barcoding expansion kit (EXP-PBC096). Real-time base-calling, trimming, and demultiplexing of reads were performed using in-house scripts using GPU version of Guppy v.3.1.54.4.1 (ONT) and Porechop v.0.2.4. Extracted reads were analyzed using Geneious prime v.2019.1.3. and mapped to reference to a sequence list containing a reference sequence of each segment and genotype of ARV. Additionally, the read quality was analyzed using Nanoq.

Results: Substitution of PCR for RPA at the barcoding step resulted in the detection of ARV within samples and decreased the library preparation time. While the RPA-produced reads were of similar quality to the PCR-produced reads, they were fewer and shorter, resulting in less genomic coverage.

Conclusions: RPA was effective for MinION library construction reducing time in library preparation, and multiple sample pooling was successful; however, it suffered from background amplification problems like lack of read uniformity, low number of reads, and non-specific amplification products. RPA provides the possibility for protocol modifications to achieve longer reads, which is the basis for further experimentation to improve RPA's usefulness for MinION library preparation and thus improving the utility of sequencing in a diagnostic setting.

Financial Support: This project was supported by Agriculture and Food Research Initiative Competitive Grant no. 2023-67015-39735 from the USDA and USPEA project #730. NIH Office of Research Infrastructure Programs, Grant Number 5T35OD010433-14. NIH T35 OD 010433 Georgia Veterinary Scholars Summer Research Program.

Notes:

P136 - Update on the molecular epidemiological assessment of beef cattle management systems

Bradly I. Ramirez[1], Hudson R. McAllister[1], Sarah F. Capik[2], Robert J. Valeris-Chacin[1], Kelsey M. Harvey[3], Brandi B. Karisch[4], Amelia R. Woolums[5], Paul S. Morley[1], Lee J. Pinnell[1], Matthew A. Scott[1]

[1]Texas A&M University VERO Program, [2]Tumbleweed Veterinary Services, PLLC, [3]Prairie Research Unit, Mississippi State University, [4]Department of Animal and Dairy Sciences, Mississippi State University, [5]Department of Pathobiology and Population Medicine, Mississippi State University. matthewscott@tamu.edu

Session: Microbiome, 2025-01-20, 6:00 - 8:00

Objectives: We hypothesized that cattle management decisions to reduce bovine respiratory disease (BRD) risk influence host immunity, cellular activity, and microbial communities. To test this, we analyzed 1) whole blood transcriptomes and 2) microbial DNA and RNA from the upper respiratory tract of cattle using a time-course, multi-omics approach.

Methods: Objective 1: We analyzed the effect of vaccination and marketing strategy on gene expression using blood samples from 73 cattle enrolled in a split-plot randomized controlled trial. Samples were collected at six time points: initial vaccination (VAX) or no vaccination (NOVAX) during the cow-calf phase (T1), seven days post-vaccination (T2), booster or no booster (T3), weaning and enrollment into auction (AUC) or direct sale (DIR) marketing (T4), backgrounding facility arrival (T5), and the end of the 45-day backgrounding period (T6). Illumina sequencing of mRNA was performed, and gene counts were analyzed for differential expression and dynamic expressional trends with edgeR, glmmSeq, and Trendy (FDR<0.10). Objective 2: We collected nasopharyngeal swabs from the same cattle at T1, T4, and T5 for bacterial and viral DNA/RNA extraction. Matched samples were selected for metagenomic and metatranscriptomic analyses via Illumina sequencing, with bioinformatic processing via k-mer-based assembly, Swiss-Prot annotation, and coverage-weighted contig-abundance estimation for both bacterial and viral reads to assess host-microbiome interactions.

Results: Objective 1: In the cow-calf phase, VAX cattle possessed 26, 47, and 32 DEGs at T2, T3, and T4, respectively, with increased expression related to stress response and immune processes, compared to NOVAX cattle. BRD cattle had 12, 10, and 51 DEGs at T2, T3, and T4, respectively, with increased expression in oxygenation and metabolism pathways. Timepoint comparisons, blocking for vaccination and later BRD development, revealed 10,397 DEGs related to innate and adaptive immunity, inflammatory mediation, and interleukin signaling. At T5, 834, 56, and 364 DEGs were associated with marketing, vaccination, and BRD development, respectively. Genes in NOVAX cattle, when compared to VAX cattle, enriched for extracellular matrix organization, neutrophil degranulation, antimicrobial peptides, interleukin signaling, and scavenging by class A receptors. BRD cattle, compared to HEALTHY cattle, demonstrated increased expression for cytokine and interferon signaling. At T6, 135, 17, and 3 DEGs were identified, with AUC and BRD-related genes enriched for cell junction and interleukin signaling. Objective 2: DNA sequencing is complete, and bioinformatic analyses are ongoing. RNA was deemed highly degraded from these extractions, and viral/host nucleic acid extraction from year-3 samples is planned.

Conclusions: Analysis for objective 1 is nearly complete, with manuscripts expected by early 2025. Sequencing for objective 2 is finished, with viral/host nucleic acid analysis to start in early 2025.

Financial Support: This work is supported by the USDA National Institute of Food and Agriculture (NIFA) Agriculture and Food Research Initiative Competitive Grant No. 2023-67015-39711. Any opinions, conclusions, or recommendations do not necessarily reflect the view of the USDA.

Notes:

P137 - In-silico detection of gene-specific markers for *Campylobacter* species utilizing pangenome and core genome analysis

E. Kuufire[1], K. Bentum[1], R. Nyarku[1], V. Osei[1], T. Samuel[1], W. Abebe[1]

[1]Department of Pathobiology, Tuskegee University. ekuufire9436@tuskegee.edu

Session: Microbiome, 2025-01-20, 6:00 - 8:00

Objective: *Campylobacter* is a leading contributor to foodborne illnesses globally, causing 1.5 million infections annually in the United States. This bacterium is associated with most human bacterial gastroenteritis and related syndromes and causes animal reproductive issues. It is known to be isolated from patients with alimentary tract infections at a frequency of about 3-4 times higher than *Salmonella* or *Escherichia coli*. Conventional culture-based detection methods for *Campylobacter* are time-consuming and labor-intensive. Consequently, there is a critical need to explore more efficient, molecular-based approaches. To this end, this study conducted a pan-genomic and core-genomic analysis of *Campylobacter* to identify gene-specific markers for its pathogenic species, subspecies, and biovars, providing a foundation for various diagnostic assays. This area of research has not been fully explored, given the continual evolution of these bacteria.

Methods: A total of 132 referenced genomes representing 33 species and 9 subspecies were obtained from the NCBI database and curated to 106 high-quality genomes using the checkM tool. These genomes were then processed through the Roary ILP Bacterial Annotation Pipeline (RIBAP) to construct the pangenome. This process involved identifying the presence and absence of genes across the genomes, including both core and accessory genes. Finally, the identified target genes were validated using Geneious Prime software.

Results: The core gene content displayed substantial genomic diversity across the different species, with 228 core genes at 60% nucleotide identity, 119 at 70%, 46 at 80%, 9 at 90%, and a single core gene at 95% identity. This finding suggests considerable genomic diversity within the genus. Furthermore, total accessory genes ranged from 25,118 to 70,165, with 60% and 95% identity, respectively. It further emphasizes the remarkable genomic heterogeneity exhibited by the microorganisms in this genus. The targets identified were 100 % sensitive and 85 to 100% specific for the 23 pathogenic species, subspecies, and biovars.

Conclusions: The findings of this study provide a comprehensive catalog of gene-specific targets for the rapid and accurate diagnosis of pathogenic *Campylobacter* species, subspecies, and biovars, which can be further validated and exploited for developing improved molecular detection tools. The robust pangenome analysis highlighted the remarkable genetic heterogeneity within this genus, indicating a polyphasic approach to accurately delineate the *Campylobacter* species diversity. It complements previous pangenome studies on *Campylobacter*, highlighting the genetic diversity of the various species

Financial Support: This study was supported by grant from USDA/NIFA/CBG 2021-38821-34710; MSU/USDA/NIFA RC113747TU.

Notes:

P138 - Microbiome-mediated colonization resistance against necrotic enteritis

Jing Liu[1], Isabel Tobin[1], Joy Scaria[2], Lamont Susan[3], Glenn Zhang[1]

[1]Department of Animal and Food Sciences, Oklahoma State University, [2]Department of Veterinary Pathobiology, Oklahoma State University, [3]Department of Animal Science, Iowa State University. glenn.zhang@okstate.edu

Session: Microbiome, 2025-01-20, 6:00 - 8:00

Objective: Necrotic enteritis (NE), caused by Clostridium perfringens, ranks among the most financially devastating diseases in poultry. Unfortunately, there are currently no effective preventive or therapeutic measures available. The intestinal microbiota plays a critical role in maintaining animal health and productivity by resisting the colonization and proliferation of invading pathogens. Inbred chicken lines are known to vary in their natural resistance to NE. The objective of this study was to explore whether the intestinal microbiomes of NE-resistant chickens offer strong colonization resistance against NE.

Methods: We first evaluated the relative resistance to NE in two highly inbred chicken lines, Fayoumi M5.1 (FM5.1) and Leghorn Ghs6, alongside Cobb broilers. We further investigated the intestinal microbiota differences among three breeds of chickens. To assess whether NE-resistant Fayoumi chickens harbor unique intestinal microbiota conferring enhanced resistance, we transplanted the cecal microbiota from different chicken breeds to naïve, day-of-hatch Cobb broilers, followed by NE induction and evaluation of the disease outcome.

Results: We found that FM5.1 chickens demonstrated the highest resistance, with no mortalities or small intestinal lesions, whereas Ghs6 and Cobb chickens exhibited mortality rates of approximately 8% and 20%, respectively in a chicken NE model. Furthermore, transplantation of the cecal microbiota from FM5.1 chickens provided 100% protection against NE in recipient Cobb chickens, which otherwise had a 35% mortality rate. Surprisingly, the cecal microbiota of Ghs6 and Cobb chickens also offered significant protection against NE. Intestinal microbiota differed markedly among the three breeds under both healthy and NE conditions. FM5.1 chickens had significantly higher levels of Bifidobacterium, Lactobacillus, Ligilactobacillus salivarius, and Limosilactobacillus reuteri in their ileum and cecum ($P < 0.05$), suggesting their potential role in NE resistance. To further identify commensal bacteria responsible for NE resistance, we screened a library of cecal bacteria from healthy feral chickens and identified Megasphaera stantonii with a strong ability to inhibit C. perfringens, while also enhancing innate immunity through the induction of host defense peptide synthesis. Notably, oral administration of M. stantonii to day-of-hatch broilers improved the survival rate to 98% in a chicken model of NE, while 52% of chickens died without intervention ($P < 0.05$). Moreover, M. stantonii significantly alleviated the severity of intestinal lesions ($P < 0.05$).

Conclusions: These findings underscore the crucial role of intestinal microbiota in conferring NE resistance and highlight the potential of exploiting commensal bacteria for NE mitigation in poultry.

Financial Support: This research was funded by the USDA-NIFA AFRI grant # 2022-67016-37208, 2024-67016-42415, and 2024-67011-42944), the Ralph F. and Leila W. Boulware Endowment Fund, and Oklahoma Agricultural Experiment Station Project H-3112.

Notes:

P139 - Does the pooling of raw samples or DNA provide good representation of the microbial communities of the upper respiratory tract of cattle?

Valeria Lugo-Mesa[1], Cory Wolfe[1], Molly McClurg[1], Lee J. Pinnell[1], Paul S. Morley[1]

[1]Veterinary Education, Research, and Outreach (VERO) Program, Texas A&M University. valerialugo@tamu.edu

Session: Microbiome, 2025-01-20, 6:00 - 8:00

Objective: The microbial communities of the respiratory tract play a critical role in the development of bovine respiratory disease (BRD), which remains one of the costliest diseases facing the cattle industry, impacting both animal welfare and economic outcomes. Sequencing-based studies can effectively characterize this microbial population, but to do so for individual animals within a large sampling cohort is both time-consuming and expensive. Sample pooling represents an effective time and cost-reducing strategy for investigating microbial communities from large sample cohorts. Here, we evaluated if the pooling of raw samples and/or pooled DNA accurately represents the respiratory microbial populations from individual cattle.

Methods: Upper respiratory tract samples were collected from feedlot cattle using a sterile, 4" cotton-tipped swab. To create raw sample pools, 4 or 5 different individual swabs were aseptically excised and combined for a total of 10 pools. DNA was isolated using the DNeasy PowerFecal Pro kit (QIAGEN). Isolated DNA from 5 different individual swabs was combined in equal proportion to create 10 DNA pools. The V3-V4 region of the 16S rRNA gene was amplified and sequenced on an Illumina NovaSeq 6000 platform. In an attempt to account for rare features in individual samples, pooled samples were sequenced at a ten-fold increase in targeted sequencing depth. Amplicon sequence variants (ASVs) were generated with DADA2 in QIIME2 and classified based on the SILVA 138.1 database. All data analyses were carried out in R version 4.3.2, utilizing the phyloseq, microbiome, btools, metagMisc, ANCOMBC, UpSetR, and vegan packages to analyze, visualize, and compare microbial diversity and composition between individual and pooled samples.

Results: Due to increased sequencing depth, both pooled sample types (raw and DNA pools) exhibited greater richness than individual samples. However, both pooled sample types showed similar evenness and diversity compared to individual samples. The majority (93.3%) of ASVs within individual samples were also found in both pooled sample types. Of the relatively small number of ASVs unique to individual samples, nearly all (94.51%) had a mean relative abundance of 0.001% or lower and were present in fewer than 5% of the samples. Observed variations in community composition between pooled and individual samples were primarily driven by differences in rare taxa. No ASVs classified at the rank of genus were identified as differentially abundant between pooled sample types and individual samples using ANCOMBC.

Conclusions: Pooled samples accurately captured most of the microbial diversity and composition in the respiratory tract of cattle. However, pooled samples may be less suitable for analyzing the rarest of microbial taxa within these populations. Both swab and DNA pooling can offer cost-effective solutions for most studies of respiratory microbial communities, but the suitability of pooling depends somewhat on the specific objectives of the research question.

Notes:

P140 - Development of baits and a variant classification system for target-enriched metagenomic mapping of prevalent liver abscess taxa in the bovine gut

Kayla H. Hazlett[1], Enrique Doster[1], Robert Valeris-Chacin[1], Matthew A. Scott[1], Paul S. Morley[1], Lee J. Pinnell[1]

[1]VERO Program, Texas A&M University. kayla.hazlett@tamu.edu

Session: Microbiome, 2025-01-20, 6:00 - 8:00

Objective: Liver abscesses (LAs) are a prevalent and costly challenge in the North American beef industry. Traditionally, the pathogenesis of LAs is attributed to the translocation of Fusobacterium from the rumen to the liver. However, emerging evidence suggests that LAs are highly polymicrobial, potentially originating from both the rumen and the hindgut. While 16S rRNA amplicon sequencing is commonly used to characterize the microbiome of LAs and the bovine gut, it has limitations in detecting and quantifying prevalent LA taxa in the gut due to their low abundance and classification beyond the genus level is limited. Therefore, the objective of this study is to develop a target-enriched metagenomic workflow that will allow for the strain-level quantification of prevalent LA taxa in the gut and allow for strain-level taxonomic classification.

Methods: Baits were designed for 4 genera of interest that are prevalent in LAs (Fusobacterium, Trueperella, Bacteroides, and Porphyromonas). First, fastANI was run on every representative genome in NCBI's RefSeq for each genus to compute whole-genome ANI (average nucleotide identity) and determine genetic similarity between genomes (with >97% ANI being considered a species). Using R version 4.3.2, 'pheatmap' was used to generate annotated heatmaps for each genera of interest. The heatmaps were then analyzed to identify genomic clusters represented by a reference genome. Clusters lacking representation were included in the resulting bait design to ensure comprehensive coverage of genetic variation within the genus, while redundant references for the same cluster were removed.

Results: Based on ANI, we designed baits for Trueperella around 7 lineages, despite the genus containing only 6 defined species. Porphyromonas had 56 unique lineages, resulting from a large number of lineages not represented by the 19 reference genomes. Baits for Fusobacterium were designed around 24 lineages, adding 5 additional lineages to the 19 NCBI representative genomes. Bacteroides is the largest genera of interest (>14,000 genomes). Due to the complexity of this large genera and the large number of distinct lineages, we opted to design the baits around the 56 representative genomes provided by NCBI.

Conclusions: We have shown that TE metagenomics massively enriches for strains in taxa of interest, and we believe its use here and the development of these baits and classification system will let us quantify the microbes prevalent in LAs and identify their probable source in the bovine gut. This will establish the most powerful tool to date for investigating strain-level populations of LA microbial taxa without the need for culture. Ultimately, this project will provide a better understanding of the pathogenesis of LAs, aiding researchers in the development of new LA prevention strategies, reducing the use of anti-microbial drugs, and resulting in more efficient, healthier cattle.

Financial Support: This research was funded by Texas A&M Agrilife Research, Animal Health and Disease Research Capacity Funding FY 24-25.

Notes:

P141 - Resistome-enriched sequencing of pooled fecal samples from commingled and non-commingled pre-weaned piglets

Jared G. Young[1], Tara N. Gaire[1], Gerardo R. Diaz[1], Claire Mitchell[1], Serena May[1], Mark Schwartz[1, 2], Randall Singer[3], Maria Pieters[1], Noelle R. Noyes[1]

[1]Department of Veterinary Population Medicine, University of Minnesota, [2]Schwartz Farms Inc, Sleepy Eye, Minnesota, [3]Department of Veterinary and Biomedical Sciences, University of Minnesota. youn2635@umn.edu

Session: Microbiome, 2025-01-20, 6:00 - 8:00

Objective: Antimicrobial resistance (AMR) represents a growing global health threat that decreases or negates the efficacy of antibiotics to treat bacterial disease processes. AMR is encoded by antimicrobial resistance genes (ARGs) which are housed in bacteria across the microbiome, termed the resistome. The resistome is dynamic and may be influenced by many factors, including antimicrobial exposure and production practices such as commingling. In swine production, commingling occurs when pigs from different litters are mixed in a common environment. While this is a common practice in swine production, its effects on the resistome are poorly understood. To investigate the effects of commingling on fecal resistome richness and composition in the context of antimicrobial drug exposure, ARG-enriched shotgun metagenomic sequencing was performed on pooled fecal samples from commingled and non-commingled pre-weaned piglets treated with antibiotics shortly after birth and before weaning in a randomized controlled trial study.

Methods: At birth, 833 piglets were enrolled into the study involving 84 litters (up to 10 piglets per litter) and 2 rooms within a single sow facility, and randomly assigned to either the commingled or non-commingled treatment group. Commingled piglets were housed with sows and piglets from other litters, while non-commingled piglets were housed only with their sow and littermates. All piglets received ceftiofur I.M. at enrollment and enrofloxacin when creep feed was introduced. Rectal fecal samples were collected from all piglets at the following timepoints: 9 and 15 days of age, one day following creep feed introduction, and immediately before weaning. All samples (N=1214) were subjected to total DNA extraction, and the DNA was then pooled by sow and time point to generate 144 pooled samples for resistome analysis using bait enrichment for ARGs, followed by metagenomic sequencing. Deduplicated resistome data was generated from sequenced samples using AMRplusplus v3, and statistical analysis was performed in R using the phyloseq, mgcv, and vegan packages.

Results: Across all samples, 2870 unique ARGs were identified, and ARGs conferring resistance to tetracycline, aminoglycoside, and betalactam antibiotics were most prevalent. For both commingled and non-commingled treatment groups, mean richness was highest at time point one (commingled = 1744 ARGs, non-commingled = 1676 ARGs) and decreased consecutively at each timepoint, reaching the lowest mean richness at time point four (commingled = 1367 ARGs, non-commingled = 1444 ARGs). A generalized additive model was fit with richness as the response variable, and differences in richness associated with treatment were not statistically significant ($p = 0.507$) while differences in richness associated with time point were ($p < 0.001$). A PERMANOVA model was fit with Bray-Curtis dissimilarity as the response variable, and differences in dissimilarity associated with the treatment group were not statistically significant ($p = 0.304$), while differences associated with time point were ($p < 0.001$).

Conclusions: Resistome richness and Bray-Curtis dissimilarity were similar between commingled and non-commingled piglets, and statistically significant differences in these metrics were associated with the sampling time point. Further resistome analysis must be performed at the gene level to elucidate specific compositional differences, if any, associated with commingling.

Financial Support: This work was supported, in part, by Agriculture and Food Research Initiative grant no. 2019-67017-29110 and grant no. 2021-68015-33499 from the USDA National Institute of Food and Agriculture.

Notes:

P142 - Fecal microbiome of horses under different housing environments in West Texas

Md.Kaisar Rahman[1], Yamima Tasnim[1], Luis Morales[1], Mohamed Fokar[1], Chiquito Crasto[1], Babafela Awosile[1]

[1]Texas Tech University School of Veterinary Medicine. babafela.awosile@ttu.edu

Session: Microbiome, 2025-01-20, 6:00 - 8:00

Objective: Several factors including host attributes have been reported to influence the microbiome of horses. However, there is limited information on housing and other environmental factors influencing the microbiome of horses in general. Therefore, this study aimed to describe and evaluate the effect of sex, breed, and different housing environments on the fecal microbial communities of healthy horses in West Texas.

Methods: Freshly voided fecal samples were collected from 70 healthy horses from six locations in West Texas including rescue center, equine center, farm, veterinary clinics, boarding stable, and household. Information on the sex (mare/filly, gelding, and stallion/colt), breed (paint, quarter, stock type), work type (breeding/stud, leisure, ranch horse, and others), premises where horses are kept (stud/breeding farm, full livery yard, field/pasture only, and private yard), and whether the horses were kept on the grass or stabled. After DNA extraction, 16S rRNA sequencing was performed using the Illumina Miseq platform to determine the fecal microbiota composition. The effects of host attributes and housing factors on microbiota composition were determined using statistical analytic methods including alpha and beta diversity, PERMANOVA, and simple percentage analyses (SIMPER).

Results: Proteobacteria (51.39%), Bacteroidetes (26.73%), Firmicutes (17.45%), and Actinobacteria (4.08%) were the predominant phyla among the horses. While Microbacter (12.46%), Acinetobacter (6.85%), and Pseudomonas (6.25%) were the predominant genera among the horses. There was a significant difference in the relative abundance of Actinobacteria between the locations, breeds, sex, premises, work types, and whether the horses were kept on the grass or stabled. Also, there was a significant difference in the relative abundance of Bacteroidetes between the locations and Chloroflexi between the breeds. No significant difference in alpha diversity existed between the locations, breed, sex, premises, work types, and whether the horses were kept on the grass or stabled. However, there was a significant difference in beta diversity between the location ($p<0.001$), breed ($p<0.001$), premises ($p<0.001$), and whether the horses were kept on the grass or stabled ($p=0.036$). Using SIMPER, 21 amplicon sequence variants (ASVs) drove the microbial community's differential abundance between the locations, 6 ASVs between the breeds, and 1 ASV between the premises. In a multivariable PERMANOVA, breed, premises, and whether the horses were kept on the grass or stabled were statistically associated with the fecal microbial diversity of horses. A significant interaction between work types and locations was also observed ($p=0.006$). These factors explained 61% of the variation in fecal microbial diversity among the horses. Information on diet was not available, therefore, a limitation of this study.

Conclusions: The results from this study indicated that the fecal microbiome of horses is influenced by both intrinsic and extrinsic factors. This information should be considered in the disease management of horses especially gut dysbiosis and other metabolic disorders.

Notes:

P143 - A comparison of temporal variation in bacterial diversity in the respiratory tracts of dairy calves receiving milk replacer alone or containing probiotics

Carol G. Chitko-McKown1, Jia W. Tan1, Susan D. Eicher[1], Janice E. Kritchevsky[2], Keith A. Bryan[3], Aaron Dickey[1], Tara G. McDaneld[1]

[1]USDA-ARS, [2]Purdue University College of Veterinary Medicine, [3]Novonesis. Carol.ChitkoMcKown@usda.gov

Session: Microbiome, 2025-01-20, 6:00 - 8:00

Objective: To characterize the bacterial populations along multiples sites of the bovine respiratory tract over time in dairy calves fed control milk replacer or milk replacer containing probiotics.

Methods: Dairy calves were fed either control milk replacer (N=10) or milk replacer with probiotics (N=10) from birth to weaning, and swabs were obtained from the nares and tonsils over 9 sampling times. Lung lavage fluids were obtained from five animals in each group on day 52. DNA was extracted, and 16S ribosomal gene hypervariable regions 1-3 were sequenced.

Results: Temporal variation in alpha bacterial diversity was observed within the nostril, tonsil, and lung lavage samples, which indicated distinct bacterial compositions among sampling time points. The three respiratory tract regions studied showed spatial variability in bacterial taxa composition, however, oral probiotic treatment did not change alpha diversity in any respiratory tissue. Differentially abundant taxa were unique to anatomical location in treated calves, however a few were common to two locations and Finegoldia was differentially abundant in all three locations.

Conclusions: The results show the potential effects of probiotics on the bovine respiratory tract microbiome and contribute to the understanding of the dynamic nature of bacterial diversity in one system.

Financial Support: USDA-ARS 3040-32000-036-000D; USDA-ARS 3040-31000-104-000D.

Notes:

P144 - Genetic analysis of whole genome and plasmid sequences show spillover of antibiotic resistance genes in ESBL-producing Enterobacteriaceae in human and dog populations

Charles Whitehead-Tillery[1], Linda S. Mansfield[1]

[1]Michigan State University. white174@msu.edu

Session: One health / Public health, 2025-01-20, 6:00 - 8:00

Objective: Extended-spectrum β-lactamase (ESBL) producing *Enterobacteriaceae* were recently shown to contribute significantly to infections in diverse healthcare and community settings including urinary tract diseases. This study assessed the distribution of ESBLs, antibiotic-resistant genes (ARGs), and other genetic traits of ESBL-producing *E. coli* from dogs, aiming to establish their genetic relationships with those observed in human cases.

Methods: We utilized *in vitro* conjugation assays to test plasmid transfer function, using 16 Uropathogenic *E. coli* (UPEC) isolates from humans as donors and *E. coli* MG1655 as the recipient. Cefotaxime (4μg/ml) and rifampicin (20μg/ml) served as antibiotic selective markers. Then we performed DNA extractions on these UPEC strains and employed Nanopore Technology to obtain plasmid sequences. Next, we collected 154 plasmid sequences from ESBL-producing isolates and 114 sets of Whole Genome Sequencing (WGS) data. Plasmid sequences were genetically analyzed using the Bacterial and Viral Bioinformatic Resource Center (BV-BRC), Comparative Antibiotic Resistance Database (CARD), plasmid Multi-Locus Sequence Typing (pMLST), and the National Center for Biotechnology Information (NCBI). WGS data was analyzed using BV-BRC, Center for Genomic Epidemiology (CGE), and Multi-Locus Sequence Typing (MLST).

Results: We categorized 105 (73%) of the 157 plasmids obtained from human (N = 133) and dog (N = 24) populations as Class A ESBLs. Specifically, CTX-M-14 and TEM-1 predominated in the human population, while CTX-M-1, 8 (33%), and TEM-1, 7 (29%) variants predominated in the dog population. Furthermore, we observed a significant presence of Class C ESBLs, notably CMY-2, in the human population 12 (54%) and the dog population 5 (21%)

Conclusions: We concluded that ESBL-producing *Enterobacteriaceae*, particularly *E. coli*, predominated in both human and dog populations. Class A ESBLs; CTX-M-14 and TEM-1, predominated in humans, while CTX-M-1, and TEM-1 predominated in dog populations. This comprehensive genomic exploration provides insights into the genetic landscape of ESBL-producing *Enterobacteriaceae* in humans and dogs, offering valuable perspectives on potential cross-species transmission and implications for public health.

Financial Support: These studies were funded with funds from the Albert C. and Lois E. Dehn Chair Endowment and a University Distinguished Professor Endowment to Linda S. Mansfield.

Notes:

P145 - Pastoralists' perceptions of climate change and copping strategies in the Borana Range lands of Southern Ethiopia

M Bayssa[1], S Betsha[1], S Yigrem[1], D Areda[2]

[1]Hawassa University, [2]Ottawa University

M.Bayssa[1], S. Betsha[1], S. Yigrem[1], A. Tolera[1], D. Areda[2]

[1]Hawassa University College of Agriculture, Ethiopia, [2]Department of Applied Sciences, Ottawa University. demelash.biffa@gmail.com

Session: One health / Public health, 2025-01-20, 6:00 - 8:00

Objective: This study explored Boran pastoralists' perceptions of climate change and its impact on Boran cattle populations, alongside the adaptation strategies of pastoral and agro-pastoral communities in the Borana rangelands of Southern Ethiopia.

Methods: Data were collected from a household survey in five districts, encompassing 365 households across 25 villages. Secondary data on climate variability were obtained from the National Meteorological Agency, while additional data came from local livestock offices and the Central Statistics Agency. Socio-economic and perception data were analyzed using correlation and regression analysis, and meteorological data were assessed with the Standardized Precipitation Index and Mann-Kendall test.

Results: The findings revealed that most respondents (83.9%) perceived low and erratic rainfall, increased temperatures, and frequent droughts (87.5% and 74.3%, respectively), as cause leading to deteriorating feed and water resources. Thirty years of meteorological data showed a decline in Boran cattle due to climate change, equating to 1,857 Tropical Livestock Units (TLU) for a 1 mm decrease in mean annual rainfall and 13,550 TLU for a 1°C increase in mean annual maximum temperature. The average decline in Boran cattle was estimated at 686,857 TLU over the last 30 years. In response to climate change, Borana communities employed strategies such as traditional grazing management, income and livestock species diversification, herd splitting, strategic destocking, and seasonal herd mobility.

Conclusions: the Boran pastoralists of Southern Ethiopia have observed a significant impact of climate change. These include reduced quantity of rainfall, rising temperatures, and more frequent droughts, which have led to a significant decline in cattle population in the study area. To mitigate this, the communities have implemented various strategies such as traditional grazing management, livestock diversification, and seasonal herd mobility to mitigate the adverse effects of climate change on their livelihoods.

Financial Support: This study was supported by the graduate program funding from Hawassa University, Ethiopia.

Notes:

P146 - Camels as biosurveillance sentinels: Risk at the human-camel Interface

Takhmina Argimbayeva[1], Dolyce Low[2], Liesbeth Frias[2], Kairat Tabynov[3], Lena Ch'ng[2], Sophie Borthwick[2], Maruf Kosumbekov[4], Ian Mendenhall[2], Yvonne Su[2], Alan Hitch[5], Zamira Omarova[1], Aslan Kerimbayev[1], Aziz Nakhanov[6], Shuhrat Jumaev[7], Mukhit Orynbayev[1], Gavin Smith[2, 8]

[1]Laboratory for Monitoring of Infectious Diseases, Research Institute for Biological Safety Problems, Republic of Kazakhstan, [2]Programme in Emerging Infectious Diseases, Duke-NUS Medical School, Singapore, [3]International Center for Vaccinology, Kazakh National Agrarian Research University, Republic of Kazakhstan, [4]Laboratory for Prevention of Foot and Mouth Disease and Animal Leukosis, Institute of Biosafety Problems and Biotechnology, Tajik Academy of Agricultural Sciences, Tajikistan, [5]Museum of Wildlife and Fish Biology, University of California, Davis, USA, [6]Laboratory for Cell Biotechnology, Research Institute for Biological Safety Problems, Republic of Kazakhstan, [7]Laboratory for Monitoring Infectious Diseases of Small Ruminants, Institute of Biosafety Problems and Biotechnology, Tajik Academy of Agricultural Sciences, Tajikistan, [8]Centre for Outbreak Preparedness, Duke-NUS Medical School, Singapore. kairat.tabynov@gmail.com

Session: One health / Public health, 2025-01-20, 6:00 - 8:00

Objective: Camels are an integral part of the economy, subsistence, and agricultural practices in many countries in Africa and Asia. In Kazakhstan, the large camel population is a valuable source of transport, clothing material, milk, and meat. Camels have also been identified as reservoirs for many zoonotic and economically important biological agents, such as Middle East Respiratory Syndrome Coronavirus (MERS-CoV), Crimean Congo Haemorrhagic Fever Virus (CCHFV), Tick-Borne Encephalitis Virus (TBEV), and West Nile Virus (WNV). This study aims to conduct serological surveillance of these viruses in Kazakhstan's camel population and develop probabilistic risk maps to inform surveillance efforts.

Methods: We will analyze 7,000 banked camel sera collected from seven oblasts in Kazakhstan using a Multiplex Microsphere Immunoassay against the antigenic proteins of the mentioned livestock pathogens. Seropositivity cutoffs for each protein will be determined using an expectation-maximization algorithm to sort output mean fluorescence intensity (MFI) values into seropositive and seronegative clusters. The study will investigate the correlation between seropositivity towards these agents against multiple independent variables such as habitat type, farm size, and geographic region using Bayesian multilevel logistic regression models.

Results: Preliminary findings from 46 camel sera indicate variable exposure levels to CCHF nucleoprotein (19.6%, n=9/46) and MERS-CoV spike protein (13.0%, n=6/46) from three of the seven tested oblasts. Results will be presented in histogram and jitter plot to present serological cutoffs clusters and individual data points with cutoffs for each viral protein. Summarized results detailing seroprevalence towards viral proteins and a risk map for geographical hotspots will be presented.

Conclusions: The serological surveillance will provide a historical overview of infections among camels in Kazakhstan, enabling the identification of high-risk areas for these agents. This information will inform the selection of field surveillance sites and strengthen Kazakhstan’s response to infectious disease outbreaks by pinpointing high-risk interfaces for spillover and guiding future surveillance efforts.

Financial Support: This work is funded by the US Defense Threat Reduction Agency (DTRA), Grant #HDTRA1-21-1-0035.

Notes:

P147 - How does companionship loss impact cognitive decline in aging dogs?

Courtney Sexton[1], Kathryn Shannon[1], Audrey Ruple[1]

[1]Population Health Sciences, Virginia Tech. sextonc@vt.edu

Session: One health / Public health, 2025-01-20, 6:00 - 8:00

Objective: Although nearly half of households in the United States are home to companion dogs - many of whom are considered members of the family - little is understood about how changes to the social fabric in these households affect dogs' welfare. While many anecdotal reports indicate other animals experience grief and forms of depression, the effects of loss of social partners have not been quantified in dogs, despite known implications of such experiences for both physical and mental health in humans. Our objective in this study is to validate anecdotal evidence of grief in dogs by examining how reported permanent loss of social companionship within the familial social structure relates to cognitive changes in aging dogs enrolled in the Dog Aging Project (DAP), a large-scale longitudinal study.

Methods: All DAP participants complete a series of surveys, HLES, at the time of project enrollment and annually update information about their dog in the DAP Annual Follow-up Survey (AFUS). Using data collected from 2019 to 2023, we evaluated narratives from owner participants who reported what they perceived to be grief in their dogs as a response to loss-related changes in family structure in free-response. These preliminary data inform the content of an interview guide, to be used in conducting 15 qualitative interviews with participants from the preliminary pool who indicated a change to their household social structure. Based on issues, questions, and themes that emerge from this analysis, we will develop a dog grief and loss survey instrument to deploy to a larger, stratified study population of DAP dog owners. These data will be used to evaluate the presence and emergence of reported cognitive and physical conditions together with owner reports of permanent changes in dogs' household social structure to investigate whether such changes are associated with health outcomes.

Results: Our preliminary data show that of those owners who reported symptoms identified with grief and loss in their dogs, 93% also reported concurrent physical and/or behavioral changes in the dog (e.g., sundowning, increased sleeping, repetitive behaviors, attention-seeking, anxiety, etc.), with 90% attributing these changes directly to loss/grief. For those who reported family additions, nearly half of changes reported were considered positive. Changes categorized by owners as behavioral were reported more often than physical. With these outcomes in mind, we hypothesize that owner-indicated loss of companionship for their dog will positively correlate with recorded symptoms associated with degraded dog cognitive health in the larger study population.

Conclusions: As peoples' relationships with dogs continue to become more and more complex, it is crucial that as caretakers we understand how experiences of companionship loss may impact physical and cognitive changes in dogs, especially as they age. As our preliminary data indicate, this study has the potential to provide evidence-based insight into how dogs' health is impacted by such experiences. This is important not only from a quality of life perspective, but also in terms of understanding clinical presentations of grief and loss, and designing and delivering appropriate veterinary care and interventions.

Notes:

P148 - Q fever passive surveillance in Georgia 2019-2023 overview

Lena Ninidze[1], Natia Kartskhia[1], Ioseb Menteshashvili[1], Tengiz Chaligava[1], Marina Donduashvili[1]

[1]National Food Agency Ministry of Environmental Protection and Agriculture of Georgia. lenaninidze@gmail.com

Session: One health / Public health, 2025-01-20, 6:00 - 8:00

Objective: Q fever (or Coxiellosis) is a zoonosis caused by Coxiella burnetii that occurs in most countries. Humans generally acquire infection through air-borne transmission from animal reservoirs, especially from domestic ruminants, but other domestic and wildlife animals (pets, rabbits, birds, etc.) can be involved. It can be found in animal blood, feces, urine, milk, and tissue. This disease can cause economic losses through spontaneous abortion: Metritis, infertility, and increased mortality among young animals. In humans, the disease exhibits a large polymorphism. Q fever occurs either as an acute form or a severe chronic form following an early infection that may go unnoticed.

Methods: Since 2019, the NFA has undertaken passive surveillance of Q fever in Georgia. This surveillance relies on notifications from state and private veterinarians, public health specialists, farmers, associations, the SLA, and the National Center for Disease Control and Public Health (NCDC). When human cases are reported by the NCDC, in the household animals undergo testing within the epidemiological unit. In instances where animals are suspected of having issues such as abortion or reproductive problems, they are first tested for brucellosis (abortus, melitensis); those found negative for brucellosis are subsequently tested for Q fever it should be underlined that based on our study all Q fever positive animals were negative on Bruceloses. Samples, carefully collected by state veterinarians in accordance with biosecurity protocols, are then submitted to the SLA. In the laboratory, these samples are analyzed using accredited methods: ELISA for initial screening, IFA for confirmation, and PCR for further validation. All data is meticulously recorded in the EIDSS system.

Results: Last years, as part of the passive surveillance program, blood samples were collected from eighteen municipalities. A total of 294 samples (85 from cattle and 209 from small ruminants) were tested using serological methods (ELISA). Of these, 61 samples (7 from cattle and 54 from small ruminants) tested positive by IFA. According to the passive surveillance data, the prevalence of Q fever was 8.24% in large ruminants and 25.84% in small ruminants. During the same period, 59 human cases were reported in Georgia, with 15 confirmed and 44 suspected cases. However, no direct link was found between the human and animal cases.

Conclusions: The primary reason for the high prevalence is that the studies focused on samples taken from animals suspected of having the disease. However, it is notable that over the past five years, cases of Q fever have increased in both humans and animals. To prevent the spread of Q fever, health and veterinary services must collaborate at both national and local levels. Enhanced surveillance systems can help detect positive animals that do not exhibit clinical signs, especially when there is information about Q fever in humans. Additionally, raising public awareness is essential, encouraging people to avoid consuming unpasteurized dairy products and to report, rather than conceal, animals showing clinical symptoms.

Financial Support: Government of Georgia

Notes:

P149 - High Crimean-Congo Hemorrhagic Fever seroprevalence in cattle indicating possible long-term transmission in areas with recent identification of human cases in Georgia 2023

Tornike Khargaladze[1], Levan Liluashvili[2], Tengiz Chaligava[1], Lia Bekauri[1]

[1]LEPL National Food Agency of Georgia, [2]National Center for Disease Control and Public Health of Georgia. tornike.khargaladze@nfa.gov.ge

Session: One health / Public health, 2025-01-20, 6:00 - 8:00

Objective: In 2022, Georgia detected its largest known Crimean-Congo hemorrhagic fever (CCHF) outbreak among humans (49 cases) since 2009, primarily in a region with widespread livestock production. We conducted a survey to estimate CCHF virus (CCHFV) seroprevalence among cattle in villages with human cases identified for the first time in 2022 and to detect previously unknown CCHFV circulation among livestock.

Methods: We tested cattle blood for anti-CCHFV IgG antibodies using double-antigen ELISA from 14 villages across three districts. We calculated a sample size of 194 cattle from 97 households using a 95% confidence interval, 80% expected prevalence, and 6% margin of error. We created age groups for cattle (1-3 years, 4-7 years, ≥8 years) and conducted a chi-square test for trend to assess a linear trend. We used EpiInfo to analyze data.

Results: Among 194 cattle included in the study anti-CCHV IgG seroprevalence was 84% (163/194; range=40-100%). Seropositive cattle were identified in all villages. Seropositivity differed by age groups: 18 (45%) among 1-3 years old, 75 (91%) among 4-7 years old, and 57 (98%) among ≥8 years old. Compared with 1-3 years old animals, we found an odds ratio (OR) of 69.7 for ≥ 8 years and OR=13.1 for 4-7 years. The chi-square test for the linear trend between seroprevalence and age groups was 42.87 (p-value <0.001).

Conclusions: We identified high CCHFV seropositivity in cattle from the selected villages. Older animals (≥ 8 years) had a higher seroprevalence than younger animals. This may suggest that in addition to recent circulation evidenced by seropositivity in animals 1-3 years old, CCHFV may have been circulating undetected in these villages for some time. These results highlight the need for increased CCHF surveillance, awareness, and prevention efforts, including tick control, to better understand CCHF in Georgia.

Financial Support: Government of Georgia

P150 - Development of a One Health informatics framework in Ontario using a stakeholder driven approach

H. Davies[1], L. Grant[1]

[1]Department of Population Medicine, University of Guelph. hdavie03@uoguelph.ca

Session: One health / Public health, 2025-01-20, 6:00 - 8:00

Objective: The overall aim of this project is to use a stakeholder driven approach to develop a One Health informatics framework for an integrated surveillance system that can monitor companion animal zoonoses in Ontario. The specific objectives are to identify and engage relevant stakeholders, convene an advisory group of key stakeholders, and use collective visioning to characterize important features of the proposed system and barriers to its successful implementation.

Methods: A stakeholder identification exercise was completed to identify and broadly group stakeholders relevant to a One Health informatics system in Ontario. Stakeholders were identified in government, academia, the private sector, and other organizations such as charities and not-for-profit groups. We then engaged stakeholders using a mix of existing connections and by identifying contact information online. Stakeholders were provided with a brief overview of the project and invited to form a provincial advisory committee to guide framework development. The first provincial advisory committee meeting was held in March 2024 in hybrid format. Participants were asked to share their vision for a One Health informatics system in Ontario in round-robin format. Using the same format, key barriers were also discussed. From these discussions, a visioning document was created.

Results: The provincial advisory committee consists of experts from government, academia, and private industry (i.e., diagnostic laboratories and corporate veterinary groups). Thoughts shared by participants were grouped into 7 areas describing operational features of the system including important elements which should be clearly defined, overall system design, data collection processes and how data should be used. Further, the vision includes key details on how to encourage participation, ensure long-term sustainability, and foster trust.

Conclusion: Creation of any One Health system requires careful balancing of the interests and motivations of actors from different sectors. A stakeholder-led approach to framework development ensures that both shared and sector-specific issues are discussed and addressed during the design phase. The visioning document described here captures an initial vision for a One Health informatics system in Ontario and will be used as the basis for further discussions. Specifically, the document has been used as an initial prompt during semi-structured interviews undertaken with members of the provincial advisory committee. These interviews provided participants with the opportunity to discuss their thoughts in more detail, and the results will be used to update the visioning document as appropriate. Interviews also sought to capture which barriers participants perceive as the most important, and the steps that could be taken to overcome these barriers. Interviews will be analyzed thematically, and results will be available in due course.

Financial Support: This project is funded by the Public Health Agency of Canada (PHAC) Infectious Disease and Climate Change fund (Grant Number 2324-HQ-000026).

Notes:

P151 - Mapping anthrax affected areas and distribution ranges of wildlife in the South Caucasus

Ioseb Natradze[1], Vakhtang Martashvili[2], Tengiz Chaligava[3], Irma Burjanadze[2], Tamar Chichinadze[4], Lilit Avetisyan[5], Astghik Pepoyan[6], Nana Bolashvili[4], Elshad Askerov[7], Alexander Bukhnikashvili[1], Lile Malania[2]

[1]Institute of Zoology of Ilia State University, [2]National Center for Disease Control and Public Health, Georgia [3]National Food Agency, [4]Institute of Geography of Tbilisi State University, [5]National Center for Disease Control and Prevention, Armenia, [6]Armenian National Agrarian University, [7]Institute of Zoology, Azerbaijan. malanial@yahoo.com

Session: One health / Public health, 2025-01-20, 6:00 - 8:00

Objective: Anthrax, a zoonotic disease caused by the bacterium *Bacillus anthracis*, produces spores that can remain viable in the soil for decades. This disease affects domestic and wild animals worldwide, such as cattle, sheep, goats, chamois, deer and other ungulates. Animals typically become infected by ingesting contaminated plants, water, or soil. To assess the potential risk of anthrax spread among wild ungulates in the South Caucasus region, we mapped anthrax cases and positive soil samples alongside the distribution ranges of ungulate species.

Methods: Our analysis encompassed 5,607 anthrax-positive records, among which 56,43% are soil-positive samples and 43,57% domesticated animal cases; Distribution by country: Georgia - 1427 positive soil samples and 624 animal cases; Armenia - 144 positive soil samples and 100 animal cases and Azerbaijan - 1593 positive soil samples and 1719 animal cases from 1948 to 2022. We evaluated over 30 geographical variables such as climate, topography, and soil, creating various digital thematic maps. The distribution range maps of all nine ungulate species were created using models of the species occurrence probability based on 1,390 records' points reported from 1876 through 2022 for the South Caucasus countries.

Results: Our findings reveal significant overlap between anthrax-affected areas and ungulate distribution ranges. In regions with active anthrax foci, including parts of Georgia (north, west, and east) and Azerbaijan and Armenia (north and south), there is a heightened risk of disease transmission through herbivores grazing in contaminated areas.

Conclusions: The management of infectious diseases in wildlife and livestock presents significant challenges, potentially leading to large die-offs, economic losses, and human health risks. Given the increased susceptibility of ungulates to anthrax and the potential for disease spread, it is crucial for relevant stakeholders in the South Caucasus to address these issues. Currently, surveillance efforts are primarily focused on farm animals and humans, leaving a gap in the study and monitoring of anthrax in wildlife.

Financial Support: The research study described in this presentation was made possible by financial support provided by the US Defense Threat Reduction Agency in the frame of Grant HDTRA11910044 - "Preparation of the atlas of zoonotic infections in South Caucasus".

Notes:

P152 - Pharmacological effects of D-3-O methylchiroinositol isolated from stem bark of *Piliostigma thonningii* in diabetic mice

Chinaka O Nwaehujor[1], Edwin A Uwagie-Ero[2], Onyedikachi A Adika[3]

[1]Department of Biochemistry, University of Calabar., [2]Faculty of Veterinary Medicine, University of Benin., [3]Department of Animal Production and Health, Federal University Oye-Ekiti. edwin.uwagie-ero@uniben.edu

Session: One health / Public health, 2025-01-20, 6:00 - 8:00

Objective: Based on the use of *Piliostigma thonningii* in African traditional medicine in the management of pain, inflammation and fever as well as the anti-inflammatory effect of its crude extract, the study evaluated D-3 O-methylchiroinositol extracted from *P. thonningii* stem back for analgesic, anti-inflammatory and antipyretic activities in diabetes using diabetic mice as a model.

Methods: Diabetes was induced with 35 mg/kg of streptozotocin, *i.p*. D-3-O methylchiroinositol was screened for analgesic activity by the acetic acid-induced writhing and tail immersion tests. Anti-inflammatory activities were evaluated by the carrageenan-induced paw edema and Freund adjuvant-induced arthritis tests while anti-pyretic activity was determined using yeast-induced pyrexia test. Standard drugs used were diclofenac, pentazocine and acetylsalicylic acid (ASA).

Results: D-3-O methylchiroinositol (10, 30 and 60 mg/kg) significantly ($P< 0.01$) reduced the number of acetic acids induced writhing. At 15, 30, 45- and 60-min post treatment, tail withdrawal times in D-3-O-methylchiroinositol (30 and 60 mg/kg) were significantly ($P<0.01$) longer compared to reaction times recorded in control group. Sub-acute phase of arthritis (18 h) paw volumes in D-3-O methylchiroinositol (30 and 60 mg/kg) were significantly ($P<0.05$ and $P<0.01$ respectively) lower compared to paw volume in the control group. Chronic phase (30 days) of arthritis, paw volumes in the D-3-O-methylchiroinositol (10, 30 and 60 mg/kg) were significantly ($P<0.01$) lower than paw volume of the control group. Rectal temperatures of the 60 mg/kg D-3-O-methylchiroinositol were significantly lower at 1, 2, 3, 4 and 5 h post-yeast injection compared to those of the control.

Conclusions: The findings of this study showed that D-3-O-methylchiroinositol has analgesic, anti-inflammatory and anti-pyretic potentials in diabetes.

Notes:

P153 - Prevalence of the *Coxiella burnetii* in beef cattle surveyed by real-time PCR and ELISA testing

Eric Cheng[1], Stephen Waldron[2], Cori Ondrashek[2], Ellyn Mulcahy[1], Roman M. Pogranichniy[1, 2], Gregg Hanzlicek[1, 2], Jianfa Bai[1, 2]

[1]Diagnostic Medicine/Pathobiology, Kansas State University, [2]Kansas State Veterinary Diagnostic Lab, Kansas State University. eacheng@ksu.edu

Session: One health / Public health, 2025-01-20, 6:00 - 8:00

Objective: Coxiella burnetii causes Q fever, a worldwide zoonotic disease. This disease is common among cattle, sheep, goats, and swine. Since 1999, Q fever has been considered as a reportable disease in the U.S., due to its threat to food safety and public health. Coxiella burnetii has been identified in a large percentage of dairy farms, however its prevalence in beef cattle herds has not been reported. The objective of this study was to identify the potential presence of the bacterium by qPCR, and compare with antibody response tested by Q-fever ELISA using paired blood and serum samples.

Method: A collection of 567 paired blood and serum samples and an additional 562 serum samples were used for the study. Animal age, breed, and bovine leukemia (BLV) status were recorded at sample collection. Whole blood samples were subjected to DNA extraction and qPCR identification using the KSVDL C. burnetii assay. Serum samples were processed and tested with a commercial antibody ELISA kit against C. burnetii infections.

Result: All 567 blood samples were negative by the qPCR test. ELISA test was performed on 1129 serum samples, and the overall positive rate was 15.4%. Descriptive statistical analysis on different cattle breeds indicated that the animal breed with the highest seroprevalence was Angus cross breed (23.4%), followed by Simmental-Angus cross (19.7%), Hereford (18.9%), Angus (17.7%) and Hereford-Angus cross (10.7%). The lowest positive rate was found in Red Angus cattle (4.3%). Animal age analysis observed that the lowest seroprevalence was found in 2.5 years and 3 years-old cattle with 5.1% and 8.3% positive rate, respectively. It was interesting to see higher positive rates were found in both younger and older age groups. A 11.9% positive rate was found in 1.5 years of age, 11.7% in 2 years, 28.6% in 5 years, 21.9% in 6 years, and 19.1% found in 8 years old cattle. A higher C. burnetii positive rate was identified in animals that were also positive to BLV (18.8% vs. 13.7%).

Conclusion: Although all blood samples were PCR negative for C. burnetii, a relatively high antibody positive rate (15.4%) was observed using a commercial ELISA kit. Those that were positive for BLV had higher C. burnetii antibody positives (18.8%) than those that were negative for BLV (13.7%). Only 2.5 and 3 years-old animals had lower ELISA positive rates, and animals younger or older than this age group both had higher positive rates indicating animal age may not be an influential factor.

Financial Support: CDC - Association of Public Health Laboratories Fellowship

Notes:

P154 - Effects of white-tailed deer-specific spike glycoprotein mutations on SARS-CoV-2 fusogenic activity

W. Dittmar[1], C.J. Thieulent[1], N. Gaudreault[2], U.B.R. Balasuriya[1], J.A. Richt[2], M. Carossino[1]

[1]Department of Pathobiological Sciences, Louisiana State University, [2]Department of Diagnostic Medicine/Pathobiology, Kansas State University. wdittm2@lsu.edu

Session: One health / Public health, 2025-01-20, 6:00 - 8:00

Objective: SARS-CoV-2 can naturally infect white-tailed deer (WTD), with sustained deer-to-deer transmission and spillback to humans. The viral kinetics, replication dynamics, and pathogenic potential of WTD-adapted SARS-CoV-2 strains are poorly understood. We hypothesize that WTD-specific mutations in the SARS-CoV-2 spike glycoprotein will provide a fitness advantage, increasing cell-cell fusion and thus facilitating transmission. The objective of this study was to investigate the effects of WTD-specific spike mutations on cell-cell fusion.

Methods: Full-length genome sequences (n=172) from SARS-CoV-2 strains derived from WTD across North America were obtained from GISAID. Sequences were aligned, WTD-specific spike mutations identified, and phylogenetic analysis was performed to determine evolutionary relationships. Spike glycoprotein sequences from four WTD-adapted SARS-CoV-2 strains were selected based on the presence of WTD-specific mutations. These sequences included a strain from Ohio, USA (hCoV-19/deer/USA/OH-OSU-0343/2021, referred to as OH-0343), where the first evidence of deer-deer transmission was identified, a strain from Ontario, Canada (hCoV-19/deer/Canada/ON-WTD-04658-2372/2021, referred to as ON-0465), corresponding to the first reported spillback from WTD to humans, and two strains from New York (hCoV-19/deer/USA/NY-WTD_119505/2021 and hCoV-19/deer/USA/NY-WTD_164412/2021, referred to as NY-119505 and NY-164412, respectively) . The Wuhan-like USA-WA1/2020, the Alpha variant B.1.1.7, the Delta variant B.1.617.2 and the mink-derived strain mDK-87 (Cluster 5) were included as controls. Spike nucleotide sequences were cloned into the pCAGGS vector under a CAG promoter and used in a cell-cell fusion assay with a split-GFP reporter system, in which VeroE6-TMPRSS2 cells were cotransfected with each pCAGGS-spike vector and plasmids containing either fragments 1-10 or fragment 11 of the GFP protein. Transfected cells were then pooled, and cells were incubated for 72 hours. Fluorescent signal generated by cell-cell fusion was recorded using the Sartorious IncuCyte Live Cell Analysis System. The ratios of GFP-positive area to confluent area were collected and ratios were compared between each spike variant at each time point using ANOVA and Dunnett's post-hoc test. The times to the peak ratio for each spike variant were compared using a Kruskal-Wallis test and a Wilcox each-pair post-hoc test.

Results: Based on phylogenetic analysis, the WTD-derived viruses were predominantly grouped into the B.1.1.7 (Alpha) and B.1.617.2 (Delta) variants. In WTD-derived spikes, there were an average of 7 non-synonymous mutations and 2 deletions/insertions with a range of one to two nucleotides. The mink-derived mDK-87 spike exhibited the highest level of cell-cell fusion, followed by ON-0465 and USA-WA1/2020. Other spike sequences examined showed a lower degree of fusion, with B.1.1.7 showing the lowest level. Notably, ON-0465, which showed a fusion profile almost identical to that of USA-WA1/2020, contained eight non-synonymous mutations in its spike sequence and was previously associated with spillback into humans. Interestingly, NY-1644, which contained the highest number of spike mutations (n=12), exhibited a peak in fusogenic activity nearly 10 hours earlier than other spike sequences analyzed.

Conclusions: The data gathered here indicates that WTD-specific spike proteins exhibit differences in cell-cell fusion dynamics in vitro. The conclusions drawn from this study will assist in the characterization of evolving SARS-CoV-2 variants in WTD as well as at the human-animal interface.

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:

P155 - Evaluating dairy farming interests of K-12 and college students utilizing virtual reality as a tool

M. Bradley[1], E. Kim[1], P. Boscan[2], I. Fraire[1], M. Jones[1], C. Tornatzky[3], M. Vans[4], W. Jiang[5], S. Rao[1]

[1]Department of Clinical Sciences, Colorado State University, [2]Veterinary Teaching Hospital, Colorado State University, [3]Colorado State University, [4]Department of Systems Engineering, Colorado State University, [5]College of Veterinary Medicine Information Technology, Colorado State University. brad1020@colostate.edu

Session: One health / Public health, 2025-01-20, 6:00 - 8:00

Objective: The lack of agriculture-focused education in the K-12 curriculum is directly impacting a student's interest in these key areas. The livestock agriculture workforce is reducing due to decreased interest among the younger generations. Therefore, it is an emerging issue in agriculture that needs special attention. Virtual reality (VR) technology as a teaching aid in the K-12 curriculum is advancing as the education system is exploring the virtual world as an educational tool. The goal of our project was to evaluate the likeability, feasibility, and applicability of interactive VR farming games for K-12 and college students.

Methods: A preliminary questionnaire (pre-test) was given to assess student's interest in a career in agriculture before the VR tool. The VR tool developed for this study employs a concept process algorithm where participants are placed in a dairy farm locker room with options to choose from a variety of clothing and protective equipment to wear. Participants need to choose the appropriate items in their correct sequence to progress further into the farm. To assess the tool's effectiveness, a second questionnaire (post-test) was administered to the participants after completing the simulation. Student data helped assess the likability and whether VR could be an effective educational training tool for animal agriculture. Statistical analysis was performed using a Signed Rank test between pre- and post-questionnaire survey data.

Results: A total of 104 participants used the VR tool, with ages ranging from 6 to 19 years. Overall, the experience was well-received, as 90.1% of participants rated it positively. Comparison between surveys indicated VR tool use was statistically significant in increasing interest in learning about animal agriculture (p-value < 0.01). Furthermore, 78.6% of respondents considered the VR tool educational, indicating they learned something from the experience. 48.8% of participants reported learning the proper attire needed before entering a dairy farm, while another 27.5% of participants reported learning the correct order of PPE equipment. Interest in pursuing a career in agriculture increased in 40.4% of participants, whereas interest stayed the same in 55.6% of participants.

Conclusions: These findings suggest favorable effectiveness of VR technology as an educational tool and to evaluate dairy farming interests among this younger population. Further data is necessary to enhance the VR training in implementing more livestock practices within the farm environment.

Financial Support: I would like to thank High Plains Intermountain Center for Agricultural Health and Safety (HICAHS) and National Institute for Occupational Safety and Health (NIOSH) for their contirbutions.

Notes:

P156 - Using spatial models to understand potential overlap of wildlife susceptible to SARS-CoV-2

Gabrielle Johnson[1], Adrian Castellanos[2], Barbara A. Han[2], Andrew M. Kramer[3]

[1]Swarthmore College, [2]Cary Institute of Ecosystem Studies, [3]University of South Florida. amkramer@usf.edu

Session: One health / Public health, 2025-01-20, 6:00 - 8:00

Objective: The persistence and spillover of multi-host zoonotic pathogens, like SARS-CoV-2, may depend on transmission between diverse animal species. It is straightforward to determine if susceptible species' ranges overlap, but forecasting cross-species pathogen transmission depends on better understanding of the likelihood of finer-scale spatial and temporal overlap. Here we use presence-only data to predict spatial overlap at the 1 km scale and preview how camera-trap data will be integrated to further refine predictions of potential transmissive interactions for mammals carrying SARS-CoV-2 in the eastern U.S.

Methods: Mammals susceptible to SARS-CoV-2 were determined from analysis of the literature. Input data include mammal occurrence records from the Global Biodiversity Information Facility and bioclimatic variables at 1 km resolution from Worldclim. Species distribution models were fit with the MaxEnt algorithm, using non-target mammal observations as background points. Model probabilities were summed to predict local species richness. Camera trap data were opportunistically obtained from deer monitoring and long-term ecological research projects in New York and analyzed using multi-species occupancy models.

Results: We found confirmation of SARS-CoV-2 susceptibility in 18 mammals commonly occurring in the eastern U.S. The relative habitat suitability could be successfully predicted for the species (mean AUC = 0.89). Large areas are expected to have high overlap of these species, with a maximum prediction of 15 species in a given grid cell. Other areas are predicted to have fewer co-occurring SARS-CoV-2 susceptible species. A total of 18 species of birds and mammals were able to be identified to species level from camera trap data.

Conclusions: There is significant variation in predicted species overlap at the local scale within the ranges of mammals susceptible to SARS-CoV-2. Nevertheless, variants with wide host range have the potential to transmit among a high number of species. Using higher-resolution data from camera traps can allow for additional precision in predictions of these species' interactions. Such information can then be integrated into dynamical models of multi-host dynamics in wild animal populations.

Financial Support: This work was funded by USDA-NIFA AFRI grant 2023-70432-40381 as part of the joint USDA-NSF-NIH-UKRI-BSF-NSFC Ecology and Evolution of Infectious Diseases program.

Notes:

P157 - Serological investigation with multiple methods revealed no evidence of natural SARS-CoV-2 infection in US cattle

Santhamani Ramasamy[1], Meysoon Quraishi[2], Swastidipa Mukherjee[1], Sonalika Mahajan[3], Lindsey LaBella[1], Shubhada Chothe[1], Padmaja Jakka[2], Abhinay Gontu[2], Sougat Misra[1], Meera Surendran-Nair[2], Ruth H. Nissly[2], Suresh V. Kuchipudi[1]

[1]Department of Infectious Diseases and Microbiology, University of Pittsburgh School of Public Health, [2]Department of Veterinary and Biomedical Sciences, The Pennsylvania State University, [3]Division of Biological Standardization, ICAR-Indian Veterinary Research Institute. sar465@pitt.edu

Session: One health / Public health, 2025-01-20, 6:00 - 8:00

Objective: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) continues to pose a significant threat to public health. Notably, SARS-CoV-2 can infect a wide range of non-human animal species, including captive and free-living animals. Earlier experimental studies revealed low susceptibility of domestic cattle (Bos taurus) to ancestral B.1 lineage; however, recent experimental findings indicate greater permissiveness of cattle to the SARS-CoV-2 Delta variant. The continued circulation of SARS-CoV-2 in free-ranging wild animals such as white-tailed deer poses a potential risk of spillover into cattle. While some studies detected SARS-CoV-2 antibodies in cattle in Italy, Germany, India, and Nigeria, currently, there is no evidence of SARS-CoV-2 infections in US cattle. In this study, we aim to detect the evidence of SARS-CoV-2 spillover in cattle using multiple serological assays.

Methods: We have screened 598 cattle serum samples, including 49 samples collected before 2020 (pre-pandemic) and after March 2020 (pandemic) collected from Pennsylvania for the presence of SARS-CoV-2 antibodies. We have employed pseudovirus neutralization assay (pVNT) using pseudoviruses expressing Spike from Delta and Omicron SARS-CoV-2 variants to detect the SARS-CoV-2 antibodies. In addition, we have also used a surrogate virus neutralization assay (sVNT), an in-house developed indirect ELISA, and standard live virus neutralization (VN) assay to detect the antibodies against SARS-CoV-2. Due to the non-availability of known SARS-CoV-2 positive cattle serum samples, we used hyperimmune serum raised in cattle immunized with SARS-CoV-2 RBD as a positive control for the pVNT and indirect ELISA test validation. In the pVNT, we have used SARS-CoV-2 Delta and Omicron spike pseudoviruses.

Results: None of the pre-pandemic serum samples tested positive with any method except for one that showed 55% inhibition in the Delta pVNT. Among the 549 pandemic serum samples, 56 exhibited >60% inhibition in Delta pVNT, and 44 in Omicron pVNT. We employed sVNT assays targeting the RBD of Delta and Omicron variants to confirm these results. Only one pre-pandemic and one pandemic sample were positive in the Delta sVNT, while one pandemic sample was positive in the Omicron sVNT. Further testing of 88 samples with >60% inhibition in pVNT using indirect ELISA revealed only one positive result. Notably, samples with >30% inhibition in Delta sVNT (n=2) and Omicron sVNT (n=1) were negative in ELISA. The sample that tested positive in ELISA showed 45% inhibition in Delta pVNT, highlighting inconsistencies between different assays. Finally, when both pandemic and pre-pandemic serum samples with >60% inhibition in pVNT were tested using the live virus neutralization (VN) assay, all tested negative for SARS-CoV-2 antibodies, indicating no evidence of natural infection. This emphasizes the need for multiple testing methods to avoid false positives.

Conclusions: Our findings reveal no serological evidence of natural SARS-CoV-2 infection or transmission among cattle in the U.S. However, caution is warranted when interpreting results from pseudovirus neutralization assays, as they may produce false positives. This study highlights the need for comprehensive evaluation and cross-validation serology assays to detect SARS-CoV-2 antibodies in cattle.

Financial Support: This work was supported by the USDA NIFA Award # 2023-70432-41334.

Notes:

P158 - Blood transcriptomic profile of North American Elk challenged with SARS-CoV-2

Bruna Petry[1], Ellie J Putz[1], Kaitlyn M Sarlo Davila[2], Alexandra C Buckley[3], Eric D Cassmann[3], Steven C Olsen[1], Paola M Boggiatto[1], Mitchell V Palmer[1]

[1]Infectious Bacterial Diseases Research Unit, National Animal Disease Center (NADC-USDA), [2]Ruminant Diseases and Immunology Research Unit, National Animal Disease Center (NADC-USDA), [3]Virus and Prion Research Unit, National Animal Disease Center (NADC-USDA). bruna.petry@usda.gov

Session: One health / Public health, 2025-01-20, 6:00 - 8:00

Objective: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a zoonotic public health concern responsible for the coronavirus disease 2019 (COVID-19) pandemic. In addition to its impact on human health, it has been shown to infect domestic and wildlife species. We have previously demonstrated that elk are susceptible to infection with SARS-CoV-2. The objective of this study was to investigate the changes in blood gene expression profile in North American Elk (Cervus canadensis) following experimental challenge with SARS-CoV-2.

Methods: Elk were challenged with an ancestral strain of SARS-CoV-2 (USA-WA1/2020), and PAX blood tubes were collected in the day 0, prior to challenge for the control group (6 animals), and days 2 (6 animals) and day 5 (5 animals) post-challenge. RNA was isolated using MagMAX™ for Stabilized Blood Tubes RNA Isolation Kit, and the RNA integrity was analyzed by Bioanalyzer, and the average RIN was 9.3. RNA library preparation was performed using Illumina mRNA kit and sequencing was done at the University of Illinois Urbana-Champaign at Roy J. Carver Biotechnology Center by Nova Seq X Plus generating 150 base pair paired ends reads. RNA sequencing analysis was performed using Nextflow nf-core/rna-seq pipeline; quality control was processed by FastQC, library adapters from sequencing were removed using TrimGalore, and sequences read were aligned against the Cervus canadensis genome assembly (ASM19320065v1) using STAR software. Gene counts were performed using featureCounts and differentially expressed gene analysis was performed using DESeq2, comparing day 2 compared to day 0 (control group), and day 5 post-challenge compared to day 0.

Results: Day 2 post-challenge revealed 2756 genes differentially expressed (DE) (p-adjusted<0.05) when compared to day 0, where 1421 genes were upregulated, and 1335 genes were downregulated. From the upregulated genes, 65 were found related to the Coronavirus disease KEGG pathway, while mostly of the genes are involved in immune systems functions, including viral defense. Comparing day 5 post-challenge to the control group, 9708 genes were differentially expressed (p-adjusted<0.05) after SARS-CoV-2 challenge, between them, 5399 genes were downregulated while 4309 genes were upregulated. From the upregulated list, 106 genes were found being involved in the Coronavirus disease KEGG pathway, and other genes involved in different viruses' infections, members of tumor necrosis factors and angiotensin-converting enzyme 2 (ACE2), an important cell receptor for the SARS-CoV-2 infection and replication.

Conclusions: These results illustrate a broad and rapid immune response of the North American Elk to SARS-CoV-2 challenge. Gene expression profiles highlight the changing expression of several genes related to important pathways for controlling viral infection and accelerating the host immune system response. These data offer an important resource for comparative studies in elk and other mammals that can be reservoirs for COVID-19, helping to understand the molecular machinery behind this infection.

Financial Support: ORISE - Oak Ridge Institute for Science and Education

Notes:

P159 - Activity of protease inhibitors against bat coronaviruses of pandemic potential

Camila Amrein Almira[1], David George[1], Alexandria Zabiegala[1], William Groutas[2], Kyeong-Ok Chang[1], Yunjeong Kim[1]

[1]Kansas State University, [2]Wichita State University. ykim@ksu.edu

Session: One health / Public health, 2025-01-20, 6:00 - 8:00

Objective: The periodic novel coronavirus outbreaks in humans, including severe acute respiratory syndrome coronavirus (SARS-CoV), Middle East respiratory syndrome coronavirus (MERS-CoV) and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), underscore the importance of strengthening pandemic preparedness to mitigate the impact on humans and animals. Bats are considered the major natural reservoir of betacoronaviruses and the source of future coronavirus pandemics. However, it is unknown whether antiviral agents developed for SARS-CoV-2 or other coronaviruses have extended effects against bat coronaviruses of pandemic potential. In this study, we determined the activity of protease inhibitors nirmatrelvir (an FDA-approved SARS-CoV-2 drug) and experimental compound GC376 and its derivatives against select betacoronaviruses. These bat coronaviruses utilize human receptors, such as human angiotensin-converting enzyme 2 (ACE2, a receptor for SARS-CoV-2) or dipeptidyl peptidase 4 (DPP4, for MERS-CoV) for virus entry, thus posing an increased risk of human spillovers.

Methods: Phylogenetic and sequence homology analysis of 3C-like proteases (3CLpro) of bat sarbecoviruses and merbecoviruses in the betacoronavirus subgenus, which can utilize human ACE2 or DPP4 receptors, and of human coronaviruses were conducted. The full 3CLpro amino acid sequences obtained from GenBank were aligned, and pair-wise percent identities were calculated using the Clustal Omega program. A phylogenetic tree was then constructed using MEGA software. The 3CLpros of SARS-CoV-2, MERS-CoV and feline infectious peritonitis virus (FIPV, a feline alphacoronavirus) and three bat coronaviruses were expressed, and their inhibitory activities against protease inhibitors were determined by the fluorescence resonance energy transfer (FRET) assay. The antiviral activity of select inhibitors was determined in cell culture against FIPV. The 50% inhibitory (IC_{50}) or effective concentrations (EC_{50}) from these tests were calculated using GraphPad Prism software.

Results: Multiple amino acid alignments and homology assessments showed that the 3CLpro amino acid sequences of sarbecoviruses are highly conserved with greater than 91% homology, while those of merbecoviruses are moderately less homologous with higher than 78% homology. The 3CLpro homology between sarbecoviruses and merbecoviruses is relatively low at around 50%. Despite the differences in 3CLpro sequences, the tested compounds exhibited broad inhibitory activity against the tested bat sarbecovirus and merbecoviruses in the FRET assay. However, the inhibition was relatively weaker against the tested bat coronaviruses with lower micromolar IC_{50} values, regardless of levels of sequence homology with SARS-CoV-2 3CLpro. When the antiviral activity was confirmed against FIPV, a Biosafety Level 2 cultivable virus, in cell culture, both the GC376 derivative and nirmatrelvir strongly inhibited the replication of FIPV, confirming the results from the FRET assay, and the GC376 derivative was five-fold more potent than that of nirmatrelvir with low nanomolar EC_{50} value.

Conclusions: The licensed 3CLpro inhibitor and the experimental compound GC376 and its derivatives have broad activity against multiple merbecoviruses and sarbecoviruses, as well as an alphacoronavirus. However, the observed discrepancy in 3CLpro sequence homology and antiviral potency may suggest that the active-site topology, where inhibitor-3CLpro binding occurs, is the major determinant of antiviral activity. This needs to be considered when developing pan-coronavirus antivirals.

Financial Support: USDA-NIFA AFRI 2019-67015-29864, NIH R01 AI161085, NIH T35OD029981

Notes:

P160 - Leptospiral shedding and seroprevalence in shelter dogs: A repeat cross-sectional/pseudo-longitudinal study

R. Lockwood[1], A. Cruz[1], M. Gutierrez[1], H. Green[1], C. LeRoy[1], T. Pulliam[1], J. Banach[1], L. Wisnieski[1], M. Gemechu[2], D. Patnayak[2], H. Naikare[2], C. Jones[1], J. Morgan[1], A. Verma[1]

[1]Richard A Gillespie College of Veterinary Medicine, Lincoln Memorial University, [2]Veterinary Diagnostic Lab, University of Minnesota. ryann.lockwood@lmunet.edu

Session: One health / Public health, 2025-01-20, 6:00 - 8:00

Objective: Leptospirosis is a significant zoonotic disease responsible for significant morbidity and mortality in both animals and humans. In a previous study (2017-2018) conducted by our lab, we found that approximately 13% of healthy shelter dogs shed leptospires in their urine, and 18% tested positive for leptospiral antibodies. This study aims to perform a repeat cross-sectional or pseudo-longitudinal study to screen urine and blood from shelter dogs for evidence of leptospiral presence.

Methods: Blood and urine samples from 230 dogs will be collected from 10 shelters in the Cumberland Gap Region of Kentucky, Tennessee, and Virginia. To date, we have collected samples from 72 shelter dogs. Leptospiral antibody levels for serovars Pomona, Hardjo, Icterohaemorrhagiae, Grippotyphosa, Canicola, Bratislava, and Autumnalis were measured using the microscopic agglutination test. DNA isolated from urine samples was tested for leptospiral DNA using a TaqMan-based qPCR targeting a highly conserved 242 bp region of the lipl32 gene.

Results: Out of the seventy-two dogs screened thus far, more than 11% (8/73) have Leptospira-specific antibodies to at least one tested serovar. Fifty percent of the positive dogs (4/8) had titers ≥1:100 against multiple serovars. The serum titers in positive dogs ranged from 1:100 to 1:6400. Leptospiral DNA was detected in the urine of three dogs (n=48). Only one dog had both serum leptospiral antibodies and leptospiral DNA present in the urine.

Conclusion: This study will provide important information on the status and trends of leptospiral infection in shelter dogs, which can pose a potential infection risk to other animals, shelter personnel, or anyone who comes in contact with them. These findings carry significant implications for both animal and public health within the region and potentially beyond, wherever these animals may be adopted.

Notes:

P161 - SARS-CoV-2 surveillance reveals broad exposure in free-ranging white-tailed deer with Delta variants in Texas

F.C. Ferreira[1], C. Rodriguez[2], W. Tang[1], R. Almeida[1], S. Sittenauer[1], A. Alamia[1], T. Hensley[2], A. Roy[3], S. Hamer[1], G. Hamer[1]

[1]Texas A&M University, [2]Texas A&M University Veterinary Medical Diagnostic Laboratory, [3]Ginkgo Bioworks. franciscocarlosfj@gmail.com

Session: One health / Public health, 2025-01-20, 6:00 - 8:00

Objective: The global spread of SARS-CoV-2 among humans facilitates infections in a wide diversity of mammals across multiple continents. Importantly, the white-tailed deer (Odocoileus virginianus, WTD) is highly susceptible to SARS-CoV-2 under experimental and natural conditions, raising concerns that it may become a long-term reservoir for the virus and potentially contribute with the generation of novel variants. Therefore, broad-scale surveillance is essential to understand virus dissemination and evolution in free-ranging deer populations in the US. Our study aimed to determine the rates of active or recent infections and previous exposure to SARS-CoV-2 in free-ranging WTD in Texas during the 2021/2022 hunting season.

Methods: We tested retropharyngeal lymph nodes (RPLN) from WTD sampled across Texas between October 12, 2021 and February 19, 2022. We tested RPLN samples using RT-qPCR to detect SARS-CoV-2 RNA, determining active/recent infections in WTD. A subset of these samples had lymph node exudate collected for the detection of neutralizing antibodies against SARS-CoV-2 via plaque reduction neutralization test (PRNT) for which both a liberal cutoff ($PRNT_{50}$) and a conservative cutoff ($PRNT_{90}$) were defined for samples with titers capable of neutralizing 50% or 90% of viral plaques, respectively.

Results: Among the 1,778 RPLN samples collected from WTD across 208 Texas counties, 22 samples from 11 counties tested positive for SARS-CoV-2 RNA. Three samples from Jackson County were initially positive by RT-qPCR, and targeted testing of 10 additional samples revealed a total of 9 out of 13 deer positive for SARS-CoV-2 harvested between November 9 and 29, 2021. Whole-genome sequencing of 9 samples, including 7 from Jackson Co., revealed that all but one virus belonged to the AY.25 Delta clade. The viral sequence from a deer harvested in Polk Co. was assigned as AY.44 Delta. Exudate was collected from 159 randomly selected deer with RT-qPCR-negative RPLN and 21 deer with positive RPLNs across 72 counties. Using the $PRNT_{50}$ and $PRNT_{90}$ criteria, seropositivity was 38.3% and 15.1% among the RT-qPCR-negative deer, and 81% and 33% among the RT-qPCR-positive deer, respectively. From 36 counties with $PRNT_{50}$-positive WTD, eight had at least five samples tested and showed seropositivity ranging from 25% to 80%. There was no difference in detection rates between juvenile and adult deer by RT-qPCR and PRNT tests. Males had higher rates of detection by RT-qPCR (18/1,049 - 1.7% vs. 4/714 - 0.5%; P = 0.047), and $PRNT_{50}$ (53/100 - 53% vs. 25/76 - 33%; P = 0.012). Road-kill WTD had higher RT-qPCR positivity (8/347 - 2.3%) compared to hunter-harvested animals (13/1,407 - 0.9%; P = 0.049).

Conclusions: Our results show active/recent infections by SARS-CoV-2 in free-ranging WTD across Texas during the 2021-2022 hunting season. Additionally, our serological results highlight the broader dissemination of WTD exposure to SARS-CoV-2 in Texas, underscoring the importance of establishing measures to mitigate transmission among human and deer. The detection of a transmission cluster within WTD in Jackson Co. supports that there are geographic foci of deer positivity. Continued genomic surveillance across Texas is essential to monitor the evolution and persistence of SARS-CoV-2 within WTD.

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:

P162 - One health surveillance for carbapenem-resistant Enterobacterales

Madison Evans[1], Madison Harr[1], Helen Johnston[2], Christopher Czaja[2], Ingrid Hewitson[2], Karlie Hoetzer[2], Jennifer Driscoll[2], Joshua Daniels[3], Elaine Scallan Walter[1]

[1]Colorado School of Public Health, [2]Colorado Department of Public Health and Environment, [3]Colorado State University. maddy.evans@colostate.edu

Session: One health / Public health, 2025-01-20, 6:00 - 8:00

Objective: To conduct enhanced surveillance for fecal shedding of carbapenem-resistant Enterobacterales (CRE) by pets owned by CRE case-patients in Colorado to better understand CRE transmission among humans and companion animals.

Methods: The Colorado Department of Public Health and Environment (CDPHE) conducts population-based surveillance for CRE statewide laboratory reporting. From January 2024, we attempted to interview all CRE case-patients in the five-county Denver metropolitan area and carbapenemase-producing CRE (CP-CRE) case-patients statewide, excluding those who were incarcerated, residents of extended care facilities, or deceased. The case interview was adapted from existing interview forms used by CDPHE as part of the Emerging Infections Program - Healthcare-Associated Infections Community Interface. We added questions about companion animal exposures (dogs, cats), including the age and sex of the animal, amount of companion animal contact preceding and following their diagnosis, the nature of their companion animal interactions, infection prevention behaviors (e.g., hand hygiene after petting), recent veterinary visits, and recent antibiotic use or travel (international or interstate) by the animal. Case patients reporting contact with a companion animal were asked to provide three fresh stool samples from each animal, collected one week apart. Companion animal samples were transported by mail on ice to the Colorado State University Veterinary Diagnostic Laboratory (CSU-VDL) for CRE detection using selective enrichment culture. Methods for whole genome sequencing (WGS) of CRE isolates from human and animal samples were established to facilitate phylogenetic comparisons, investigate genetic relationships, and understand potential zoonotic transmission pathways.

Results: From January to October 2024, 156 case-patients met the interview criteria, including 113 CRE and 46 CP-CRE cases. Of these, 26 refused an interview and 38 could not be contacted. Of 66 completed interviews, 46 (70%) case patients reported contact with one or more companion animals. Of these, 28 (61%) agreed to sample their pet(s) and 10 (22%) submitted samples for a total of 20 pets. To date, three sampling rounds have been completed for 10 pets and 1-2 samples have been tested for an additional 10 pets, for a total of 43 samples. No pet samples have tested positive for CRE; however, genetic sequencing protocols and analytical frameworks are in place for future analysis if CRE isolates are identified. Several strategies have been put in place to increase response rates and encourage increased sample submission, including the use of priming texts before making the initial phone call attempt and offering gift card incentives and pick up options for each pet sample submitted.

Conclusions: This One Health surveillance project aimed to advance our understanding of CRE transmission among humans and companion animals. The surveillance model and methodology established here could serve as a blueprint for broader applications in tracking other zoonotic pathogens, promoting a more integrated approach to public health and veterinary medicine. One of the key challenges has been consistent participant follow-through with companion animal specimen collection. Further research and continued data collection will be necessary to fully assess the potential transmission pathways and inform future antimicrobial stewardship efforts.

Finacial Support: This work was funded in part by the Colorado Integrated Food Safety Center of Excellence, which is supported by the Epidemiology and Laboratory Capacity for Infectious Disease Cooperative Agreement (CK19-1904) through the Centers for Disease Control and Prevention.

Notes:

P163 - Parasite surveillance in pigs with access to outdoor non-concrete areas across Ohio and Indiana farms

Kara E. Flaherty[1, 2], Abby K. Waldrop[1, 3], Andreia G. Arruda[2], Antoinette E. Marsh[2], Talita P. Resende[1]

[1]Department of Animal Sciences, The Ohio State University, [2]Department of Veterinary Preventive Medicine, The Ohio State University, [3]Herbert College of Agriculture, The University of Tennessee, Knoxville. flaherty.177@osu.edu

Session: Parasitology, 2025-01-20, 6:00 - 8:00

Objective: An increasing number of producers across the United States are adopting alternatives to traditional intensive farming reflecting the evolving consumer desire for sustainable and animal-centric practices. In this context, it is imperative to explore and understand aspects of non-intensive pig rearing, including its impact on pig health and broader implications for public health. In outdoor environments, pigs are exposed to factors that differ from intensive farming making them more prone to get internal parasite infections, some of which have zoonotic potential. The objective of this study was to describe the occurrence of intestinal parasites of pigs raised with access to outdoor non-concrete areas across Ohio and Indiana.

Methods: Farm recruitment occurred through the Ohio State Extension network and internet consultation of an online directory for pasture raised-pig pork products. Criteria for farm enrollment include the following: a) pigs must have access to outdoor, non-concrete areas, and b) each farm must complete a brief survey detailing management practices. Fecal samples were preferably collected directly from the rectal ampulla, with freshly dropped feces being collected as an alternative. Samples were maintained under refrigeration until testing at The Ohio State University, Veterinary Clinical Parasitology Diagnostic Laboratory. Double centrifugal fecal flotation using sucrose flotation media technique was used to qualitatively screen the fecal samples for parasite eggs and oocysts. The management survey collected information on farm routine practices and health management of the pigs.

Results: A total of 98 fecal samples were collected from 10 farms from March to August 2024, with six farms located in Ohio and four farms located in Indiana. The number of pigs per farm ranged from 3 to 70 and the age ranged from 8-10 weeks to 12 years old. Out of 10 farms, six reported not having any kind of deworming protocol, two reported that they deworm pigs regularly, and two deworm pigs as needed. When asked if there was a Veterinary-Client-Patient-Relationship (VCPR), one producer reported having a VCPR with an annual veterinary visit, seven producers responded that they had a VCPR and the attending veterinarian would provide services as needed, and two producers reported not having a VCPR in place. Of the tested animals, 85.71% were positive for coccidia with all farms having at least one positive sample; 38.78% were positive for Ascaris suum, and 14.29% were positive for Trichuris sp. Additionally, 54.08% of animals tested positive for strongyle-type eggs. Although less prevalent, some animals were positive for Cryptosporidium sp. and Giardia, 4.08% and 2.04%, respectively.

Conclusions: Results highlighted the prevalence of certain intestinal parasites among pigs raised with access to outdoor non-concrete areas in Ohio and Indiana farms. All farms were positive for at least one type of parasite; with six distinct parasites observed across all samples. While the parasites observed may negatively impact pig health and pose certain risks to farm workers, the observed parasites pose no threat to humans through consumption of properly cooked pork products.

Financial Support: We would like to recognize the staff at The Ohio State University's Veterinary Clinical Parasitology Diagnostic Laboratory and Mike Kauffman at the Center for Food Animal Health.

Notes:

P165 - Temporal changes in the GI trichostrongyle communities of North American bison using deep amplicon sequencing

Jeba R.J. Jesudoss Chelladurai[1], Theresa A. Quintana[1], Jameson Brennan[2], Jeff M. Martin[2]

[1]Auburn University College of Veterinary Medicine, [2]South Dakota State University. jrj0073@auburn.edu

Session: Parasitology, 2025-01-20, 6:00 - 8:00

Objective: In North American plains bison, infections caused by parasitic gastrointestinal nematodes (GIN), primarily consisting of trichostrongyles, significantly contribute to reduced health outcomes, decreased production, and even mortality. This study aimed to evaluate the temporal changes in the diversity of trichostrongyle GIN in plains bison using deep amplicon sequencing. The hypothesis was that there are significant fluctuations in trichostrongyle gastrointestinal nematode community structure over time in bison, influenced by seasonal variations in environmental conditions.

Methods: We performed deep amplicon sequencing of the ITS2 region of trichostrongyle third-stage larvae obtained by coproculture of bison feces from seven herds sampled in the fall of 2021, 2022 and 2023, totalling 367 samples in the sequencing dataset. ITS2 amplicons were library-prepped and sequenced on an Illumina MiSeq. Sequencing data were analyzed using the DADA2 pipeline and diversity metrics were calculated.

Results: Analysis revealed 269 amplified sequence variants (ASVs), belonging to six genera and 13 species. A significant proportion of individual bison and herds had multiple infections with ≥ 2 trichostrongyle infections. Cooperia oncophora, Ostertagia ostertagi, Cooperia punctata and Haemonchus placei were the most abundant species. Changes in the composition of trichostrongyle populations were observed in all herds. In several herds, average species richness, Shannon diversity and Inverse Simpson diversity increased over time, while in other herds, diversity values decreased.

Conclusions: The nemabiome of gastrointestinal trichostrongyles of North American bison assessed in seven herds over three years varied temporally in species composition and diversity metrics.

Financial Support: This study was funded by a grant from the Center of Excellence for Bison Studies, South Dakota State University (to J.R.J.C) while employed at the Kansas State University College of Veterinary Medicine.

Notes:

P166 - Molecular understanding of the role of cathepsin L-like proteases in the pathogenesis of Histomonas meleagridis

Alessandro J. Rocchi[1], Aaron Forga[2], Kasey Matusik[2], Billy M. Hargis[2], Danielle Graham[2], Zhicheng Dou[1]

[1]Department of Biological Sciences, Clemson University, [2]Department of Poultry Sciences, University of Arkansas – Fayetteville. zdou@clemson.edu

Session: Parasitology, 2025-01-20, 6:00 - 8:00

Objective: Histomonas meleagridis, the causative agent of "blackhead" disease in turkeys, leads to mortality rates as high as 80-100%, resulting in substantial economic losses in the poultry industry. Due to concerns over consumer health, the drugs previously used to treat Histomonas infections have been banned, highlighting the urgent need for new prophylactic and therapeutic strategies. Recent proteomic studies of a European strain revealed a significant upregulation of cathepsin L-like proteases (HmCPLs) in virulent strains, indicating that these proteases may act as key virulence factors. This study aims to elucidate the roles of HmCPLs throughout different stages of the Histomonasinfection cycle, identify inhibitors of their enzymatic activity for novel antibiotic development, and evaluate their potential as vaccine targets.

Methods: We performed a transcriptomic analysis to identify the most abundant HmCPLs in Histomonas. Live cell imaging was used to examine their subcellular localization and activity, particularly within the food vacuole. Multiple HmCPL genes were cloned and expressed in both prokaryotic and eukaryotic systems, allowing for the production of recombinant proteins used to generate polyclonal antibodies. Additionally, a quantitative PCR-based assay was developed to monitor Histomonas growth by quantifying parasite genome copies in culture. To assess the potential of HmCPLs as vaccine candidates, we conducted turkey trials, with ongoing data analysis to determine vaccine efficacy.

Results: In our study, we performed transcriptomic analysis on an American Histomonas strain (PHL) isolated from Arkansas, identifying three highly transcribed cathepsin L-like proteases: HmCPL1, HmCPL2, and HmCPL3. Fluorescent staining with LysoSensor and BODIPY-LHVS probes in purified Histomonas parasites indicated a highly active food vacuole (a lysosome-like organelle) crucial for nutrient acquisition. The parasites express abundant cathepsin L-like proteases within the food vacuole, likely functioning as hydrolytic enzymes. The top three HmCPL genes were cloned into the pQE30 prokaryotic vector and Pichia eukaryotic expression systems, with a 6xHis tag added at their N-termini for affinity purification using a fast protein liquid chromatography system. These recombinant proteins were used to immunize rabbits, successfully generating highly sensitive and specific polyclonal antibodies against HmCPL3, which do not cross-react with HmCPL1 or HmCPL2. This specificity facilitates distinguishing the localization and abundance of HmCPL3 in parasites. Indirect immunofluorescence revealed that HmCPL3 localizes to the food vacuole, as confirmed by co-localization with the lysosomal dye Lysotracker Red DND-99. This is the first identification of a protein marker for the food vacuole in Histomonas, suggesting HmCPL3's key role in hydrolysis within this organelle. Additionally, we developed a quantitative PCR-based growth assay to assess Histomonas proliferation in response to cysteine protease inhibitors. Preliminary data indicate an EC_{50} of approximately 0.35 mM for chloroquine, which neutralizes acidic organelles, against in vitro growth. Recently, two turkey trials were conducted to evaluate HmCPL3 as a potential vaccine candidate, with data analysis currently underway.

Conclusions: Our results revealed that Histomonas cathepsin L-like proteases play a critical role in parasite growth and infection. Future studies will focus on elucidating the molecular mechanisms regulating differential HmCPL expression in virulent versus attenuated strains to clarify their roles across various life stages of Histomonas.

Financial Support: This project was supported by Agriculture and Food Research Initiative Competitive Grant no. 2024-67016-42416 from the USDA National Institute of Food and Agriculture.

Notes:

P167 - *Salmonella* infections in cattle: Antimicrobial resistance, biofilms, and virulence factors

Nada A. Fahmy[1], Sumin Karna[1], Angel Bhusal[1], Ajran Kabir[1], Steve Locke[2], Erdal Erol[2], Yosra A. Helmy[1]

[1]Department of Veterinary Science, University of Kentucky, [2]Veterinary Diagnostic Laboratory, University of Kentucky. Nada.adel@uky.edu

Session: Salmonella, 2025-01-20, 6:00 - 8:00

Objective: *Salmonella enterica* (*S. enterica*) infection is estimated to impose the highest economic burden among all foodborne diseases in the US, with annual costs exceeding $3.7 billion. Antibiotic therapy is frequently used as the initial strategy to manage infections caused by *Salmonella* spp. Nevertheless, the extensive misuse of antibiotics has raised significant concerns regarding the emergence of antimicrobial resistance (AMR). Our study aims to comprehensively characterize *Salmonella* isolates from necropsied cattle by analyzing their antimicrobial resistance profiles, biofilm-forming abilities, and distribution of AMR and virulence genes. This investigation will characterize *Salmonella* isolates from cattle, focusing on the distribution of virulence and antimicrobial resistance genes, antimicrobial profiles, and biofilm formation. Ultimately, mitigating *Salmonella* in cattle will enhance food safety, safeguarding public health from foodborne and zoonotic infections.

Methods: From January 2022 to October 2023, a total of 1008 samples were collected from necropsied cattle at the UK Veterinary Diagnostic lab. Genotyping and serotyping characterization confirmed 23 isolates as *Salmonella enterica* subspecies. Further characterization was conducted to detect the profiles of AMR and virulence genes. The AMR patterns were identified against 11 antibiotics and the results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. The biofilm-forming ability of the isolated bacteria was assessed using a crystal violet assay. The motility phenotypes of the isolates were assessed on soft-agar plates. The diameter of the motility swarm and the swarming area were measured and compared to the wild-type strain.

Results: A Total of 23 *Salmonella* isolates were collected from cattle, ages ranging from 3 days to 12-year and categorized as neonatal, calf, juvenile and adult. *Salmonella* isolates revealed various serotypes, Dublin, Typhimurium, Muenster, Cerro and Thompson. For the virulence genes, the outer protein (sopB) and (spvC) operons were detected in 94.7%, 86.96% of isolates. This was followed by the invasion gene (Hil A) (82.6%) and the pathogenicity islands (Spi A) (73.7%) and (Spi D) (65.2%) that are associated with invasion, and systemic virulence. The highest resistance percentages were observed to tetracycline and chloramphenicol (100%), followed by to azithromycin, imipenem, marbofloxacin (95.65%) and piperacillin/tazobactam (69.6%). Likewise, lower resistances were detected in all isolates against doxycycline, ampicillin and meropenem. These isolates also harbor AMR genes such as Sal_{CatB} (65.21%), BlaCMY (30.43%), Bla_{CTXM} (26.08%), $Bla_{TEM\text{-}1B}$ (21.73%) and tet_B (4.34%). Additionally, these isolates produced biofilm at different levels of intensity, classified as strong (47.8 %), moderate (39.1%) and weak or non-biofilm producers (13.04%). Eighteen isolates (78.26%) exhibited swarming motility characterized by a smooth, featureless morphology; eight isolates (44.44%) displayed high swarming activity. Twenty-two (95.65%) isolates displayed swimming motility with either featureless or bull's-eye morphology, and sixteen isolates (72.72%) displayed high swimming activity.

Conclusions: This study reveals significant AMR and virulence gene prevalence in *S. enterica* isolates from necropsied cattle. Furthermore, the observed correlation between virulence gene distribution and AMR raises concerns regarding the emergence of highly virulent and resistant strains, posing significant challenges for effective treatment and control. These findings are critical for developing strategies to enhance food safety and public health.

Financial Support: The authors would like to extend our sincere gratitude to the University of Kentucky for the financial support and sponsorship of this research project.

Notes:

P168 - Mutation in Wzz(fepE) linked to altered O-antigen biosynthesis and attenuated virulence in rough *Salmonella* Infantis variant

N.V. Iduu[1], S. Kitchens[1], S. Price[1], C. Wang[1]

[1]Auburn University. nvi0001@auburn.edu

Session: Salmonella, 2025-01-20, 6:00 - 8:00

Objective: *Salmonella enterica* serovar Infantis (*S.* Infantis) has emerged as a prevalent foodborne pathogen in poultry with significant implications for public health worldwide. To better understand its virulence, this study investigates the molecular characteristics influencing virulence in a *S.* Infantis rough variant collected from a poultry farm in the USA.

Methods: Whole genome sequencing, comparative genomics, and Sanger sequencing were performed on both a smooth and a rough *S.* Infantis isolate to examine their genetic features. A chicken embryo lethality assay was conducted to assess their virulence. Comparative genomics between isolates was analyzed using Mauve pairwise Locally Collinear Blocks to measure genetic conservation between strains. Embryo survival rates were compared using Kaplan-Meier curves.

Results: High genomic conservation was observed between the two isolates. However, a frameshift mutation was detected in the *Wzz(fepE)* gene of the rough variant, which is crucial for the O-antigen chain length. The chicken embryo lethality assay revealed that the smooth strain had a significantly higher lethality rate compared to the rough strain ($P < 0.05$). This study identifies the frameshift mutation in the *Wzz(fepE)* gene, leading to protein truncation, which likely impacts O-antigen biosynthesis and contributes to the attenuated virulence of the rough *S.* Infantis variant.

Conclusions: These findings enhance our understanding of *S.* Infantis pathogenesis and suggest that targeting the *Wzz(fepE)* gene or related pathways could be a promising strategy for developing effective vaccines and therapeutic interventions.

Financial Support: This work was funded by the USDA Agricultural Research Service Program (58-6040-9-017).

Notes:

P169 - Molecular clock analysis of a persistent strain of *Salmonella* Hadar (REPTDK01) linked to backyard poultry and commercial poultry

Lingzi Xiaoli[1], G. Sean Stapleton[1], Zachary Ellison[1], Kaitlin Tagg[1], Hattie Webb[1], Taylor Griswold[1], Lee Katz[1], Katharine Benedict[1], Jessica Chen[1]

[1]Centers for Disease Control and Prevention. own3@cdc.gov

Session: Salmonella, 2025-01-20, 6:00 - 8:00

Objective: Since 2021, CDC has monitored a persistent *Salmonella* Hadar strain (REPTDK01) characterized by related isolates within 0-26 allele differences using core genome multi-locus sequence typing (cgMLST). This strain was first detected in 2019 by PulseNet and caused five multistate outbreaks between 2020 and 2023: three associated with backyard poultry (BYP), one with commercial poultry (CP) food products, and one with both vehicles simultaneously. Epidemiologic and traceback investigations were unsuccessful in determining the linkages between the strain found in both BYP and CP food products. To understand the molecular epidemiology and evolution of this persistent strain, we analyzed genomes of *S.* Hadar collected from outbreak investigations, routine surveillance, and research projects.

Methods: A rapid comparison of bulk *S.* Hadar genomes from 1990 to 2023, including REPTDK01 isolates, was conducted to gain a broad understanding of the genetic landscape. Various subsampled datasets were generated using different randomized selection strategies to evaluate the suitability for molecular clock analysis. A final subsampled dataset focusing on REPTDK01 isolates stratified by year was selected for further high-quality phylogenetic single nucleotide polymorphism (SNP) analysis, and the SNP alignment along with isolation dates, was used for molecular clock analysis.

Results: Rapid clustering of over 3000 S. Hadar genomes, including non-REPTDK01 isolates, using Mashtree, revealed multiple genetic subclusters. The subsampled dataset consisting only of REPTDK01 isolates exhibited a stronger temporal signal for further investigation, showing a higher positive correlation of genetic distances against sampling dates (R^2=0.6493). Molecular clock analysis in BEAST2 of 404 subsampled REPTDK01 isolates estimated that the most recent common ancestor of this strain emerged in early 2018. Two major clades were identified: a smaller clade (n=95) with clinical isolates linked exclusively to CP food products and a larger clade (n=308) with clinical isolates linked to BYP contact as well as isolates from BYP, CP food products such as turkey and chicken, pork, or beef food products. Comparison between the two clades revealed two clade-specific SNPs: a synonymous mutation in pdxA (encoding the dehydrogenase) and an early stop in yihS (encoding the isomerase for O-antigen assembly).

Conclusions: The persistent strain designated REPTDK01 is estimated to have emerged in early 2018 and exhibits two distinct clades with different genetic and epidemiological characteristics. Clade-specific SNPs detected here have the potential to serve as molecular markers for identifying possible transmission vehicles in future investigations of REPTDK01 clusters, thereby improving the timeliness of public health interventions. The presence of REPTDK01 in both BYP and CP food products in the large clade suggests ongoing transmission across commercial and backyard poultry industries. Future research combining epidemiologic data collection, detailed traceback information, and advanced genetic analysis will be crucial for monitoring and investigating outbreaks associated with transmission through both food products and animal contact.

Notes:

P170 - Detection of *Salmonella* in chicken rinsate as early as six hours of enrichment using ONT flongle sequencing

Anand B. Karki[1, 2], Elise Delaporte[1], Hailey Hall[1], Suhani Sharma[1], Maya Sous[1], Mohamed K. Fakhr[1]

[1]The University of Tulsa, [2]Sam Houston State University. mohamed-fakhr@utulsa.edu

Session: Salmonella, 2025-01-20, 6:00 - 8:00

Objective: The rapid detection of foodborne pathogens in retail meat is vital to food safety. Chicken products are consumed more frequently than other meat products in the USA and are often contaminated with foodborne bacterial pathogens which results in outbreaks of food-related illnesses. The objective of this study was to optimize the ONT Flongle sequencing for the rapid detection of *Salmonella enterica, Campylobacter spp., Listeria monocytogenes,* and *E. coli* O15:H7 in whole chicken rinsate.

Methods: Whole chicken carcasses were rinsed with sterile 400 ml buffered peptone water, filter sterilized, and then spiked with various inoculum concentrations of each tested pathogen. A total of 30 ml of each inoculated chicken rinsate was mixed with 30 ml of the appropriate enrichment media for each pathogen and incubated at the appropriate conditions for up to 24 hours. All samples were processed in triplicates. For each spiked sample, 2 ml from each enrichment media were collected at 0, 6, 12, 18, and 24 hrs and mixed for DNA isolation using Qiagen DNeasy Blood and Tissue Kit and then used for Native Barcoding library preparation and loaded on ONT Flongle flow cell.

Results: Up to 12 barcoded libraries were loaded in a single Flongle sequencing run which generated enough sequence reads within 24 hours using fast Basecalling to identify bacterial species when EPI2ME Fastq WIMP was run concurrently. Species level identification, with 1% minimum abundance cutoff value, was successful for all spiked samples. Using ONT flongle sequencing, *Salmonella enterica* were identified as early as 6 hrs of enrichment and at a concentration as low as 0.83 CFU/ml of chicken rinsate. *E. coli* O157:H7 was detected as early as 6 hrs but its relative abundance was higher than the rest of the tested pathogens starting at 12 hrs. *Listeria monocytogenes* and *Campylobacter* species (*C. jejuni*, *C. coli*, and *C. lari*) were detected as early as 18 hrs but their relative abundance was higher at 24 hrs.

Conclusions: ONT Flongle sequencing was able to detect *Salmonella* in chicken rinsate as early as 6 hours of enrichment in the presence of several other foodborne bacterial pathogens. The success of ONT Flongle sequencing in accurately identifying multiple foodborne pathogens in chicken rinsate within 24 hours of enrichment and in a single sequencing run is very valuable and could serve as a rapid detection method replacing the laborious time-consuming conventional culture methods.

Financial Support: This work was supported by the USDA National Institute of Food and Agriculture, AFRI grant # 2023-67018-39049.

Note:

P171 - Genotypic and phenotypic antimicrobial resistance in cattle-associated *Salmonella* Dublin

Sophia Kenney[1], Kelli Maddock[2], Dayna Harhay[3], Erika Ganda[1]

[1]Dept. of Animal Science, Penn State University, [2]North Dakota State University Veterinary Diagnostic Lab, [3]Meat Safety and Quality, USDA Agricultural Research Service. smk459@psu.edu

Session: Salmonella, 2025-01-20, 6:00 - 8:00

Objective: *Salmonella enterica* serovar Dublin is a cattle-adapted zoonotic pathogen recognized for generally greater antimicrobial resistance (AMR) relative to other serovars. Given its ability to cause invasive disease in both cattle and humans, effective antibiotic intervention when applicable is crucial. AMR surveillance programs for pathogens like S. Dublin integrate sequence-based and in vitro AMR-typing methods. While sequence-based methods have research and surveillance utility, clinical relevance lies primarily in accurate phenotype prediction. Most studies examining this relationship understandably focus on clinical strains, leaving gaps in our understanding of AMR in strains that pose occupational and foodborne hazards. To this end, we sought to examine the AMR genotype-phenotype relationship within and between groups of clinical and environmental S. Dublin strains.

Methods: Forty-four *Salmonella* Dublin strains collected from clinical (N=26) or environmental cattle-associated sources (N=18) were identified and whole-genome sequenced on the Illumina NovaSeq platform. After raw read trimming and classification by Kraken2, reads classified as Enterobacteriaceae or lower ranks were extracted and used in de novo genome assembly with Unicycler. Following dual in silico serotype confirmation, strains were screened for antimicrobial resistance genes through the AMRFinderPlus pipeline. Antimicrobial susceptibility testing (AST) via broth microdilution was performed with the Sensititre EQUIN1F panel. Where applicable, breakpoints were interpreted according to the National Antimicrobial Resistance Monitoring System's *Salmonella* breakpoints. Concordance between AMR genotype and AST results was evaluated. Proportion of resistant strains in clinical and environmental groups was compared using a one-tailed, two-proportion t-test for each antibiotic. Multiple hypothesis testing corrections were performed with the Benjamini-Hochberg procedure.

Results: Multi-drug efflux pump genes, mdsA/B, were present in all strains. Other AMR genes identified include genes conferring resistance to aminoglycoside, beta-lactam, quinolone, phenicol, sulfonamide, and tetracycline drugs. Most (76.7%) strains were multidrug resistant (resistance to >3 drug classes). Three environmental strains were pan susceptible, and all were susceptible to imipenem. Clinical strains were more resistant to ampicillin (p.adj=0.044), ceftiofur (p.adj=0.005), chloramphenicol (p.adj=0.022), doxycycline (p.adj=0.004) and tetracycline (p.adj=0.004). Concordance accuracy varied (33%-84%) but was highest in tetracyclines, third-generation cephalosporins, and ampicillin. Relative to clinical strains, accuracy was numerically higher in environmental strains.

Conclusions: AMR concordance varied depending on antibiotic and strain source, concordance was generally moderate. While higher proportions of resistance in clinical strains is concerning, the relatively lower resistance in environmental strains suggests a potentially higher chance of successful intervention for occupationally acquired or foodborne infection. Discrepancies in genetic and in vitro AMR point to underlying mechanism warranting further investigation.

Financial Support: Funding support was provided by the USDA APHIS NBAF Scientist Training Program Fellowship (AP23VSD&B000C001); in part by the intramural research program of the U.S. Department of Agriculture, National Institute of Food and Agriculture, Accessions #7007417, #1023328, and #102244.

Notes:

P172 - Genomic characterization of *Salmonella* Mbandaka carrying the colistin resistance gene mcr-9 in horses

Ajran Kabir[1], Erdal Erol[1], Yosra A. Helmy[1]

[1]Department of Veterinary Science, University of Kentucky. ajran.kabir@uky.edu

Session: Salmonella, 2025-01-20, 6:00 - 8:00

Objective: *Salmonella* is a zoonotic bacterial pathogen causing foodborne illnesses worldwide. *Salmonella* Mbandaka has been as a major serotype responsible for outbreaks identified by the Centers for Disease Control and Prevention. In Europe, *Salmonella* Mbandaka ST413 ranks among the top 10 serotypes causing human infections. Horses can be one of the major hosts of this *Salmonella* serotype and can spread this through feces. Additionally, the emergence of multidrug resistance (MDR) in *Salmonella* is a significant public health concern. Colistin, a last-resort antibiotic, is used to treat severe infections caused by MDR bacteria. However, *Salmonella* Mbandaka harboring the plasmid-borne colistin resistance gene mcr-9 poses a potential threat to both human and animal health, as it can spread through horizontal gene transfer. This study aimed to uncover the genomic characteristics of *Salmonella* Mbandaka from foals in Kentucky, focusing on their virulence and antimicrobial resistance profiles.

Methods: *Salmonella* isolates were obtained from both live and deceased horses and were confirmed using PCR and MALDI-TOF. Serotyping was performed based on Kauffmann-White classification scheme and antimicrobial resistance profiling was performed by broth microdilution method against 11 antibiotics. Colistin resistance isolates (n=3) were selected for whole-genome sequencing (WGS) on the Illumina MiSeq System. Adapter trimming from paired-end reads (n = 1,442,773) was performed using Trimmomatic version 0.40, and de novo assembly was performed on SPAdes version 4.0. The quality of the raw reads was evaluated using FastQC version 0.12.1. Assembled contigs were annotated using PGAP version 6.7 (NCBI Prokaryotic Genome Annotation Pipeline). Sequence typing was performed on mlst version 2.23.0. Resistance genes and plasmids were detected on ABRicate version 1.0.1 using the NCBI database and PlasmidFinder database, respectively. Virulence factor genes were identified using VFanalyzer. Unless otherwise noted, all the aforementioned software was run with its default settings.

Results: These isolates were found resistance to five group of antibiotics including aminoglycosides, penicillin, cephalosporins, chloramphenicol, and sulphonamides. Whole genome sequencing revealed that sequenced isolates belong to pathogenic sequence type ST413 responsible for several outbreaks in Africa, Europe, and potentially other continents. These isolates were identified as *Salmonella* enterica subsp. enterica serotype Mbandaka on serotyping. Virulence profiling revealed more than 127 genes linked to host cell invasion, immune evasion, biofilm formation, and systemic infection, indicating significant pathogenic potential. Alarmingly, our isolates harbor genes conferring resistance to five antibiotic classes, including colistin which is considered a last-resort antibiotic for human infections.

Conclusions: The multidrug-resistant *Salmonella* Mbandaka isolates identified in this study pose a significant public health risk. The presence of the mcr-9 gene is particularly concerning due to its potential for environmental and human transmission to other isolates.

Notes:

P173 - Examining the differentiation and clustering of *Salmonella enterica* serovars based on relatedness of whole genome sequences

Brennen Hunt[1], Enrique Doster[1], Keith E. Belk[2], Brandy A. Burgess[3], Robert Valeris-Chacin[1], Cory A. Wolfe[1], Lee Pinnell[1], Paul S. Morley[1]

[1]Texas A&M University, VERO program, [2]Colorado State University, [3]University of Georgia. brennenohunt@tamu.edu

Session: Salmonella, 2025-01-20, 6:00 - 8:00

Objective: *Salmonella enterica* is a major pathogen of both humans and animals, with over 2,600 documented serovars, all of which are capable of causing disease in their hosts. Therefore, identifying *S. enterica* and distinguishing between different serovars and strains is critical. Traditionally, serovar typing has been conducted using antisera that recognized specific variants of the O cell surface antigen and the H flagellar antigen. Our group investigated a method of classifying isolates based upon the relatedness of the whole genome sequences to determine if genetic classification can provide differentiation and clustering of *Salmonella enterica* serovars.

Methods: All genome data belonging to *Salmonella* enterica subsp. enterica were downloaded from the annotated RefSeq database from NCBI and analyzed with fastANI to calculate the average nucleotide identity (ANI) among all genomes. The matrix ANI distances among isolates into RStudio version 4.1.0+ for further analysis. The R package "complexheatmap" was used to generate heatmaps labeled by serovar and colored by ANI which demonstrated hierarchical clustering of the serovars. Secondarily, we selected a subset of 30 genomes from 8 serovars belonging to 5 serogroups (Typhimurium, Anatum, Newport, Dublin, 4,[5],12:i:-, Agona, Infantis, and Enteritidis) and examined their clustering in detail to investigate genomes where the serovar annotation downloaded from RefSeq did not match the genetic clustering. SeqSero2 was then used to investigate genomic characteristics related to serovar classification for these non-matching genomes.

Results: ANI classification successfully characterized subtle differences among genomes that allowed clustering of genomes with >99% ANI similarity that also separated clusters that had 95-99% ANI similarity. Although genetic determinants for H and O antigens represent a small portion of S. enterica genome, ANI clustering largely differentiated genomes that were annotated as belonging to different serovars within the same genotype. However, our preliminary work also identified a small minority of genomes that did not cluster with others of the same serotype annotations as expected. We investigated the method reported by authors for serotype classification of these minority genomes and also analyzed their genomes using a bioinformatic tool (SeqSero2) designed to classify the serovar of isolates. Results of this analysis found that serovar classification of these minority isolates based on genomic sequence did not match the serovar classification available from the RefSeq database. This highlights the challenges faced as we explored this complicated area of study.

Conclusions: ANI is a useful method for classification of *Salmonella* enterica isolate relatedness at the strain level and is generally capable of predicting the phenotypic serovar classification based upon genetic relatedness. This method of genetic clustering may be useful in identifying strains that are candidates to be used as representatives for a large number of related isolates.

Financial Support: Texas A&M University

Notes:

P174 - Genomic comparison of *Salmonella* Typhimurium and *Salmonella* Dublin isolates from bovine sources in the U.S.

Kingsley Emmanuel Bentum[1], Emmanuel Kuufire[1], Rejoice Nyarku[1], Viona Osei[1], Temesgen Samuel[1], Woubit Abebe[1]

[1]Department of Pathobiology, Tuskegee University, Center for Food Animal Health and Food Safety Laboratory. kbentum8786@tuskegee.edu

Session: Salmonella, 2025-01-20, 6:00 - 8:00

Objective: The cattle-adapted serovar *Salmonella* Dublin and the generalist serovar *Salmonella* Typhimurium are important zoonotic pathogens. Although both are commonly isolated from cattle, disease presentation in cattle and other hosts is more severe with *Salmonella* Dublin. This study investigates the genomic variations that may contribute to this pathogenicity and the population dynamics among the two serovars.

Methods: Whole genome sequence data on 1337 *Salmonella* Dublin and 787 *Salmonella* Typhimurium isolates from bovine sources in the United States were retrieved from Enterobase (a publicly available database) and analyzed. The Achtman 7-gene MLST scheme was used for isolate sequence typing. Clonal groups among isolates were assigned using the Hierarchical Clustering of Core Genome Multi-Locus Sequence Typing (HierCC-cgMLST) plug-in and neighbor-joining GrapeTrees based on the CgMLST V2 + HierCC V1 scheme were constructed. All isolates were screened for antimicrobial resistance genes, virulence genes, and plasmid replicons using the CARD and AMRFinderPlus databases, the virulence factor database (VFDB), and the plasmidfinder database respectively. For the presence and absence of unique genes among isolates of the two serovars, genomic annotation was first performed with Prokka, and the results were parsed to Roary to generate our results.

Results: Few population variations existed with *Salmonella* Dublin as 99.25% of the isolates belonged to the MLST ST10. On the contrary, more variation existed with *Salmonella* Typhimurium populations, although 92.63% of the isolates were assigned to the MLST ST19. The two serovars: *Salmonella* Dublin (79.66%) and *Salmonella* Typhimurium (22.49%) mostly simultaneously carried resistant genes against drugs belonging to antibiotic classes aminoglycoside, beta-lactam, sulfonamide, tetracycline, fluoroquinolones, phenicol and antimicrobial peptides. The IncX1 plasmid was found in 93.19% of *Salmonella* Dublin isolates whereas only 0.51% of *Salmonella* Typhimurium isolates carried this plasmid. On the other hand, 39.01% of *Salmonella* Typhimurium isolates possessed the IncFIB(S) plasmid which was missing in all the 1337 *Salmonella* Dublin isolates. Similar observations existed with virulence genes where the sspH2, sseK2, and gogB genes were detected in over 80% of the *Salmonella* Typhimurium isolates but found in less than 1% of the *Salmonella* Dublin isolates. Finally, there were also 182 and 141 proteins unique to *Salmonella* Dublin and *Salmonella* Typhimurium respectively. A large proportion of these were hypothetical proteins and for both serovars, the functions of some of the annotated proteins were mostly related to DNA binding, transport, and oxidoreductase activities.

Conclusions: As a bacterium, it is quite evident that, in the journey from being generalist serovars to developing some host-adapted and host-restricted serovars, *Salmonella* might have engaged in some gene trading, both gaining and losing some and this may have influenced the survival and pathogenicity of certain serovars in their hosts. More comparative studies are needed to unravel some of these key markers that could be exploited for tailored therapeutics, identification, and serovar differentiation. Host-related factors are also important to be considered in such studies and for *Salmonella* Typhimurium and *Salmonella* Dublin, the cattle host may be an ideal subject because, in this single host, a host-adapted serovar and a generalist serovar are both commonly found.

Financial Support: The study was supported by a grant from USDA/NIFA/CBG 2021-38821-34710, MSU/USDA/NIFA RC113747TU.

Notes:

P175 - Prevalence of *Salmonella* enterica in beef-on-dairy cattle: Investigating management factors in calves and growers

H.B. Sridhar[1], R.G. Amachawadi[1], S.N. Dasari[1], H. Wang[2], T. Mahmood[1], H. Salih[1], H. Alneaemy[1], P.A. Lancaster[1], A.J. Tarpoff[3], P.R. Broadway[4], K.E. Hales[5], T. Barnhardt[6], M.E. Theurer[7], C.A. Cull[8], T.G. Nagaraja[9]

[1]Department of Clinical Sciences, Kansas State University, [2]Department of Statistics, Kansas State University, [3]Department of Animal Science & Industry, Kansas State University, [4]United States Department of Agriculture-Livestock Issues Research Unit, Lubbock, Texas, [5]Department of Animal and Food Sciences, Texas Tech University, [6]Consulting Veterinarian, Southwest Kansas, [7]Veterinary Research and Consulting Services LLC, [8]Midwest Veterinary Services, [9]Diagnostic Medicine/Pathobiology, Kansas State University. haribala30@vet.k-state.edu

Session: Salmonella, 2025-01-20, 6:00 - 8:00

Objective: *Salmonella* enterica is a foodborne pathogen of major public health concern. In the US, non-typhoidal *Salmonella* is estimated to cause *annually* about 1.35 million infections, 26,500 hospitalizations, and 420 deaths. Currently, there are no studies on the prevalence of *Salmonella* in beef-on-dairy cross cattle. Our objectives were to conduct a comprehensive analysis of feeding and management practices, including data on morbidity and mortality, from birth to harvest to determine their associations with *Salmonella* prevalence in feces and lymph nodes.

Methods: A total of 529 pen floor fecal samples were collected randomly from feedlots in 5 different states across US. *Salmonella* isolation and identification were done by culture method, agglutination and PCR detection. Responses to a survey questionnaire related to farm management practices were collected from these feedlots and analyzed by LASSO logistic regression. A logistic regression model was fitted with *Salmonella* prevalence as the response variable and the main and interaction effects between production stage and State as predictors.

Results: The overall prevalence of S. enterica in fecal samples was found to be 14.5% (77/529), with calves and growers having prevalence rate of 7.3% (22/303) and 24.33% (55/226), respectively. Among the five states, Kansas (8.7%) and Texas (62.1%) had high prevalence of *Salmonella* among calves and growers respectively. Similarly, Texas had the highest cumulative *Salmonella* prevalence (23.58%) across all five states. However, states like Colorado (5%) and Nebraska (2.5%) showed very low *Salmonella* prevalence among calves. No positive *Salmonella* isolates were observed in Indiana among growers. Interestingly, odds of *Salmonella* prevalence were found to be significantly associated ($P<0.001$) with geographical region and production stage. Four different management practices were also shown to potentially affect the prevalence of *Salmonella* in beef-on-dairy cattle.

Conclusions: Our study provides valuable insights into the geographical and age-related variations in the prevalence of *Salmonella* enterica among beef-dairy cross cattle. These findings emphasize the need for region-specific control strategies in US to mitigate spread of *Salmonella* across food chain.

Financial Support: Research coordinated by the National Cattlemen's Beef Association, a contractor to the Beef Checkoff.

Notes:

P176 - Crimean-Congo hemorrhagic fever seropositive cases among livestock in the Tavush Region of Armenia

Armen M. Danelyan[1], Pertsh G. Tumanyan[1], Ashkhen A. Hovhannisyan[1]

[1]Reference Laboratory of Especially Dangerous Pathogens. armen.danelyan.85@gmail.com

Session: Tick-borne diseases, 2025-01-20, 6:00 - 8:00

Objective: Crimean-Congo hemorrhagic fever (CCHF) is a vector-borne viral zoonosis. The virus circulates in a tick-vertebrate-tick cycle but can also be transmitted horizontally and vertically within the tick population. As animals do not develop clinical signs, CCHF has no economic impact connected with animal production, however animals play a crucial role in the life cycle of ticks, and in the transmission and amplification of the virus. The major route of infection for humans is represented by the bites of infected ticks, but also by the exposure to the blood of infected animals. In 2022, it was previously reported that CCHF virus (CCHFV) was detected by polymerase chain reaction (PCR) in ticks collected in the Tavush Region of Armenia. The aim of this study was to determine the presence of antibodies to CCHFV in cattle and small ruminants in the Tavush Region.

Methods: The study was conducted in 2023 from a random sampling of 120 blood samples (75 cattle and 45 small ruminants) submitted to the Reference Laboratory for Especially Dangerous Pathogens (RLEDP) that were previously submitted for other investigations. Samples were tested by the enzyme-linked immunosorbent assay (ELISA) method using a multi-species commercial assay kit for the detection of antibodies against CCHFV.

Results: Out of the 120 samples, 7 samples (5.8%) tested positive for CCHFV antibodies. The 7 positive cases by species were 5/75 (6.6%) in cattle and 2/45 (4.4%) in small ruminants. The 7 cases were distributed in 4 villages within the Tavush Region: 3 cases were from Koghb, 2 from Sevqar, 1 from Berd, and 1 from Azatamut. The distance between these 4 villages ranges from 10-55 km.

Conclusions: While this initial pilot study provides limited information and sample size, it does identify the presence of CCHFV antibodies in cattle and small ruminants in the Tavush Region of Armenia. We recommend the expansion of this study to the other regions of Armenia, coupled with additional molecular diagnostics, to improve our understanding of CCHFV in Armenia. Future studies will be important to include animals and CCHFV vectors so that we can better understand the presence of CCHFV in Armenia and the epidemiology of CCHF.

Notes:

P177 - Inhibiting Anaplasma marginale adhesion: Exploring Msp1a and Msp1b as potential targets for preventing bovine anaplasmosis

Roberta Koku[1], Susan M Noh[2]

[1]Department of Verterinary Microbiology and Pathology, Washington State University, Pullman Washington, [2]Animal Disease Research Unit, United States Department of Agricultural, agricultural Research Service, Pullman Washington. roberta.koku@wsu.edu

Session: Tick-borne diseases, 2025-01-20, 6:00 - 8:00

Objectives: Bovine anaplasmosis, caused by *Anaplasma marginale*, is an often severe, production limiting disease of cattle which disproportionally affects small holder farmers in low and middle-income countries. Better methods to prevent this disease are needed. *A. marginale* is an obligate, intracellular bacterium. Consequently, for successful tick transmission, it must attach, invade and replicate in tick cells. Developing methods to block pathogen attachment and thus entry into these host cells could serve as method to prevent bovine anaplasmosis. Msp1a and Msp1b mediate attachment of *A. marginale* to tick cells and form a heterodimer of the surface of the bacterium. When added separately as recombinant protein to tick cells, each competes with *A. marginale* for host cell binding and consequently reduces *A. marginale* entry. While the binding domain of Msp1a is in the N-terminus of the protein, the binding domain of Msp1b is unknown. Msp1a and Msp1b may serve as components in a vaccine to block tick transmission. To guide antigen selection and vaccine formulation, the goals of this work are to: 1) Determine if the ability of Msp1a and Msp1b to block *A. marginale* binding to tick cells is dose dependent; 2) Determine if Msp1a and Msp1b in combination enhance blocking of *A. marginale* entry; 3) Identify the binding domain of Msp1b and determine its level of conservation across strains.

Methods: Concentrations varying from 0.5 to 3.0 uM of recombinant Msp1a or Msp1b or 2.0 uM of each combined were added to *Dermacentor andersoni* tick cells. *A. marginale* was then added at an MOI of 1:100 to infect the tick cells and allowed to replicate for 48 hours. The cells were harvested, and *A. marginale* levels measured using RT-qPCR. One-Way ANOVA was used to determine differences between groups. Protein-binding prediction software was used to identify Msp1b binding domains, which will be followed by experimental validation. To determine the amino acid identity of the predicted binding domain, Msp1b was PCR amplified from strains from different geographic regions, sequenced and aligned using Clustal Omega.

Results: There was a 0.6 to 4.0-fold decrease ($p < 0.0001$) and a 2.0 to 7.2-fold decrease ($p = 0.0062$) in *A. marginale* levels dependent on the concentration of Msp1a or Msp1b, respectively. When Msp1a and Msp1b were used in combination, there was up to a 12-fold decrease ($p < 0.0001$) in *A. marginale* levels. Glycoprotein binding domains are predicted in the N and C terminus of Msp1b, with a high confidence level in the C terminus. There was 1.8-fold decrease in *A. marginale* entry using the N terminus of Msp1b, indicating the binding domains may be conformational or span the N and C termini. There is 65% amino acid conservation of predicted binding domains across strains.

Conclusions: Msp1a and Msp1b significantly reduce *A. marginale* entry into tick cells in a dose-dependent manner. Importantly the proteins combined yielded greater blocking than either protein alone. Additional work is required to identify the Msp1b binding domain and determine whether antibodies targeting Msp1a and Msp1b can prevent tick transmission of *A. marginale*.

Financial Support: Animal Disease Research Unit, United States Department of Agricultural, Agricultural Research Service, Pullman Washington.

Notes:

P178 - Vector borne diseases surveillance in Georgia

Ana Gulbani[1], Marina Donduashvili[1], Vasil Basiladze[1], Tengiz Chaligava[1]

[1]State Laboratory of Agriculture, Georgia. ana.gulbani@sla.gov.ge

Session: Tick-borne diseases, 2025-01-20, 6:00 - 8:00

Objective: Bluetongue (BT) is a vector-borne viral disease that affects wild and domestic ruminants. In many parts of the world infection has a seasonal occurrence. The vertebrate hosts for BTV include both domestic and wild ruminants. Virus may be introduced to a free area via infected insects, live ruminants or in contaminated products that are then transmitted to susceptible ruminants. West Nile fever is a disease caused by West Nile Virus (WNV), which is a flavivirus It causes disease in humans, horses, and several species of birds. Currently, we do not know distribution of both diseases and main objective of the study is Ascertain the presence, distribution, and prevalence BT and WNV among the hosts -domestic animals and - Identify vectors of BT and WNF. Last Bluetongue outbreak in Turkey was reported to the OIE in 2019 and West Nile fever outbreak was reported in Armenia 2024.

Methods: In project participates two agency National Food Agency (NFA)- responsible for collecting the vectors and samples from domestic ruminants and State Laboratory of Agriculture of Georgia (SLA)- responsible for identification of vectors and investigation of animal samples by serological (ELISA) and molecular biology methods (RT-PCR).

Results: A total of 740 blood and 740 serum samples were submitted for testing for BT, from different regions of Georgia. Samples were tested by RT-PCR, and by ELISA (INGezim BTV DR R.12.BTV.K0), all samples were negative. To test on WNF included blood and serum samples from 227 horses, tested by PCR and ELISA method, results - all negative. Culicoides species that were endemic in Georgia and also those species that only migrated to Azerbaijan were identified.

Discussion: Because vectors have been identified for bluetongue in Georgia, it can be assumed that ecological changes, as well as the expansion of tourism and trade relations, may lead to the introduction of vector-borne diseases from neighboring countries, and therefore, we continue to collect samples at the borders of neighboring countries. This study would greatly strengthen our knowledge of vector borne infections, promote collaborations, and help to develop prevention and control strategies for vector borne infections in two agencies in Georgia. The research study was made possible by support provided by US Defense Threat Reduction Agency (DTRA).

Financial Support: The research study was made possible by support provided by US Defense Threat Reduction Agency (DTRA).

Notes:

P179 - Evaluation of the use of sub-immunodominant RAP-1 family antigens of Babesia bovis as subunit vaccine components

Manuel J. Rojas[1,2], Reginaldo G. Bastos[1,3], Jinna Navas[1], Heba F. Alzan[1,4], Jacob M. Laughery[3], Paul A. Lacy[3], Massaro W. Ueti[1,3], Carlos E. Suarez[1, 3]

[1]Washington State University, [2]Universidad Nacional de Colombia, [3]USDA, [4]National Research Center Egypt. reginaldo.bastos@usda.gov

Session: Tick-borne diseases, 2025-01-20, 6:00 - 8:00

Objectives: Bovine babesiosis caused by the tick-borne apicomplexan parasite *Babesia bovis*, remains a threat for cattle worldwide, and new vaccines are needed. A plausible promising approach is based on the use of immune-subdominant (ISD) antigens, such as the RAP-1 Related Antigen (RRA) and the Rhoptry Associated Protein-1 NT (RAP-1 NT) fragment as vaccine candidates using a FliC based adjuvant.

Materials: We first obtained purified recombinant RRA, RAP-1NT, and FliC from a prokaryotic expression system. The three recombinant antigens were used for iELISA, immunoblots, and cattle immunizations.

Results: *B. bovis*-protected animals demonstrated high antibody responses against the known immunodominant recombinant (r) rRAP-1 CT antigen, but significantly lower levels against the rRAP-1 NT and rRRA antigens, confirming the immune-sub dominance of these two antigens. Next, a group of cattle (n=6) was vaccinated with purified rRRA, rRAP-1 NT using a FliC-Emulsigen mix as adjuvant, and a control group (n=6) with the adjuvant mix alone. All but one, immunized animals demonstrated elicitation of strong humoral immune responses against the two ISD antigens. Acute babesiosis occurred in both groups of cattle upon challenge with virulent *B. bovis*, but a significant delay in the average rate of decrease of hematocrit in the vaccinated group, and an early monocyte response (a correlate for protection against acute bovine babesiosis), was found in half of the vaccinated animals.

Conclusions: We confirmed the immune-sub dominance of rRRA, rRAP-1 NT and the ability of FliC to increase immunogenicity of ISD antigens and generating useful information towards developing future subunit-vaccines against *B. bovis*.

Notes:

P180 - Pathogen sharing in a diverse tick community found on wild birds and bird workers in South Texas (USA)

Julia Gonzalez[1], Mark Conway[2], Sarah A. Hamer[1]

[1]Texas A&M University, [2]Master Bird Bander. julsglezglez@gmail.com

Session: Tick-borne diseases, 2025-01-20, 6:00 - 8:00

Objective: The Rio Grande Valley (RGV) is part of the natural border between South Texas and Mexico, a key location for many vector-borne pathogen transmission cycles due to its tropical climate and animal migration passage. We explored the presence of tick-borne pathogens by testing ticks collected opportunistically during bird banding activities in 2019-2024.

Methods: Birds were captured using mist nets and examined to collect attached ticks; in addition, ticks attached to humans or crawling around the area were collected. Tick species were identified morphologically and molecularly. Applying PCR-DNA sequencing approach, ticks were tested for Ehrlichia and Rickettsia species targeting dsb and 17KD genes, respectively. For confirmatory purposes, ticks that screened positive were then tested using specific PCRs for the pathogens E. chaffeensis and E. ewingii, and we also amplified and sequenced the gltA and ompA genes of Rickettsia.

Results: Eight tick species were identified among the 375 ticks collected, including species regarded as locally established (Amblyomma inornatum, A. maculatum, A. mixtum, A. tenellum, Dermacentor variabilis), neotropical species imported by migratory birds (A. geayi, A. longirostre) and for the first time in Texas, Ixodes keiransi, formerly the North American lineage of Ixodes affinis. Amblyomma tenellum was the most abundant (89.3%). Screening for tick-borne pathogens resulted in 1.1% (4/375) of the ticks positive for an Ehrlichia species (Clopper-Pearson exact 95% CI: 0.3%-2.7%). Ehrlichia chaffensis was detected in three unfed A. tenellum ticks found crawling, and one E. ewingii positive in an A. inornatum nymph collected off a Clay-colored Thrush (Turdus grayi). Both bacteria can cause human ehrlichiosis, a tick-borne disease infrequently reported in Texas. In addition, 3.5% (13/375) of the ticks were positive for a Rickettsia species (Clopper-Pearson exact 95% CI: 1.8%-5.8%). The potentially pathogenic species of the spotted fever group Rickettsia amblyommatis was identified in nine ticks: eight A. inornatum ticks, seven of which were collected off two Long-billed Thrashers (Toxostoma longirostre), and an A. longirostre engorged nymph from an Acadian flycatcher (Empidonax virescens). We also detected putative Rickettsia endosymbionts in four ticks that need to be further investigated.

Conclusions: Our results highlight the importance of migratory birds as one of the main routes of tick dispersal and warn of occupational tick exposure in this area to establish measures of prevention. There is also a critical need to investigate the roles of the relatively neglected human-biting A. tenellum and the bird-imported ticks A. inornatum and A. longirostre in the transmission of emerging and neglected tick-borne diseases.

Financial Support: Schubot Center for Avian Health, Texas A&M University.

Notes:

P181 - Surveillance for Theileria orientalis and the invasive tick Haemaphysalis longicornis in three Missouri beef herds

Rosalie Ierardi[1, 2], Savannah Chance[3], Celeste Morris[4], Jacqueline Nunnelley[1, 2], Solomon Odemuyiwa[1, 2], Angela Royal[1, 2], Loren Schultz[1, 4], Zhenyu Shen[1, 2], Jordyn Young[5], Ram Raghavan[1, 2, 6]

[1]Veterinary Medical Diagnostic Laboratory, University of Missouri, [2]Department of Veterinary Pathobiology, University of Missouri, [3]Division of Animal Sciences, University of Missouri, [4]Department of Veterinary Medicine and Surgery, University of Missouri, [5]College of Veterinary Medicine, University of Missouri, [6]Department of Public Health, University of Missouri. smcbd8@umsystem.edu

Session: Tick-borne diseases, 2025-01-20, 6:00 - 8:00

Objective: Theileria orientalis is a protozoan hemoparasite of cattle vectored by the rapidly emerging invasive longhorned tick (Haemaphysalis longicornis). Theileria-associated bovine anemia (TABA) is easily mistaken for bovine anaplasmosis, which can lead to delayed diagnosis in areas where bovine anaplasmosis is endemic and TABA is newly emerging. Our objective was to surveil for infestation of cattle by H. longicornis and infection with T. orientalis on three Missouri cow-calf operations in counties where H. longicornis is known to be established.

Methods: A total of 147 apparently healthy adult cows from 3 herds were inspected for ticks. Whole blood was collected for Theileria orientalis and Anaplasma marginale real-time PCR and was also used for immediate preparation of blood smears and measurement of packed cell volumes.

Results: A total of 527 ticks were collected from the cows and taxonomically identified to the species level. Eighteen H. longicornis, including 9 adult females and 9 nymphs, were collected from 16 cows (Farm A, 2 cows; Farm B, 4 cows; Farm C, 10 cows). Intraerythrocytic T. orientalis organisms were presumptively identified on blood smears from 10 cows. PCR screening of blood samples with primers designed to amplify all T. orientalis genotypes detected 11 positive samples (Farm A, 7 cows; Farm B, 3 cows; Farm C, 1 cow). Positive samples were re-tested with probes specific for the Ikeda, Chitose, and Buffeli genotypes, which detected the Chitose genotype in 10 samples and the Ikeda genotype in 1 sample. Anaplasma marginale was detected by PCR in 67 out of 147 samples (45.6%). Nine cows were PCR-positive for both T. orientalis and A. marginale.

Conclusions: Detection of T. orientalis with concurrent infestation of cows by H. longicornis within these 3 herds, along withcollection of H. longicornis from vegetation on the premises, supports local tick-borne transmission of this emerging pathogen.

Financial Support: This work was supported by the USDA National Institute of Food and Agriculture, Animal Health Formula Fund, project 7003929.

Notes:

P183 - Vaccine production using Bryophyllum pinnatum extracts against Mycobacterium avium isolated from poultry birds

ThankGod Onuoha[1]

[1]Novena University. onuohakelechi6@gmail.com

Session: Vaccinology, 2025-01-20, 6:00 - 8:00

Objectives: (1) to extract glycosylated antigen85A (G-Ag85A) from Bryophyllum pinnatum (Miracle leaf) and non-glycosylated antigen85A (NG-Ag85A) in Escherichia coli system using recombinant technique. (2) to evaluate the immune response and potential protective effectiveness of G-Ag85A, NG-Ag85A, and the Bacille Calmette- Guerin (BCG) vaccine by comparing their reactions to the Mycobacterium avium sub specie Avium (MAA CVCC275) strain in a poultry bird model.

Methods: A total of 40 poultry birds were purchased from Delta State University, Abraka, Nigeria. Approval was obtained from the Ethics Committee under the Animal Care and Use Committee Act of Nigeria. Gel electrophoresis technique (SDS-PAGE) was used to extract and purify glycosylated antigen 85A (G-Ag85A) from Bryophyllum pinnatum harvested from Novena University environment and confirmed by a botanist. A non-glycosylated form of Ag85A (NG-Ag85A) were produced in an Escherichia coli recombination system. The MAA CVCC275 strain was obtained from the National Tuberculosis Research Institute, Nigeria, and the Bacillus Calmette-Guerin (BCG) vaccine were obtained from the Pasteur Institute (Pasteur 1173P2). The ability to elicit immune response was compared between the two forms of Ag85A and the BCG vaccine. The 40 birds were divided into four experimental groups: 1) (n=10) receiving the G-Ag85A; 2) (n=10) receiving the NG-Ag85A; 3) (n=10) receiving the BCG vaccine; and 4) (n=10) with no treatment. All birds were inoculated with the MAA CVCC275 strain. After 152 days, tissues were harvested from the birds, and analyzed by flow cytometer. The statistical package for social sciences (IBM SPSS version 22) was used to conduct the analysis. Distinctions among the four groups was examined through an unpaired Student's T-Test. Tukey's multiple comparison tests were used to analyze the data from these groups after one-way ANOVA. The data were reported as means (± standard deviations, SDs), with statistical significance determined at $p< 0.05$.

Results: It was discovered that the G-Ag85A induced a robust gamma interferon reaction with a value of 88±0.1 compared to that of the NG-Ag85A with a value of 25±1.1 and BCG with a value of 44±2.1, indicating host immune recognition of G-Ag85A when there is an infection with MAA. The pilot experiments carried out in birds, the plant source vaccine (G-Ag85A) showed a protective and a better immune response at a significant value of $p< 0.05$ against Avian Tuberculosis compared to the currently used BCG vaccine.

Conclusion: The study, therefore recommends the use of G-Ag85A from Bryophyllum pinnatum since it induced a robust gamma interferon reaction against Avian Tuberculosis when compared to the available BCG vaccine and suggest further research for the development of a new plant-based AT vaccine.

Financial Support: I knowledge myself and families especially my wife for assisting me financially during this project work.

Notes:

P184 - Electron beam (eBeam)-killed multivalent vaccines to control *Clostridium perfringens* and *Mycoplasma gallisepticum* in chickens

Palmy Jesudhasan[1], Annie Donoghue[1], Komala Arsi[1], Jeff Evans[1], Ali Nazmi[2], Suresh, D. Pillai[3], Joseph L. Purswell[1], Anna Assumpcao[4]

[1]USDA-ARS, [2]The Ohio State University, [3]Texas A&M University, [4]University of Arkansas. palmy.jesudhasan@usda.gov

Session: Vaccinology, 2025-01-20, 6:00 - 8:00

Objectives: (a) To prepare a multivalent Clostridium perfringens (CP) (mixture of 6 or more strains) vaccine using eBeam technology and determine its efficacy in protecting broiler chickens when challenged with homologous and heterologous strains of CP. (b) To prepare a multivalent Mycoplasma gallisepticum (MG) (mixture of 6 or more strains) vaccine using eBeam technology and determine its efficacy in protecting layer chickens when challenged with homologous and heterologous strains of MG.

Methods: All CP poultry isolates will be grown independently in anaerobic conditions at 37˚C using a Fluid Thioglycolate (FTG) medium. All MG strains will be grown independently in aerobic conditions at 37˚C using Frey's medium and pooled in equal proportions. Both CP strains and MG strains will be exposed to lethal eBeam exposure at Texas A&M University. The eBeam-exposed-CP and MG cells will be tested in vitro and in vivo assays to test the effects of eBeam. We will conduct bird studies to test the efficacy of eBeam-inactivated CP and MG vaccines.

Results: We have obtained results from in vitro assays of CP and MG strains exposed to lethal eBeam dose. Results indicate that the lethal eBeam dose did not affect cell membrane of both CP and MG strains.

Conclusions: This novel approach using eBeam technology offers a promising avenue for developing effective and safe vaccines against CP and MG. Future bird studies will evaluate the efficacy of these vaccines in protecting poultry against homologous and heterologous challenges.

Financial Support: This project was supported by Agriculture and Food Research Initiative Competitive Grant no. 2024-67015-42629 from the USDA National Institute of Food and Agriculture.

Notes:

P185 - Rationally designed *Mycoplasma gallisepticum* vaccine using a recombinant subunit approach

Jeremy Miller[1], Rosemary Ozyck[1], Patrick Pagano[1], Esmeralda Hernandez[1], Megan Davis[1], Anton Karam[1], Jessica Malek[1], Arlind Mara[1], Edan Tulman[1], Steven Szczepanek[1], Steven Geary[1]

[1]Department of Pathobiology and Veterinary Science, The University of Connecticut. steven.geary@uconn.edu

Session: Vaccinology, 2025-01-20, 6:00 - 8:00

Objective: Mycoplasma gallisepticum (MG) is the primary etiologic agent of avian mycoplasmosis, which predisposes infected poultry to subsequent co-infections with other pathogens leading to a pathological condition known as chronic respiratory disease. The objective of our work is to develop an MG subunit vaccine to protect from disease caused by MG that retains the safety profile of a bacterin with no possibility of reversion to virulence, by targeting key protective antigens.

Methods: We developed a subunit vaccine consisting of the primary adhesin GapA, the cytadhesin-related molecule CrmA, and four early-phase-expressed Variable Lipoprotein Hemagglutinins (VlhAs) (3.03, 3.06, 4.07, 5.05) of the virulent strain R_{low}. Recombinantly produced proteins are administered at a dose of 50 µg per protein combined with different adjuvant formulations utilizing a prime-boost schedule with three weeks between doses. Four-week-old specific pathogen free female white leghorn chickens were vaccinated subcutaneously between the back and right wing with 500 µL of saline for negative control or of the specified vaccine formulation. Three weeks later, chickens were boost -vaccinated with the same vaccine dose and route. Blood was collected 20 days later from the wing vein or jugular vein. At three weeks post-boost vaccination, chickens were challenged intratracheally on D0 with $1*10^8$ CFU of Mycoplasma gallisepticum given in 200 µL of Hayflick's media. Chickens were challenged a second time using the same dose and route on D2. Chickens were infected in sets of five chickens per group, cycling between the different groups. Chickens were sacrificed on D14. Chickens were sacrificed in sets of five chickens cycling between the different groups. Three sections 1 cm in length each were removed from the proximal, middle, and distal portions of the trachea (three total section per trachea) and placed together into tubes containing 3mL of Hayflick's media. The tubes were then placed on ice. Upon completion of the necropsies, tubes were vortexed 4x for 30 seconds each time and incubated at 37ºC for 3 hours. Samples were then filtered through .45 uM filters. 200 µL of sample was loaded in duplicate into a 96 well plate. Five-fold serial dilutions were performed into fresh Hayflick's medium. The plates were sealed, wrapped in Parafilm, and incubated at 37ºC for 28 days. The tubes were also incubated at 37ºC for 28 days. Growth was determined by a medium color shift from red to yellow. The duplicate wells were averaged to determine color changing units.

Results: All recombinant proteins were immunogenic. No gross lesions were found at necropsy in vaccine groups that showed efficacy. Alum-, MPLA-, CpG ODN-, and Pam2CSK4-Adjuvanted-Vaccines reduced bacterial recovery. CpG ODN-Adjuvanted-Vaccine reduced average tracheal thickness.

Conclusions: We have demonstrated that our subunit vaccine formulation containing the CpG ODN 2007 adjuvant provides protection against MG disease in chickens as determined by significant decreases in recoverable MG load from the trachea and significant decreases in both average tracheal thickness and thickest tracheal section. Our work highlights the value of our biologically informed and rational approach to developing a vaccine to combat MG disease.

Financial Support: This work was supported by USDA-NIFA grant #2022-67016-37222.

Notes:

P186 - Broadly protective bovine parainfluenza 3 virus and bovine viral diarrhea virus vaccine

Waithaka Mwangi[1], Huldah Sang[1], Tae Kim[1], Rakshith Kumar[1], Jayden McCall[1], Brandon Plattner[1], Katherine Bauer[1]

[1]Kansas State University. wmwangi@vet.k-state.edu

Session: Vaccinology, 2025-01-20, 6:00 - 8:00

Objective: Develop broadly protective Bovine Parainfluenza 3 Virus (BPI3V) and Bovine Viral Diarrhea Virus (BVDV) vaccines.

Methods: A prototype vaccine containing mosaic antigens designed from twenty BVDV genomes, conferred protection against BVDV1-2 strains. To improve efficacy, mosaic envelop (E2) and non-structural (NS2-5) antigens, designated E2-NS2-5, were designed from >200 genomes. Similarly, mosaic BPI3V antigens, designated F2 and HN2 (F2-HN2), were designed. Attenuated BPI3V genotype C (BPI3Vc) vector was generated by introducing mutations responsible for the attenuation of the BPI3Va commercial vaccine. The vector was used to generate viruses expressing the F2-HN2 (BPI3Vc-F2-HN2) or the E2-NS2-5 (BPI3Vc-E2-NS2-5) mosaic antigens. A recombinant virus expressing an irrelevant antigen (BPI3Vc-TMSP67) was also generated. Animal studies were conducted to test the hypothesis that immunization of calves with the BPI3Vc-F2-HN2 or the BPI3Vc-E2-NS2-5 virus constructs, would confer protection against BPI3V and BVDV strains. In the first study, calves were immunized with the BPI3Vc-F2-HN2 virus. Calves immunized with a commercial BPI3Va vaccine served as a positive control, whereas sham treated calves served as negative controls. Vaccine safety and immunogenicity was evaluated and challenge with wildtype BPI3Vc strain was used to determine protection. Sera was tested for cross-neutralization of disparate BPI3Va-c strains. In the second study, calves were immunized with the BPI3Vc-E2-NS2-5 virus and calves immunized with a commercial BVDV vaccine served as a positive control, whereas BPI3Vc-TMSP67-immunized calves served as negative controls. Safety and immunogenicity of the vaccine was evaluated and challenge with wildtype BVDV1b strain was used to determine protection. Sera was tested for cross-neutralization of BVDV1-2 strains. At study termination, tissue samples were analyzed and scored for pathological lesions. The significance of the differences in immune readouts, viremia, clinical scores, and pathological lesions between the treatment and controls were analyzed and compared.

Results: A modified BPI3Vc vector was generated by introducing mutations responsible for the attenuation of the BPI3Va commercial vaccine. A recombinant BPI3Vc-GFP virus was generated and used to confirm virus attenuation as judged by temperature sensitivity. Transfection of HEK293A cells with a plasmid construct encoding a novel chimeric F2-HN2 mosaic polypeptide, designed using all characterized BPI3a-c proteomes, expressed the transgene as judged by IFA and Western blots using tag-specific mAbs and authenticated using convalescent serum. Similarly, tag-specific and BVDV-specific mAbs as well as hyperimmune sera, confirmed protein expression and authenticity by genes encoding novel E2-NS2-5 mosaic polypeptides. Recombinant viruses, BPI3Vc-F2-HN2 and BPI3Vc-E2-NS2-5, were generated and shown to express the encoded antigens. Immunization of calves with either the BPI3Vc-F2-HN2 or the BPI3Vc-E2-NS2-5 virus constructs, elicited strong antibody responses. Compared to calves immunized with commercial vaccines, the BPI3Vc-F2-HN2 vaccinees had significantly higher neutralizing antibodies against representative BPI3Va-c viruses, whereas the BPI3Vc-E2-NS2-5 vaccinees had higher neutralizing antibodies against representative BVDV1-2 strains. The prototype vaccines conferred protection upon challenge.

Conclusions: Two promising novel prototype vaccines, BPI3Vc-F2-HN2 and BPI3Vc-E2-NS2-5, were developed and shown to safely confer protection. Importantly, sera from the vaccinees neutralized diverse BPI3V and BVDV-1&2 strains. Development of broadly protective vaccines for control of diverse BPI3V and BVDV strains will improve BRDC control.

Financial Support: This project is supported by Agriculture and Food Research Initiative Competitive Grant no. 2023-67015-39736 from the USDA National Institute of Food and Agriculture.

Notes:

P187 - Mucosal adjuvants potentiate the efficacy of mannose conjugated chitosan nanoparticle-based oral *Salmonella* subunit vaccine in broilers

Raksha Suresh[1], Shekoni O Comfort[1], Sara Dolatyabi[1], Jennifer Schrock[1], Mithilesh Singh[1], Renukaradhya J Gourapura[1]

[1]Center for Food Animal Health, The Ohio State University. suresh.138@osu.edu

Session: Vaccinology, 2025-01-20, 6:00 - 8:00

Objective: Salmonellosis continues to be one of the major public health food safety concerns worldwide causing the gastrointestinal disease. Poultry meat and eggs are recognised as the major source of *Salmonella* food poisoning in humans. Our study evaluated the protective efficacy of adjuvanted mannose-conjugated chitosan-nanoparticle (mChitosan-NP) based oral subunit vaccine consisting of immunogenic outer membrane proteins and flagella (FLA) of *Salmonella* Enteritidis [mChitosan (OMP+FLA)/FLA-NP], against the bacterial colonization in the intestines of broiler chickens. The mucosal adjuvants used in the study were, c-di-GMP (stimulator of interferon gene agonist) and whole cell lysate (WCL) of Mycobacterium smegmatis.

Methods: Three concentrations of WCL and cyclic-di-GMP (2.5µg, 10µg, and 50µg) encapsulated in mChitosan-NP were tested separately with mChitosan (OMP+FLA)/FLA-NP vaccine containing fixed single dose of combined OMP+FLA proteins (20µg/dose). The broiler birds were vaccinated orally at age 3 days and 3 weeks and challenged at 5 weeks with 5×10^7 CFU of S. Enteritidis and euthanized at day post challenge (DPC) 4 and 10. Cecum was evaluated for challenge bacterial load and the immune responses were determined at local mucosal and systemic sites.

Results: Physical characteristics of mChitosan (OMP+FLA)/FLA-NP, mChitosan-GMP/FLA-NP and mChitosan-WCL/FLA-NP formulations revealed a high positive charge (Zeta potential +20-25mV), size 235-260nm, and polydispersity index 0.35-0.52, conducive for oral delivery. Our data showed that mChitosan (OMP+FLA)/FLA-NP WCL 10µg/dose group consistently reduced the S. Enteritidis load by over 0.5 $\log_{10}$ comparable to a commercial live vaccine at both DPC 4 and 10. Immunologically, enhanced systemic and mucosal antibody and cellular (B cells frequency) immune responses were induced by adjuvanted mChitosan-NP *Salmonella* subunit vaccine. Additionally, observed upregulation of immune gene expression of cytokines IFN-γ and TGF-β. Statistical differences among groups were determined by one-way ANOVA followed by Tukey's multiple comparison test.

Conclusion: Overall, mucosal adjuvant WCL enhanced the efficacy of mChitosan (OMP+FLA)/FLA-NP vaccine by inducing effective immune responses in broilers.

Financial Support: This work was supported by the USDA National Institute of Food and Agriculture (USDA-AFRI 2022-67017-36559).

Notes:

P189 - MVA-vectored vaccines expressing equine rotavirus A VP7 elicit antibody responses with limited neutralization

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]Dept. of Pathobiological Sciences, Louisiana State University, [2]Louisiana Animal Disease Diagnostic Laboratory, Louisiana State University, [3]Viral Vector Core Facility, 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, 2025-01-20, 6:00 - 8:00

Objective: Equine rotavirus A (ERVA) is a non-enveloped virus with a genome comprised of eleven double-stranded RNA segments. It is a major cause of diarrhea in foals, leading to significant economic losses to the equine breeding industry. ERVA strains are classified into G-genotypes based on the VP7 glycoprotein, which contains neutralizing epitopes. Genotypes G3 and G14 are responsible for the majority of ERVA outbreaks worldwide. However, current vaccines are limited to the G3 genotype and do not completely protect against heterologous strains (i.e., G14 strains). The aim of this study was to develop a vector-based vaccine to address this gap.

Methods: We developed a modified vaccinia virus Ankara (MVA)-vectored vaccine expressing the VP7 glycoprotein from ERVA genotypes G3 and G14 through homologous recombination and demonstrated successful expression of VP7 via Western blotting and immunofluorescence. Subsequently, we vaccinated two groups of eight-week-old male and female BALB/c mice, both intramuscularly (IM) and intraperitoneally (IP), with a two-dose regime at 14-day intervals containing $1´10^7$ PFU of either MVA-G3, MVA-G14, or a combination of MVA-G3+MVA-G14. Additionally, we vaccinated a group of BALB /c mice with formalin-inactivated and adjuvanted preparations of ERVA G3 strain H2, and ERVA G14 strain MCBI. Antibody responses were determined via indirect immunofluorescence and virus neutralization tests against ERVA G3 and G14 strains at 7dpi, 14dpi, and 21dpi for IP, and up to 35dpi for IM vaccination. The geometric mean titers of serum IgG and neutralizing antibodies were analyzed in GraphPad Prism 9 using the Kruskal-Wallis test, with $p < 0.05$ considered significant.

Results: Recombinant MVA viruses successfully expressed the ERVA G3 and G14 VP7 proteins (~37 kDa). No adverse clinical signs were observed in immunized mice following vaccination. Both vectored vaccines, as well as the combination MVA-G3+MVA-G14, induced VP7-specific serum IgG antibodies when administered through the IP or IM routes, with a geometric mean titer of 101.59 and 54.10 at 7 days post-vaccination, to 223.72 and 166.84 at 21 days after the first vaccine dose. In contrast, neutralizing antibody titers were low, with a geometric mean of 16 and no further increase despite the booster dose. Vaccination with inactivated ERVA G3 H2 and ERVA G14 MCBI elicited significantly higher neutralizing antibody titers (1:512 to 1:2048) compared to VP7-vectored vaccines.

Conclusions: The MVA-vectored ERVA VP7 vaccines demonstrated successful expression of VP7 and strong, specific IgG responses. However, these vaccines did not exhibit significant neutralizing activity, regardless of the route of inoculation. This is probably explained by the retention of VP7 within the endoplasmic reticulum, impairing the proper exposure of neutralizing epitopes. Therefore, the inclusion of additional viral glycoproteins (e.g., VP4) may be necessary to induce strong neutralizing antibody responses. Further improvement of this vectored vaccine is warranted.

Financial Support: This study was supported by a Grayson Jockey Club Research Foundation award (Award #863; GR-00011211), USDA 1433 Formula funds (GR-00010866; School of Veterinary Medicine, Louisiana State University) and start-up funds by the School of Veterinary Medicine to Mariano Carossino (PG009641).

Notes:

P190 - Generation of avian paramyxovirus type 1 (AMPV-1) recombinants that protect against Marek's disease

Stephen Spatz[1], Lei He[1], Qingzhong Yu[1]

[1]USDA ARS. Stephen.Spatz@usda.gov

Session: Vaccinology, 2025-01-20, 6:00 - 8:00

Objective: A structural-based vaccine design will be used to create vaccine candidates against Marek's disease virus (MDV) using an AMPV-1-based vector (TS09) that has been proven to be temperature stable and safe for *in-ovo* administration. TS09 recombinants expressing the prefusion conformation of glycoprotein B of MDV with or without the dual expression of the chicken cytokine granulocyte-macrophage colony-stimulating factor (GM-CSF) will be generated. The recombinants will be tested for safety, genetic stability, and protective efficacy in challenge models.

Methods: The gene encoding glycoprotein B was modified with nucleotide substitutions needed to lock the expressed protein in a prefusion conformation as designed by computational modeling. This construct was codon-optimized for chicken usage and synthesized commercially along with a DNA fragment containing a P2A linker upstream of a codon-optimized gene encoding chicken GM-CSF. These two DNA fragments were amplified using PCR and cloned into the pTS09 infectious clone as independent transcription units (ITUs) to express either the gB protein alone or with the expression of GM-CSF. This was accomplished using fusion cloning and insertion between the P and the M genes. Recombinant viruses were reconstituted by transfecting MVA/T7 virus infected HEp-2cells with these constructs along with the supporting plasmids expressing the NP, P, and L proteins of APMV-1. At 72h post-transfection, the rescued viruses, harvested by 2X freeze-thawing the transfected cells, were amplified by inoculating the transfected cell lysates into the allantoic cavities of 10-day-old SPF embryonated chicken eggs (ECE). After 4 days of incubation, the allantoic fluids (AF) were harvested and passed through 0.22 µm filters. The hemagglutination (HA) assay positive allantoic fluids were then propagated in ECE twice. After an additional passage in ECE, the allantoic fluids were harvested, aliquoted, and stored at -80^{o}C.

Results: Immunofluorescence assays confirmed the expression of gB in cells infected with both recombinant viruses. Comprehensive safety assessments, including median death time, intracerebral pathogenicity, and 50% eggs infectious dose, demonstrated that both recombinant viruses retained their non-virulent pathotype and exhibited log reductions in titers ($TCID_{50}$) relative to the parental virus (TS09, 1.76 X 10^8; TS09-gB, 3.12 X 10^7; and TS09-gB/GM-CSF, 1.76 X 10^6).

Conclusions: Two recombinant Newcastle disease virus (NDV) strains, expressing the Marek's disease virus (MDV) gB or gB and GM-CSF, were created using reverse genetics. These recombinant viruses maintained their non-virulent pathotype with lower titers relative to the parental APMV-1. IFA confirmed the expression of MDV gB in infected cells, but GM-CSF expression was not detected due to the lack of a specific antibody. The results suggest that these recombinant viruses, rTS09/MDV-gB and rTS09/MDV-gB/GM-CSF, are promising vaccine candidates for *in-ovo* vaccination against Marek's disease.

Financial Support: The research was supported by a grant from the USDA-NIFA Agriculture and Food Research Initiative.

Notes:

P191 - Safe and broadly cross-protective live attenuated influenza virus vaccines for use in swine

Vanessa Mendieta-Reis[1], Matias Cardenas[1], Sasha Compton[1], Tavis Anderson[2], Amy Baker[2], Daniel Perez[1], Daniela Rajao[1]

[1]University of Georgia, [2]USDA-ARS. m.cardenas@uga.edu

Session: Vaccinology, 2025-01-20, 6:00 - 8:00

Objective: Swine producers and veterinarians continue to be challenged by the rapid evolution of influenza A viruses (FLUAV). Although vaccination is an effective tool for the prevention and control against influenza, available vaccines for use in swine result in limited protection amongst all the antigenically distinct IAV 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 either 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) or rearranged genome (termed Flu-RAM) containing the M2 open reading frame (ORF) downstream of PB1. To further improve the immune stimulation of Flu-att or Flu-RAM, we have modified the hemagglutinin (HA) and neuraminidase (NA) gene segments of swine-adapted H3N2 (A/turkey/Ohio/313053/2004, OH/04) virus to express the porcine IgA-inducing protein (IGIP; Flu-IGIP) or an interleukin immune stimulator (Flu-IL). 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 LAIV backbones. Naïve pigs were vaccinated intranasally with $2x10^5$ $TCID_{50}$ and antibody and cell-mediated immune responses evaluated at 7 days post vaccination (dpv), 7 days post boost (dpb), and 21 dpb.

Results: Virus rescue with all unique molecular markers and individual modified surface genes (Flu-IGIP and Flu-IL) were successful in the Flu-RAM backbone, while rescue with double modified surface genes (Flu-IGIP-IL) was only successful in the Flu-att backbone. In vitro stability was confirmed for a minimum of 5 passages, and in vitro testing for reassortment potential using co-infection assays is ongoing. Strains were tested in pigs for their safety, transmission, and immune responses and immune characterization is ongoing. 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:

P192 - Probiotic Lactobacilli enhances cytokine production in broilers and acts as an adjuvant to oral *Salmonella* vaccine

Sara Dolatyabi[1], khaled Abdelaziz[2], Comfort Olaitan Shekoni[1], Raksha Suresh[1], Jennifer Schrock[1], Ronna Wood[3], Sudhir Yadav[1], Megan Tenney[1], James Sanko[4], Tonima Rahman[1], Renukaradhya J. Gourapura[1]

[1]Department of Animal Science, The Ohio State University, [2]Department of Animal and Veterinary Sciences, Clemson University, [3]The Ohio State College of Veterinary Medicine, [4]Department of Biology, The Ohio State University. dolatyabi.1@buckeyemail.osu.edu

Session: Vaccinology, 2025-01-20, 6:00 - 8:00

Objective: *Salmonella enterica* serovar enteritidis (SE) substantially threatens food safety worldwide, with poultry products being a major source of human infections. This study investigates the immunomodulatory role of Lactobacillus acidophilus as a probiotic supplement in broiler chickens receiving an experimental *S. enteritidis* subunit nanovaccine. Birds were administered with the probiotic in-ovo and/or post-hatch, and the probiotic's effects on cytokine production and immune gene expression were analyzed to understand its role in modulating both mucosal and systemic immunity. The objective is to determine whether probiotic-induced secretion of different cytokines in mucosal lymphoid tissues enhances immune activation, promotes regulatory immune mechanisms, and supports induction of effective immune responses to subunit vaccine against SE challenge infection.

Methods: In this two-phase study, broiler chickens were administered L. acidophilus P42 probiotic in-ovo and/or post-hatch. In the first phase, fifty embryonated eggs were injected into the amniotic cavity with 1x10^6 CFU/egg of L. acidophilus at embryonic day 18 (ED18). After hatching, some birds received additional oral probiotic supplementation (1x10^7 CFU/bird) three times. Birds were sacrificed on day 10 post-hatch, and lymphoid tissues (spleen, bursa of Fabricius, and cecal tonsils) were collected for different cytokines gene expression analysis. In the study's second phase, a similar in-ovo and post-hatch probiotic regimen was followed. Additionally, birds received two doses of an experimental *Salmonella* subunit nanovaccine on days 3 and 21. On day 35, the birds were challenged with SE and sacrificed at 4 days post-challenge to assess immune responses by using quantitive PCR.

Results: Cytokines' gene expression was assessed in lymphoid organs of broilers supplemented with L. acidophilus. In the pre-challenge (phase 1) study, In the cecal tonsils, IL-4 expression was significantly upregulated in the in-ovo group compared to the mock group, indicating enhanced Th2-driven anti-inflammatory and humoral responses. In the bursa of Fabricius, IL-17 production was higher in both the in-ovo and post-hatch received group than in the group that received only the in-ovo probiotic, highlighting a stronger Th17 response, enhancing mucosal defense. In the spleen, IFN-γ production was significantly higher in the in-ovo and post-hatch group compared to the mock group, reflecting robust systemic Th1-driven immunity. In Phase 2, which included vaccination and challenge, the combination of probiotic and nanovaccine resulted in increased IL-4, IFN-γ, and TGF-β compared to the mock + challenge group and commercial vaccine group, though not significantly. Notably, IL-10 and IL-17 were significantly upregulated in the probiotic + vaccine group 4 days post-challenge, suggesting enhanced regulatory and mucosal responses against *Salmonella*.

Conclusions: The findings from this study demonstrate that L. acidophilus supplementations significantly enhance cytokines gene expression in broiler chickens. This immunomodulatory effect is evident through the upregulation of key immune cytokines such as IL-4, IL-10, IFN-γ, and TGF-β in the cecal tonsils, bursa of Fabricius, and spleen. The probiotic-induced modulation of both mucosal and systemic immune responses suggests that L. acidophilus plays a critical role in promoting anti-inflammatory and immune regulatory pathways enhancing specific cellular immunity. These results support the potential of probiotic supplementation as a promising strategy to enhance immune resilience against *Salmonella* in broilers contributing to improved effective prevention against *Salmonella* enterica serovar enteritidis food poisoning.

Financial Support: This work was supported by the USDA National Institute of Food and Agriculture USDA-AFRI 2022-67017-36559.

Notes:

P193 - A pig model to investigate maternal passive immunity after influenza vaccine immunization

John Byrne[1], Diego Leal[1], Danielle Meritet[1], Michael Rahe[1], Tatiane TN Watanabe[1], Juliana B Ferreira[1], Christopher Beverly[2], Susan Johnson[3], Sean N Tucker[3], Stephanie Langel[4], Elisa Crisci[1]

[1]Department of Population Health and Pathobiology, North Carolina State University, [2]Duke Human Vaccine Institute, Duke University School of Medicine, [3]Vaxart, South San Francisco, [4]Center for Global Health and Diseases, Case Western Reserve University School of Medicine. ecrisci@ncsu.edu

Session: Vaccinology, 2025-01-20, 6:00 - 8:00

Objective: Influenza A virus can cause severe complications for pregnant women and infants. New vaccines and strategies are being implemented to increase global access to vaccination in these vulnerable populations. Additionally, there are no influenza vaccines approved for infants younger than six months. The aim of the study was to evaluate the capacity of a hemagglutinin (HA) (A/California/2009(H1N1)) human Ad5 vector vaccine coupled with a TLR3 agonist as adjuvant to induce specific passive immunity in pregnant and lactating pigs using different routes of administration. Pigs were used as a translational model to investigate the protective level of passive maternal antibodies in infants.

Methods: Influenza naïve pregnant pigs were vaccinated via oral, intranasal and intramuscular routes three weeks prepartum and boosted four weeks later (one week postpartum). Serum, colostrum and milk samples, as well as samples from the nasal mucosa and saliva were collected to measure the level of HA-specific antibodies induced by the vaccine over time in different mucosal tissues. Antibody detection was performed via in-house HA-specific ELISA using HA proteins from different A/California/2009 H1N1 strains. Serum, colostrum and milk IgG and IgA antibodies were isolated using a modified column chromatography protocol using Pierce Protein G and Capture Select IgA respectively. IgG and IgA antibodies were detected in saliva, nasal secretions, and piglets' serum without isolation. Antibody levels were evaluated by Optical Density 450nm, as mean ± SD, N=4 per group. One-way ANOVA followed by Šídák's multiple comparisons test was performed using GraphPad Prism (GraphPad Software, Boston, USA).

Results: Intranasal (IN) and intramuscular (IM) immunized pigs showed significant levels of HA-specific antibodies in serum, colostrum, milk when compared to control animals at three weeks post-partum. In milk IM and IN showed approx. one-fold-increase HA-specific IgG compared to controls ($p<0.05$). In serum IN and IM showed approx. four-fold increase of HA-IgG and one-fold-increase of IgA compared to controls ($p<0.05$). The oral route of immunization in pigs elicited poor HA-specific antibodies in all the tissues tested. Piglets nursing from intranasally and intramuscularly vaccinated sows showed a significant level of HA-specific IgG and IgA in serum at 2-3 weeks post-partum. Influenza specific antibody levels varied between the different HA proteins used.

Conclusions: Pregnant and lactating pigs can work as models to evaluate lactogenic immunity for intranasal and intramuscular huAd5-based vaccines. Intramuscular route of immunization of pregnant/lactating pig with a HA-Ad5 vector vaccine showed the highest level of lactogenic immunity and transferred antibodies to suckling piglets.

Financial Support: This work was supported by the Bill & Melinda Gates Foundation [INV-22595D].

Notes:

P194 - Developing a vaccine against spotty liver disease caused by *Campylobacter hepaticus* in commercial layer chickens

Subhashinie Kariyawasam[1], Chaitanya Gottapu[1], Lekshmi Edison[1], Thomas Denagamage[1], Gary Butcher[1]

[1]University of Florida. skariyawasam@ufl.edu

Session: Vaccinology, 2025-01-20, 6:00 - 8:00

Objective: Spotty Liver Disease (SLD) has recently emerged in the US as a significant cause of morbidity and mortality in commercial layer chickens. While antibiotics are currently used for treatment, the presence of transferable plasmids with resistance markers in some strains poses a challenge. Moreover, antibiotics are not suitable for antibiotic-free egg production, emphasizing the need for alternative treatments. Presently, the layer industry depends on autogenous killed vaccines, but the slow and challenging growth characteristics of *C. hepaticus* and uncertain efficacy have limited their use. To address these limitations, we aim to identify vaccine candidates using phage display technology and develop an avian pathogenic *Escherichia coli*-vectored vaccine to protect chickens against SLD and colibacillosis.

Methods: The phage display reverse vaccinology was used to select candidate vaccine antigens. First, antibodies against killed whole-cell *C. hepaticus* were raised in specific pathogen-free white Leghorn chickens. Next, the Ph.D.-12 phage display peptide library kit from New England Biolabs and hyperimmune serum raised against *C. hepaticus* were used to identify protective antigen epitopes ("mimotopes"). DNA sequencing, BLAST analysis, and subcellular localization prediction were used to prioritize the identified mimotopes. Then, a surface display system was developed using *E. coli* outer membrane protein C (OmpC) as the anchoring motif.

Results: We discovered 16 mimotopes that corresponded to 13 different *C. hepaticus* proteins as potential vaccine candidates. We confirmed the expression of these mimotopes on the surface of *E. coli* using an OmpC-mediated surface display system.

Conclusion: Mimotopes identified in the study have the potential to prevent SLD. *In vivo* studies are needed to validate the immunogenicity and efficacy of the mimotopes.

Financial Support: This work was supported by Agriculture and Food Research Initiative Competitive Grant no. 1031150 from the United States Department of Agriculture (USDA) National Institute of Food and Agriculture (NIFA).

Notes:

P195 - The International Veterinary Vaccinology Network - Working together to develop vaccines for livestock diseases

Madeleine Clark[1], Simon Graham[2], Timothy Connelley[1]

[1]International Veterinary Vaccinology Network, The Roslin Institute, University of Edinburgh, [2]International Veterinary Vaccinology Network, The Pirbright Institute. madeleine.clark@ed.ac.uk

Session: Vaccinology, 2025-01-20, 6:00 - 8:00

Objective: The International Veterinary Vaccinology Network (IVVN) is an open access network platform open to the breadth of the veterinary vaccinology community. The vision of the network is to provide a multidisciplinary and interconnected vaccinology community which addresses the challenges impeding vaccine development for livestock and zoonotic disease affecting low-and-middle-income countries (LMICs). The objectives of the network are to: Provide an interactive and multidisciplinary network to facilitate dissemination and exchange of knowledge and 'state-of-the-art' technologies between members of the veterinary vaccinology community, with a focus on the upstream research that facilitates vaccine development. Use the network to identify and fund collaborative teams with complementary expertise that, through application of novel approaches, can effectively address critical challenges in vaccine research and development for LMIC-relevant pathogens. Provide support and opportunities for early career researchers and the next generation of veterinary vaccinologists. Facilitate engagement between the veterinary vaccinology community with external stakeholders (e.g., industry, funders, human vaccinology communities). This presentation will provide an insight into IVVN activities and how to become involved.

Methods: The IVVN's funded activities are focused on upstream research in veterinary vaccinology and underpinning immunology that help develop these vaccines. The activities are centred around four key work areas: facilitation of networking between members supporting scientific collaboration training early career researchers promoting gender balance in veterinary vaccinology research. The network brings a large but widely dispersed community together through international conferences and workshops. Members are kept informed about the network's activities through the network website, a monthly newsletter, X and LinkedIn. The IVVN is committed to supporting the training and development of early career researchers through conference scholarships, skills-based workshops, peer-to-peer fellowship scheme and online training courses. The IVVN catalyst funding schemes award collaborative research to tackle key bottlenecks in veterinary vaccine research. The IVVN has also supported a fellowship programme for female postdoctoral researchers in collaboration with Canada's International Development Research Centre. The IVVN African Schools Outreach Programme equips a network of African women scientists with the knowledge and tools needed to host interactive workshops in local schools.

Results: The network has over 2000 members from 93 countries working across the veterinary vaccinology landscape. IVVN has awarded 17 pump prime grants and 11 lab exchanges to collaborative international teams focused on bottlenecks in livestock and aquaculture vaccine development. These grants have produced substantial outcomes addressing veterinary vaccinology research gaps and needs. IVVN has provided a diverse range of skill and research-based training for early career researchers to support career development within the field.

Conclusions: The IVVN is a broad and diverse global community spanning the veterinary vaccinology landscape. The network is a hub for veterinary vaccinology news, job adverts and funding and has been able to support new knowledge generation within the field, addressing key research and innovation gaps and needs. The network remains open and we welcome anyone with an interest in veterinary vaccinology to be part of the community. To find out more information on the network, it's activities and become a member please visit: https://www.intvetvaccnet.co.uk/.

Financial Support: This research network is funded by UK Research and Innovation through the Medical Research Council (MRC) and the Biotechnology and Biological Sciences Research Council (BBSRC).

Notes:

P196 - Prevention of Porcine Circovirus Type 3 (PCV3) with directed suicidal vaccines

Wenjuan Fang[1], Md-Tariqul Islam[1], Augustina Arjarquah[1], Sheela Ramamoorthy[1]

[1]North Dakota State University. sheela.ramamoorthy@ndsu.edu

Session: Vaccinology, 2025-01-20, 6:00 - 8:00

Objectives: Porcine circovirus type 3 (PCV3), a new member of the Circoviridae family, has a strong epidemiological association with respiratory and reproductive disease and generalized systemic inflammation in pigs. PCV3 has a global prevalence worldwide and is often co-detected with porcine reproductive and respiratory syndrome virus (PRRSV). Currently, very little is known about PCV3 pathogenesis or prevention. Therefore, this study targets the development and testing of a novel, suicidal vaccine against PCV3.

Methods: The entire genome of PCV3 strain USMN 2016 was synthesized and cloned into a shuttle vector using an enzyme with a single cut in the viral genome. To develop a suicidal vaccine, selected codon changes were introduced in the infectious clone, such that the propensity to accumulate stop codons during vaccine viral replication in the host was significantly increased. To evaluate vaccine efficacy, 3-week-old piglets (N=7) were vaccinated with the suicidal vaccine and subsequently challenged at 28 days post vaccination with virulent PCV3 virus, or dually challenged with PCV3 and PRRSV.

Results: The recombinant PCV3 virus encoding the desired suicidal mutations was successfully rescued and found to consistently replicate to high titers. The mutations remained stable over 7 serial passages. As expected, the suicidal vaccine virus was cleared from most of the vaccinated pigs by 2 weeks post vaccination. Significant levels of PCV3 specific binding and virus neutralizing antibody responses were detected in vaccinated pigs. Vaccinated pigs were completely protected against PCV3 viremia due to challenge in both the single and dual challenge groups. Statistical analysis of data was carried out by ANOVA combined with Student's T tests at a significance level of 0.05.

Conclusions: The data indicates that the suicidal PCV3 vaccine induces strong antibody responses which correlated with clearance of the challenge virus and protection against PCV3 in both the single and dual challenge models. Therefore, the approach of directed suicidal vaccine virus replication is a promising approach to improve both the safety and efficacy of vaccines. Currently, protection against PCV3 challenge induced pathological lesions and cell mediated immune responses are being assessed.

Financial Support: This project was supported by an Agriculture & Food Research Initiative Competitive Grant 2021-67015-34419 from the USDA National Institute of Food and Agriculture.

Notes:

P197 - Use of reverse genetics system to develop first inactivated vaccine for cattle against HPAI H5N1 B3.13

Aleksandar Masic[1], Suman Mahan[1], Chelsea Waite[1], Evin Hildebrandt[1], David Asper[1], Mahesh Kumar[1]

[1]Zoetis Veterinary Medicine Research and Development. aleksandar.masic@zoetis.com

Session: Vaccinology, 2025-01-20, 6:00 - 8:00

Objective: In 2024, Highly pathogenic avian influenza (HPAI) virus from the Goose Guangdong lineage (Gs/Gd), clade 2.3.4.4b, subtype H5N1 strain B3.13 emerged in the Southwestern United States and began causing disease in dairy cattle. The infections in lactating dairy cattle have caused significant economic impact due to milk production losses and culling associated with agalactosis. Affected dairy animals became clinically abnormal and developed an overall acute decrease in herd level milk, lower feed consumption, abnormal tacky or loose feces, lethargy, dehydration, and fever (rectal temperature of ≥104F). In dairy cattle, respiratory signs of clear nasal discharge were also associated with HPAI H5N1 B3.13 virus infection. Due to increase spread of HPAI infections within predominantly dairy herds in US and increased risk of cattle to human transmission there is an increasing need to develop efficacious vaccine that would limit spread of the virus and reduce clinical signs associated with HPAI infections in the field.

Methods: We utilized reverse-genetics approach to generate 6+2 reassortant virus that could be used as vaccine candidate. Six internal genes (PB1, PB2, PA, NP, M and NS) were derived from laboratory reference strain A/Puerto Rico/8/1934 (H1N1). The immunodominant HA gene segment was de novo synthesized, and it contains the sequence of the circulating highly pathogenic avian influenza (HPAI) virus A/turkey/Indiana/22-003707-003/2022 (H5N1), clade 2.3.3.4, except the cleavage site which was altered from HPAI virus to low pathogenic avian influenza (LPAI). To assure vaccine DIVA compatibility, the NA gene sequence originated from LPAI virus N2 type A/chicken/Egypt/D5490B/2012 H9N2 was also de novo synthesized. Eight plasmids encoding each of above stated viral genes were transfected in 293T/MDCK co-culture cells and after 48h of incubation, reassortant virus with properties of LAPI was rescued. This new LPAI with DIVA phenotype was further propagated, inactivated, and used as a vaccine candidate to test its ability to generate HAI titers in cattle against HAPI infection.

Results: Eight plasmids encoding each of viral genes described above were transfected in 293T/MDCK co-culture cells and after 48h of incubation, reassortant virus with properties of LAPI was rescued. Virus presence was determined by CPE and IFA against NP protein. Further characterization was carried out to confirm lack of HPAI phenotype (removal of polybasic cleavage site). Rescued LPAI virus was able to grow only with the presence of trypsin reaching titers of 7.3 log $TCID_{50}$. Sequencing results demonstrated that both LPAI and DIVA phenotype were preserved within rescued virus. This LPAI with DIVA phenotype was further expanded, inactivated, and used as a vaccine candidate to test its ability to generate HAI titers in cattle against HAPI infection. Serology results from cattle study are pending.

Conclusions: Reverse genetics approach showed to be reliable and rapid method to generate vaccine candidates against HPAI infections.

Notes:

P198 - A bovine herpesvirus-vectored subunit Rift Valley Fever Virus vaccine is protective against RVFV in sheep

Shafiqul Chowdhury[1], Selvaraj Pavulraj[1], Rhett Stout[1], Ali Mazloum[1]

[1]Louisiana State University. Chowdh@LSU.edu

Session: Vaccinology, 2025-01-20, 6:00 - 8:00

Objective: Previously, we have constructed a bovine herpesvirus type 1 (BoHV-1) quadruple genes-deleted virus (BoHV-1qmv) in which partial or complete, UL49.5, glycoprotein G(gG), gE cytoplasmic tail (gE-CT), and US9 gene sequences are deleted. BoHV-1qmv is highly attenuated because it lacks i) virulence, ii) anterograde neuronal transport, and iii) immunosuppressive properties. Subsequently, we incorporated a chimeric Rift Valley fever virus (RVFV) envelope protein coding gene codon-optimized for bovine containing i) Gn fused to bovine GM-CSF (granulocyte-macrophage colony-stimulating factor), ii) 2A cleavage peptide (P2A) and iii) Gc in the gG-deletion locus to generate a BoHV-1qmv Subunit (Sub)-RVFV vectored subunit vaccine. Further, we determined that calves vaccinated with the BoHV-1qmv Sub-RVFV vaccine generated RVFV-specific protective neutralizing antibody and cellular immune response. In the current study, we aimed to repurpose the BoHV-1qmv Sub-RVFV vaccine for sheep and test its safety and protective RVFV-specific immunogenicity in sheep.

Methods: To repurpose the bovine codon-optimized BoHV-1qmv Sub-RVFV for sheep, the subunit chimeric protein gene RVFV Gn-ovine GMCSF-P2A-Gc was codon-optimized for ovine and incorporated in the gG-deletion locus of BoHV-1qmv. The resulting BoHV-1qmv Sub-RVFV (ovine codon-optimized) vaccine was then used to test its protective immunogenicity in sheep against RVFV. To this end, one group of five sheep (vector control group) and the second group of ten sheep (prototype vaccine group) were immunized (both intranasally and subcutaneously) with BoHV-1qmv and BoHV-1qmv Subunit-RVFV, respectively. At 29 days post-primary immunization, five sheep in the prototype vaccine group were boosted by intranasal inoculation of BoHV-1qmv Sub-RVFV. Hereafter, the sheep receiving the booster vaccination with BoHV-1qmv Sub-RVFV is designated as the prototype prime-boost group and the five -remaining sheep in the prototype vaccine group are designated as the prototype prime vaccine group.

Results: Both the vaccine vector, BoHV-1qmv, and the prototype vaccine, BoHV-1qmv Sub-RVFV, were safe and highly attenuated in sheep. A single intranasal and subcutaneous inoculation of BoHV-1qmv (vector) and BoHV-1qmv Sub-RVFV induced moderately high levels of BoHV-1-specific and RVFV-specific serum neutralizing antibody (SN) titers in sheep. Notably, despite a moderate level of preexisting BoHV-1 (vector)-specific SN antibodies, a booster-intranasal BoHV-1qmv Sub-RVFV vaccination at day 29 post-vaccination induced a robust ($p < 0.001$), memory RVFV-specific-neutralizing antibody response. Significantly, following primary vaccination, the peripheral blood mononuclear cells isolated from the immunized sheep, when stimulated with heat-killed RVFV MP12 antigen, produced six-fold increased levels of interferon-gamma transcripts compared with the unvaccinated control.

Conclusions: A single dose of live BoHV-1qmv vectored Sub-RVFV prototype subunit vaccine induced a moderate level of RVFV-specific SN antibody in sheep. Notably, intranasal booster immunization induced a strong memory immune response against RVFV. Therefore, BoHV-1qmv Sub-RVFV is a promising candidate vaccine and a better replacement for the currently available modified live attenuated MP12 and inactivated RVFV vaccine in the field regarding vaccine safety and efficacy. A future BoHV-1qmv Sub-RVFV vaccination and virulent RVFV challenge experiment in sheep remains to be conducted to validate our protective vaccine immunogenicity data.

Notes:

P199 - It's EpiCC! A web-based tool for vaccine strain selection and prediction of vaccine efficacy

Soorya Seshadri[1], Anne De Groot[1], Andres H. Gutierrez[1], Matt Ardito[1], William D. Martin[1], Guilhem Richard[1]

[1]EpiVax Inc. sseshadri@epivax.com

Session: Vaccinology, 2025-01-20, 6:00 - 8:00

Objective: Swine on commercial swine farms are significantly impacted by viral pathogens of ever-increasing diversity, making it difficult to determine whether available vaccines will have a protective effect. T cell Epitope Content Comparison (EpiCC), a web-based tool designed to assess vaccine efficacy by evaluating T cell epitope content shared between commercial vaccines and field isolates, thereby facilitating the selection of vaccines with broader cross-reactive immune responses and efficacy against variant strains. EpiCC has been used to develop web applications for specific pathogens, including porcine circovirus type 2 (PCV2) [1] and swine influenza A virus (sIAV, [2]). The PCV2 website "CircoMatch™" compares capsid protein sequences from field isolates with four commercial vaccines, generating EpiCC scores and visual representations of T cell epitope coverage. For swine H1 influenza A, the FluMatch website can analyze hemagglutinin (HA) protein sequences across different phylogenetic clades to identify optimal vaccine candidates based on epitope coverage. The tool has also been employed to evaluate PRRS viral evolution in response to vaccination and herd immunity and to assess the predictive efficacy of PRRSV vaccines to analyze existing data in a live challenge study.

Methods: One case study will be presented: Selection of the best swine influenza virus strain based on evaluation of circulating strains. For this project, the HA protein sequences of field isolates were collected, and the putative SLA class I and II T cell epitope content in the input sequences was identified using PigMatrix. EpiCC was used to compare the T cell epitopes contained in HA protein sequences between field isolates and to identify the vaccine strain with the highest coverage as a proposed 'new vaccine strain'.

Results: EpiCC revealed clade-specific T cell epitope coverage for swine H1 influenza A HA protein. Strains with the highest EpiCC scores (greater relatedness and higher T cell epitope coverage) were selected as vaccine strains. This approach to vaccine strain selection is highly scalable, allowing for comparisons for a wide range of commercial and autologous vaccines for various swine pathogens.

Conclusion: EpiCC may serve veterinarians as a first estimate of vaccine efficacy and enable vaccine companies to select the best vaccine for new outbreaks. Additionally, as individual veterinarians report strain sequence data into the tool to obtain the EpiCC coverage information, collection of this clinical data may enable vets to identify new outbreaks as they occur.

[1] Bandrick, Meggan, et al. "T cell epitope content comparison (EpiCC) analysis demonstrates a bivalent PCV2 vaccine has greater T cell epitope overlap with field strains than monovalent PCV2 vaccines." Veterinary immunology and immunopathology vol. 223 (2020): 110034. doi:10.1016/j.vetimm.2020.110034; [2]Tan, Swan, et al. "H1N1 G4 swine influenza T cell epitope analysis in swine and human vaccines and circulating strains uncovers potential risk to swine and humans." Influenza and other respiratory viruses vol. 17,1 (2023): e13058. doi:10.1111/irv.13058

Notes:

P201 - Characterizing de novo virus gene expression in cells transfected with BoHV-1 genomic DNA

Jeffery Ostler[1]

[1]Oklahoma State University, College of Veterinary Medicine. jostler@okstate.edu

Session: Virology, 2025-01-20, 6:00 - 8:00

Objective: Bovine Alphaherpesvirus 1 (BoHV-1) remains a major cattle pathogen domestically and globally, causing Infectious Bovine Rhinotracheitis, reproductive failure, and genital tract disease. Furthermore, BoHV-1 is a major risk factor for Bovine Respiratory Disease (BRD) complex, which causes annual losses in excess of US$1 billion annually for domestic dairy and beef producers. Controlling the spread of BoHV-1 has seen moderate success in recent years, but is complicated by under vaccination, vaccine-induced complications, and the notoriously difficult to control herpesvirus latency-reactivation cycle. Even in countries that have eliminated BoHV-1, preventing reintroduction continues to draw resources. The mechanisms driving reactivation from latency remain elusive, largely due to limited models for study. BoHV-1 has proven an excellent model for dissecting these mechanisms as we can directly interrogate latently infected neurons within the native host. In latently infected calves, I.V. dexamethasone (DEX) treatment reliably induces productive infection and virus shedding. In vitro work has shown that DEX activates the Glucocorticoid Receptor (GR) to drive virus gene expression, along with stress-induced host gene activation. However, the resources required to fully investigate the mechanisms driving virus gene expression during the early stages of reactivation *in vivo* are staggering. However, our lab has demonstrated that in certain cell types, transfecting BoHV-1 genomic DNA can produce a productive infection in some cells. This system mimics the environment of reactivation, lacking virus proteins found in the tegument and virion during acute infection, and relies exclusively on host factors to initiate virus gene expression. We have used this system to demonstrate that GR and DEX together promote productive infection, and now are investigating the early steps of *de novo* BoHV-1 gene expression.

Methods: BoHV-1 is propagated through bovine kidney (MDBK) cells. Genomic DNA from virions is isolated from infected cells and their growth medium by ultracentrifugation through a sucrose cushion and collecting the pellet. Virions pass through the 30% sucrose but larger cell debris cannot. This genomic DNA is purified and then transfected into cultured cells using lipofection. Rabbit skin cells, mouse neuroblastoma cells (Neuro-2A), and bovine turbinate (BT) cells have proven permissive to BoHV-1 infection by transfected DNA. Transfected cells are harvested at several time points in Trizol to isolate RNA.

Results: We have successfully identified virus transcripts from transfected Rabbit skin cells using RT-PCR and virus specific primers. BoHV-1 glycoprotein B (gB) RNA has been detected by 48 hours post transfection and increases significantly at later time points, consistent with lytic spread of the virus between cells. ICP0 and ICP4, key virus transcriptional activators have been detected at 24 hours post transcription.

Conclusions: This model is effective in identifying BoHV-1 de novo gene expression initially driven solely by host factors in transfected cells. Further work is ongoing to better characterize the timing of virus gene expression and identify host proteins associated with the virus DNA at different time points.

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, and Oklahoma State University College of Veterinary Medicine faculty startup funds.

Notes:

P202 - Deletion viral genome diversity amongst Bovine Viral Diarrhea Virus (BVDV) 1a and 1b strains

David Holthausen[1], Darrell Bayles[1], John Neill[1], Rohana Dassanayake[1], Shollie Falkenberg[2], Harish Menghwar[1, 3], Eduardo Casas[1]

[1]USDA ARS, [2]Auburn University, [3]ORISE. david.holthausen@usda.gov

Session: Virology, 2025-01-20, 6:00 - 8:00

Objective: Bovine Viral Diarrhea Virus (BVDV) is a pervasive respiratory pathogen of economic concern for the cattle industry. Deletion Viral Genomes (DelVGs) are naturally occurring products of the viral replication process by which viral genomic transcripts are generated with truncations of various size and cannot or severely impede self-replication.

Methods: We used a bioinformatic pipeline to discover the presence of BVDV DelVGs. These deletion viral genomes were found by analyzing Illumina MiSeq reads from 74 BVDV1 field isolates from two closely related sub-genotypes, and in an *in vitro* passage of a BVDV1a virus at two different multiplicities of infection (MOI).

Results: After identifying DelVGs, we assessed their phylogenetic linkage to begin elucidating potential roles in the viral life cycle and persistence. BVDV1a viruses queried generate significantly more DelVGs, with 52% of 5' and 3' junctions occurring in the core/capsid (C) region and a major NS2-NS5B deletion species. In contrast, BVDV1b viruses generated significantly fewer DelVGs, especially reduced C region deletions. *In vitro* passage of the BVDV1a Singer virus demonstrates that MOI significantly impacts the generation of DelVGs with the higher MOI condition generating more DelVGs and a different deletion profile.

Conclusions: Here we report that BVDV1a and BVDV1b sub-genotypes generate diverse species of DelVGs. These DelVGs may have key roles in BVDV evolution and the establishment of persistence during transplacental infection.

Financial Support: USDA ARS CRIS Project 5030-32000-229-00D

Notes:

P203 - Investigating the effects of intramammary inoculation of bovine-origin H5N1 influenza in lactating ferrets

P.H. Baker[1], M.C. Moyer[1], C. Lee[2], S.P. Kenney[2], S.N. Langel[1]

[1]Case Western Reserve University, [2]The Ohio State University. pxb461@case.edu

Session: Virology, 2025-01-20, 6:00 - 8:00

Objective: Highly pathogenic avian influenza (HPAI) H5N1 clade 2.3.4.4b has been detected in lactating dairy cattle in the U.S. and has now spread across 14 states. The ability of HPAI H5N1 to transmit to multiple mammalian species raises concerns for mammalian adaptation that may lead to increased risk of human transmission. The virus appears to have an apparent tropism towards the mammary gland, displaying high levels of viral RNA and infectious virus in milk from infected cows. The ability of the virus to affect the mammary gland suggests possible implications to the mother-infant dyad via breastfeeding. To understand influenza transmission and pathogenesis in the mammary gland, we investigated bovine-origin H5N1 influenza infection and transmission utilizing a lactating ferret model.

Methods: Briefly, lactating ferrets (n=2) were intramammary inoculated (3 glands/ferret) with 105 EID50 of A/Bovine/Ohio/B24OSU-439/2024 at two weeks postpartum. Two-week-old ferret kits (n=3/litter) were housed with the nursing mother before and after inoculation. Ferret kits had nasal, oral and fecal swabs, weight, temperature, and serum collected on 0-, 2-, and 4-days post-infection (DPI) and ferret dams were sampled on 0, 2, 4, 6 DPI and in addition, milk was collected on those days.

Results: By day 4 post-infection, all ferret kits succumbed to illness or malnutrition and were necropsied for various tissues. Ferret kits exposed to H5N1-positive milk experienced marginal weight gain ($P = 0.13$) from 0 to 2 DPI, however experienced significant weight loss by day 3 and day 4 ($P \leq 0.01$). Ferret dams showed lethargy, reduced feed intake, and weight loss. Weights significantly decreased by 6 DPI ($P < 0.0001$) and dams were euthanized on day 6 due to a humane endpoint weight cut-off (20% of original weight). Additionally, we observed a decrease in milk at 4 DPI with abnormal milk displaying thick colostrum-like appearance in one mammary gland of one ferret dam while other mammary glands from both ferrets displayed watery secretions, indicating possible signs of mastitis. Tissues were collected at necropsy from all animals for viral titration via RT-qPCR and plaque assay. Nasal, oral, fecal, milk and serum samples will be also analyzed for identification of H5N1 RNA and infectious virus using real-time PCR and plaque assay, respectively.

Conclusions: Lactating ferrets can be utilized as a small animal model for intramammary H5N1 2.3.4.4b infection studies. Studies can focus on how the virus affects both the lactating mother and her offspring. This experimental model will be used to further study H5N1 pathogenesis in the mother-infant dyad and be used to assess potential therapeutic interventions against infection and disease.

Financial Support: This research was supported by startup funds provided to the principal investigator (S.N. Langel) by Case Western Reserve University School of Medicine.

Notes:

P204 - Global phylodynamics of highly pathogenic avian influenza virus H5N1 - Patterns of transmission between species and geography

Darrell Kapczynski[1], Samantha Lycett[2], Lu Lu[2], Will Harvey[2], Paul Digard[2]

[1]USDA, [2]Roslin Institute. darrell.kapczynski@usda.gov

Session: Virology, 2025-01-20, 6:00 - 8:00

Objective: A highly pathogenic avian influenza (HPAI) virus lineage with H5N1 subtype emerged in China in 1996, which has caused severe disease in poultry and wild bird species. In recent years, this lineage of HPAI H5 viruses have diversified through reassortment and swapping genomic segments with various local and migratory birds. In 2014/2015, outbreaks of HPAI H5N8/X devastated poultry in North America, Europe and Asia. Subsequently, in the 2016/2017 and 2020-2024 autumn/winter seasons H5NX again caused outbreaks poultry in Asia, Europe and North America. The objectives of this USDA-NIFA research are to examine models of viral evolution to produce spread and species risk maps with predictive capability that can be used to inform vaccination and other control strategies.

Methods: Whole genome sequence datasets were compiled from worldwide H5Nx HPAI virus sequences. Using phylodynamic and phylogeographic methods including BEAST (Bayesian Evolutionary Analysis Sampling Trees), the H5NX spreading pattern across and within continents has been reconstructed. BEAST utilizes sequence data, time scales, population scales, and discrete traits to develop time-measured inferred phylogenetic relationships. Reconstructing the spatial spread with continuous trait phylogeography together with the transmission between bird species types, we demonstrate long-range transmission mediated by migrating wild bird species.

Results: Considering the phylogenies of all the segments in time and space, we can estimate the origin and track several reassortant viruses generated within a season. We find that the H5N1 HPAI viruses undergo frequent reassortment with other co-circulating low pathogenic viruses in the wild bird population, and that H5N1 may persist in wild populations between breeding and wintering seasons. In addition, the resurgence of H5N5 HPAI have recently been detected in wild birds from Europe that contain short stalk deletions in the neuraminidase (NA) gene which has previously been a marker for adaption of the virus to poultry.

Conclusions: This work provides insight into the global phylodynamics and transmission patterns of avian influenza viruses that can be utilized in model development for predictive purposes. The detection and establishment of emerging gene pools with enhanced adaption to poultry is a further concern for the poultry industry.

Financial Support: This work was funded by USDA-NIFA AFRI grant # 2021-67015-34032 as part of the joint USDA-UKRI-NSFC Ecology and Evolution of Infectious Diseases (EEID) program and USDA-ARS CRIS # 6040-32000-081-00D.

Notes:

P205 - Entry of SARS-CoV-2 Omicron variants into cells expressing human or animal ACE2 to assess host susceptibilities

Alexandria Zabiegala[1], Yunjeong Kim[1], Kyeong-Ok Chang[1]

[1]Kansas State University. zabiegalaa@vet.k-state.edu

Session: Virology, 2025-01-20, 6:00 - 8:00

Objective: Since the start of the Covid-19 pandemic, emergence of SARS-CoV-2 variants has been closely monitored. First reported in November 2021, the Omicron variant and its subvariants have been the dominant strains in circulation as of October 2024. With the continuing emergence of new strains, it is imperative to monitor the biologic interactions between the virus and host. Our lab has previously assessed the ability of older variants such as Alpha, Beta, and Gamma to enter cells expressing human or animal ACE2. In this study, we assessed the entry of lentiviral-based pseudotyped viruses expressing the spike protein (S) of SARS-CoV-2 Omicron subvariants BA.1.1, XBB.1.9.1, BA.4/5, and JN.1 into CRFK cells expressing human and animal ACE2. As of October 2024, the JN.1 lineage and related sublineages are the prominent strains being transmitted.

Method: Lentiviral-based pseudoviruses containing a green fluorescent protein (GFP) reporter plasmid were generated to express S of SARS-CoV-2 Omicron subvariants BA.1.1, XBB.1.9.1, BA.4/5, and JN.1. CRFK cells stably expressing ACE2 from human, horse, dog, cat, Syrian golden hamster, mink, white-tailed deer, cow, and camel were briefly plated until cells reached approximately 80% confluency before being transduced with the pseudoviruses. After 48 hours, the entry of the pseudoviruses was measured using a luciferase reporter system.

Results: We found that all the Omicron subvariants tested were able to efficiently enter the cells expressing many of the animal ACE2 when compared to human ACE2. An increase in the entry of all tested Omicron subvariants was consistently observed in hamster, white-tailed deer, cow, and camel ACE2-expressing cells, in comparison to human ACE2-expressing cells. However, the entry of Omicron subvariants varied in the horse, dog, and cat ACE2-expressing cells. Additionally, mink ACE2 expressing cells consistently showed limited entry of all variants tested.

Conclusion: Compared to the previous variants, Omicron has been shown to be highly transmissible, though the severity of the disease appears significantly decreased with less mortalities and hospitalizations. The Omicron variant is characterized by a high number of mutations, and there is no clear link to SARS-CoV-2 strains circulating at the time of Omicron emergence. Although the origin of the Omicron variant is still unclear, there is a possibility that Omicron may have arisen from a rodent reservoir following reverse zoonosis of SARS-CoV-2 to rats or mice. As viruses circulating and mutating in a rodent or other animal reservoir would likely go unnoticed until they spread back to humans, it is critical to monitor potential reservoir hosts. While a number of factors contribute to successful viral infection, using a pseudovirus entry assay allows for the detection of variants that may have better binding affinity to ACE2 of different species, which can allow for better surveillance in animal species that may serve as reservoirs.

Financial Support: NIH R01 AI130092, NIH AI161085, USDA-NIFA AFRI 2019-67015-29864

Notes:

P206 - Disruption of influenza A virus transmission in gene-edited pigs

Taeyong Kwon[1], Igor Morozov[1], Chester D. McDowell[1], Eu Lim Lyoo[1], Bianca L. Artiaga[1], Yonghai Li[1], Patricia Assato[1], Stephen N. White[2], Benjamin P. Beaton[2], Gustavo Delhon[3], Natasha N. Gaudreault[1], Juergen A. Richt[1]

[1]Kansas State University, [2]Genus plc, [3]University of Nebraska-Lincoln. tykwon@vet.k-state.edu

Session: Virology, 2025-01-20, 6:00 - 8:00

Objective: Influenza A virus (IAV) infection in pigs causes significant economic losses in swine industry. More importantly, pigs could serve as an intermediate host for generating reassortant viruses with pandemic potential. Previously, gene-edited pigs lacking the transmembrane serine protease 2 (TMPRSS2) gene were generated and tested for susceptibility to IAV infection. Experimental infection resulted in limited nasal shedding, posing the question about the ability of TMPRSS2 knockout pigs (TMPRSS2 KO pigs) to transmit IAV and further potential benefits of commercial use of TMPRSS2 KO pigs. Therefore, this study evaluated IAV transmission in TMPRSS2 KO pigs.

Methods: To determine the effect of TMPRRS2 deletion on IAV transmission in pigs, sentinel pigs were co-mingled on 2 days post-challenge with donor pigs that were intratracheally challenged with H1N1. Three groups of pigs were evaluated for IAV transmission: (i) KO-KO transmission, (ii) KO-WT transmission, and (iii) WT-WT transmission. Nasal swabs were collected during the course of infection, and tissue samples and bronchioalveolar lavage fluids were obtained at necropsy. Serum samples were collected for serology

Results: In the WT-WT transmission group, all principal-infected WT pigs shed infectious viruses at 4 and 5 DPC. The sentinel WT pigs started nasal shedding at 5 DPC and all became virus-positive at 8 to 12 DPC, with virus shedding being undetectable by 15 DPC. The time between exposure and start of virus shedding of sentinel WT pigs was calculated as 5.33 ± 0.52 days (average ± standard deviation). In the KO-WT transmission group, nasal shedding was detected in two principal KO pigs after intra-tracheal challenge. Sentinel WT pigs started to shed the infectious viruses from 9 DPC, and all sentinel WT pigs eventually became positive with a peak of virus titer at 12 DPC. The virus was first cleared from the sentinel WT pigs at 13 DPC, and ultimately, all sentinel WT pigs became virus-negative at 21 DPC. The days between exposure and start of virus shedding of sentinel WT pigs was calculated as 8.67 ± 1.03 days, indicating a significant delay of transmission, when compared to WT-WT transmission. In KO-KO transmission group, the virus was isolated from one principal-infected KO pig only at 1 DPC, while the rest of the principal - infected pigs remained virus-negative in this study. There was no virus shedding and no seroconversion in sentinel KO pigs, demonstrating that principal KO pigs did not transmit the virus to sentinel KO pigs.

Discussion: Previously, we generated and established TMPRSS2 KO pigs which were resilient to IAV infection. However, the role of TMPRRS2 deletion on IAV transmission is still not known. The present study illustrates a significantly impaired IAV transmission in genetically modified TMPRSS2 KO pigs as compared with WT pigs. Overall, the results support the potential benefits of commercial use of TMPRSS2 KO pigs to reduce IAV infection and transmission.

Financial Support: Genus PLC, the Center on Emerging and Zoonotic Infectious Diseases (CEZID) of the National Institutes of General Medical Sciences under award number P20GM130448, and the NIAID supported Center of Excellence for Influenza Research and Response (CEIRR) under contract number 75N93021C00016.

Notes:

P207 - Strain-specific replication of Rotavirus A in extraintestinal tissues

Sergei Raev[1], Maryssa Kick[1], Maria Chellis[1], Linda Saif[1], Talita Pilar Resende[1], Anastasia Vlasova[1]

[1]The Ohio State University. raevsergey33@gmail.com

Session: Virology, 2025-01-20, 6:00 - 8:00

Objective: Rotavirus A (RVA) is known to spread to some extraintestinal organs such as lungs, salivary glands (SG), pancreas, spleen and other tissues. Replication of RVA in the SG and respiratory tract is important as it may constitute an additional route of viral transmission. However, the mechanism of RVA extraintestinal spread and whether its replication outside the intestine is strain-specific remain largely unknown. We hypothesized that RVA strains with higher levels of intestinal replication would spread in extraintestinal tissues more efficiently compared to those with less efficient replication levels.

Methods: To validate this hypothesis, we used porcine RVA, OSU G5P[7] which replicates to higher titers in vitro and in vivo compared to two other porcine RVA strains (Gottfried G4P[6] and RV0084 G9P[13]) and a human RVA (Wa G1P[8]). Gnotobiotic (Gn) piglets (6-8 days of age) were inoculated orally ($1x10^6$ ^FFU/piglet). After the RVA challenge, rectal swabs were collected daily for four days to assess RV shedding. Piglets were euthanized at dpi 1-4 (n=3-6), intestinal contents and various tissues (lungs, SG, ileum, jejunum, spleen, liver, pancreas) were collected[VAN1] . RVA detection and quantification were performed by in situ hybridization (ISH, tissues) and cell culture immunofluorescence assay (CCIF, rectal swabs/intestinal contents), respectively. To determine sialic acid (SA) expression profiles, a panel of lectins specific to different SAs and non-sialylated glycoconjugates was used.

Results: Significantly higher titers of G5P[7] compared to all other strains were detected in rectal swabs and intestinal contents. Using ISH, we demonstrated that Gn pig lungs and SGs were the two main extraintestinal sites of RVA replication. However, in these tissues RVAs replicated less efficiently than in the gut. Noteworthy, RVA presence in the lung/SG tissues was detectable as early as post-infection day 1. For two strains, G5P[7] and G9P[13], a higher frequency of RVA replication in lungs/SG was observed compared to G1P[8] and G4P[6]. Additionally, despite the lower levels of intestinal replication of G9P[13] vs. G5P[7], its spread to the lungs was observed more frequently than that of G5P[7] indicating that other factors may contribute to enhanced extraintestinal spread of RVA. We also observed low SA diversity in the lungs and SG compared to the gut. Considering the complexity of RVA/host cell interactions, these findings suggest that less efficient RVA replication in the extraintestinal tissues may result from the limited glycan profile.

Conclusions: Taken together, our data indicate that the high prevalence of G9P[13] detection in extraintestinal tissues may be an important contributing factor to the global spread and dominance of this genotype in humans and pigs. Additionally, extraintestinal RVA replication appears to be regulated in a strain-specific way.

Notes:

P208 - Role of Marek's disease virus encoded US3 serine/threonine protein kinase in replication and pathogenesis

Sanjay Reddy[1], Yifei Liao[1], James Cai[1], Blanca Lupiani[1]

[1]Texas A&M University. sreddy@tamu.edu

Session: Virology, 2025-01-20, 6:00 - 8:00

Objective: Marek's disease virus (MDV) is a potent oncogenic alphaherpesvirus that elicits a rapid onset of malignant T-cell lymphomas in chickens. All MDV types, including GaHV-2 (MDV-1), GaHV-3 (MDV-2) and MeHV-1 (HVT), encode a US3 protein kinase. We earlier showed that MDV-1 US3 is important for efficient virus growth in vitro and viral pathogenesis. The US3 from MDV-2 and HVT can partially complement the kinase function in MDV-1 US3 null mutant. Since US3 plays an important role in various aspects of viral replication, pathogenesis, and virus-host interactions, we examined the role of US3 in regulating cellular and viral proteins.

Methods: Construction of the MDV US3 recombinants was performed by two-step Red-mediated recombination using a 686-BAC derived from a very virulent strain. To study the role of US3 in regulating MDV replication in chicken, lymphoid organs and skin samples were quantitatively examined. To evaluate US3 role in pathogenesis, survival rate and tumor development were determined in chicken inoculated with parental, chimeric and revertant viruses. Standard molecular biology techniques like Western-blot, immunoprecipitation, immunofluorescence and immunohistochemistry were used to examine protein-interaction, and protein localization studies.

Results: Histone deacetylases (HDAC) are a class of enzymes that remove acetyl groups from lysine residues on histone proteins. HDACs allow histones to wrap the DNA more tightly to down-regulate gene expression. We have identified chicken histone deacetylase 1 (chHDAC1) as a common US3 substrate for MDV-1, MDV-2, and HVT, while only US3 of MDV-1 and MDV-2 phosphorylate chHDAC2. Biochemical studies showed that MDV US3 mediated phosphorylation of chHDAC1 and 2 affect their stability, transcriptional regulation activity, and interaction network. We further showed that Meq co-localizes with chHDAC1 and chHDAC2 in the nuclei of MDV lymphoblastoid tumor cells and demonstrated that this interaction mediates the degradation of chHDAC1 and chHDAC2 via the proteasome-dependent pathway. CREB is a cellular transcription factor that binds to certain DNA sequences called cAMP response elements (CRE), thereby regulating gene transcription. We showed that MDV-1 US3 increases CREB phosphorylation, leading to the recruitment of phospho-CREB (pCREB) to the promoter of CREB-responsive genes and activating CREB target gene expression. Furthermore, using biochemical studies, we demonstrated that MDV US3 interacts with Meq in a phosphorylation-dependent manner.

Conclusions: MDV is a potent oncogenic herpesvirus that induces T-cell lymphomas in infected chickens. Deletion of US3 from MDV-1 attenuated the virus, which could be partially compensated by MDV2 and HVT US3. This kinase was involved in regulating gene expression through HDAC pathway. In addition, US3 interacts with MDV encoded Meq oncoprotein suggesting its role in T-cell transformation.

Financial Support: USDA NIFA grant 2022-67015-36334

Notes:

P209 - Seroprevalence of Cache Valley Virus in sheep population in the south-central Appalachian Region

Preston H. Morris[1, 2], Lauren Wisnieski[2, 3], Samantha Smith[1, 2], Kathyrn Hall[2], Victoria J. Morris[1, 2]

[1]Center for Infectious, Zoonotic and Vector-borne Diseases, [2]Richard A. Gillespie College of Veterinary Medicine, Lincoln Memorial University, [3]Center for Animal and Human Health in Appalachia. preston.morris@lmunet.edu

Session: Virology, 2025-01-20, 6:00 - 8:00

Objective: Cache Valley virus (CVV), a mosquito-borne orthobunyavirus, is an important cause of embryonic loss and fetal malformations in sheep. While anecdotal reports suggest its presence in the Southeastern United States' ovine population, no prevalence data is currently available. The objective of this ongoing study is to determine the seroprevalence of CVV in sheep populations in the South-Central Appalachian region.

Methods: Sera from a total of 350 sheep across 24 local farms will be collected and tested for the presence of CVV neutralizing antibodies using a serum neutralization assay. The inclusion criteria include animals that have been on the premises for at least 60 days and are at least one year old. Samples were collected 60-90 days post-peak mosquito season for the geographical area.

Results: In the first phase of testing, sera from 128 sheep across eight farms were tested. Fifty-one out of 128 samples tested positive (39.84%; 95% CI: 15.01-64.68%) for CVV neutralizing antibodies. We anticipate testing the remaining samples by December 2024.

Conclusions: Preliminary data indicate exposure to CVV among the region's ovine population. Prevalence data will help guide future research questions and inform interventional strategies.

Financial Support: This study was funded through an institutional research grant from LMU-CVM

Notes:

P210 - Mechanisms underlying ACE2 based species susceptibility to SARS-CoV-2

Jessie Lee Cunningham[1], Mohammed Nooruzzaman[1], Diego G. Diel[1]

[1]Department of Population Medicine and Diagnostic Sciences, Cornell University. mn496@cornell.edu

Session: Virology, 2025-01-20, 6:00 - 8:00

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. We aim to gauge the current risks that are posed to companion animals, livestock, and wildlife while also identifying the potential for animal species to function as reservoirs for the virus.

Methods. Using a library of cells stably expressing ACE2 orthologues from over 20 species, viral entry-, infectivity-, and fusogenicity assays were performed utilizing live variants of SARS-CoV-2 virus and their corresponding recombinant chimeric viruses that contain the S proteins of such variants.

Results. While low-binding in silico predictions supported the low number or lack of cases of natural infection in some animal species, such as swine, here we demonstrate that this is not due to the inability of S protein to engage with ACE2. Furthermore, Omicron-BA.1.1 entry into target cells was shown to be faster in multiple species compared to D614G-B.1, but the increased replication efficiency and overall increased fusogenicity of D614G-B.1-S virus can compensate for an initial slower entry process, emphasizing the possible repercussions of the more recent Omicron variants that exhibit higher fusogenicity.

Conclusions: An extensive and comprehensive 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 animal species to SARS-CoV-2 infection.

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

Notes:

P211 - Bovine respiratory syncytial virus infection challenge using different doses to study the range of clinical disease

Grace Beddingfield[1], Erin Blanchard[1], Cassandra Barber[2], Kenzie McAtee[2], Santiago Cornjeo Tonnelier[2], Merrilee Thoresen[2], Carrie Vance[1], Amelia Woolums[2], Florencia Meyer[1]

[1]Department of Biochemistry, Nutrition and Health Promotion, Mississippi State University, [2]Department of Pathobiology and Population Medicine, Mississippi State University. gb950@msstate.edu

Session: Virology, 2025-01-20, 6:00 - 8:00

Objective: Bovine Respiratory Disease (BRD) is a disease complex that affects cattle. It requires a susceptible host, one or more pathogens, and a stressor. Bovine Respiratory Syncytial Virus (BRSV) is one of the main viral pathogens associated with BRD. BRSV infects the lower respiratory tract targeting bronchi, bronchioles, and alveoli, resulting in difficulty breathing, excessive coughing and interstitial pneumonia. In this study, BRSV was used as a model pathogen to infect calves with varying levels of BRSV. The objective of this study was to induce respiratory disease ranging from subclinical to clinical and use biochemical information about host biofluids together with extensive clinical scoring to design predictive diagnostic models that can detect subclinical infection.

Methods: Calves were transported to Mississippi State University one-week post birth and raised on-site in research pens. When calves were approximately 12 weeks old, 22 calves were enrolled into the study and divided into four pens. Calves were challenged with approximately 10*5, 10*4, or 10*3 TCID50 units (high, medium, or low dose) of BRSV by aerosol; the control group received aerosol of cell culture medium alone. Clinical signs were assessed daily for 14 days using two different scoring systems: the Wisconsin scoring system and the modified Gershwin score. Each animal was also evaluated daily for 14 days by transthoracic ultrasound to characterize lung lesions. Nasal and oral secretions, and blood were also collected daily.

Results: After challenging **t**he Gershwin scores ranged from 0 to 300 based on the severity of the observed clinical signs. The scores from the control group remained below 100 during the study period. The low dose group peaked under 200, medium dose peaked slightly above 200, and the high group peaked between 200 and 300. A similar increase between groups was also observed in the Wisconsion scores. Ultrasound scores followed the same pattern as the clinical scores for each dosage group, Data analysis to determine statistically significant differences is in progress.

Conclusion: The BRSV challenge proceeded as expected, with peak clinical signs on days 9 and 10, and resolving by day 14. As determined by the Gershwin and Wisconsin scores, a gradient of clinical signs was successfully created. Ultrasound scores agreed with the trend observed in the clinical scores. PCR testing and analysis of biofluids using near-infrared spectroscopy (NIRS) and immunoassays will support the development of a predictive model of subclinical BRSV infection.

Financial Support: This project was supported by Agriculture and Food Research Initiative Competitive Grant no.2022-67016-36978.

Notes:

P212 - Comparative transcriptomics during persistence infection of Senecavirus A

Salman Butt[1], Nicholas Vazquez[1], Maureen H.V. Fernandes[1], Diego G. Diel[1]

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

Session: Virology, 2025-01-20, 6:00 - 8:00

Objective: Persistent viral infections are of major biomedical and agricultural importance, often posing significant challenges to disease control and eradication efforts. Senecavirus A (SVA), an emerging picornavirus causing major vesicular disease (VD) outbreaks in pigs can establish persistent infections (pi) in pigs. Here we performed both bulk RNA sequencing (RNA-Seq) and single cell RNA sequencing (scRNA-Seq) to elucidate the molecular mechanisms underlying SVA persistence in porcine kidney cells (PK-15) and porcine tonsil cells (PTOs).

Methods: SVA infected (5 & 10 MOI for PK-15 and 5 MOI for PTOs) PK-15 and PTOs were maintained for 28 days post-nfection to establish persistence SVA infection. The supernatant and cell lysates from piPK-15 and piPTOs were collected and subjected to virus titrations in H1299 cells. RNA extracted from piPK-15 and piPTO cells was subjected to bulk RNA-Seq and scRNA-Seq, respectively to investigate differential gene expression (DGE) of bulk and single cell transcriptome of persistently infected cells. The antiviral effect of IFIT1, IFIT3, MX2 and RSAD2 during acute SVA infection (0.1 MOI) was further investigated by using a reporter SVA virus (eGFP-SVA) infection (12 hrs and 24-hrs) and transient expression of these ISGs in H1299 cells.

Results: The virus titers were relatively higher in piPTO supernatant as compared to the cell lysates. Gene ontology (GO) analysis of bulk RNA-Seq data from piPK-15 cells demonstrated that piSVA modulated several biological pathways such as upregulation of genes which negatively regulate apoptosis (LCN, TIMP2), and positively regulating defense response against virus (OASL, OAS1, DHX58), and downregulation of genes associated with autophagy (CFTR) and inflammation (C5, RBP1, PTGFR). The scRNA-Seq data from piPTOs showed that only a sub-population of piPTOs had SVA transcripts and multifunctional genes in addition to ISGs had differential gene expression among these infected and the bystander cells within infected group. Notably, while transcription of housekeeping gene (GAPDH) and SVA viral receptor (ANTRX1) were similar in mock and piPTo cells, DGE of several genes related to interferon response (ISGs; IFIT1, ZBP1, RSAD2, IFIT3, MX2, DDX60) and cell cycle pathways (Tyrp-1, ERBB4, DUSP-10) was observed in piPTo cells when compared to the mock infected group. The function assay of ISGs (IFIT1, IFIT3, MX2 & RSAD2) on SVA replication cycle showed that the virus titers were significantly lower in cells with transient expression of these ISGs as compared to un-transfected cells at 12-hrs and 24-hrs post infection.

Conclusions: Here we have identified the potential host modulators of SVA infection during acute and persistence infection in piPK15 and piPTOs and provides insight into SVA and host interaction during persistence infection.

Financial Support: This work was supported by USDA NIFA grant 2024-67015-42737.

Notes:

3MT01 - Fueling dairy cows: A simple fat for better health and performance

Ursula Abou-Rjeileh[1]

[1]Michigan State University. abourje2@msu.edu

Session: 3MT Competition, 2025-01-18, 4:05 - 4:10

Imagine being a dairy cow. You've just given birth, your body is in overdrive, producing enough milk for your calf (and humans). But there's a catch—your energy reserves are quickly running low. To meet the massive energy demands, your body starts breaking down fat stores through a process called lipolysis. Seems like the perfect solution—except, there's a problem. Too much fat breakdown can send your body into stress mode, compromising your immune system and overall well-being. This strain can lead to illness and long-term health issues, affecting not just your body but your quality of life.This is the challenge dairy cows face during the early lactation period. While they need energy to produce milk, excessive fat breakdown leads to a buildup of harmful molecules known as reactive oxygen species (ROS). These ROS can cause oxidative stress, damaging cells and making the cow sick. So, the pressing question becomes: How can we help cows produce milk without compromising their health?That's where my research comes in. We're investigating a simple solution: adding fat to the cow's diet. But not just any fat—oleic acid, a healthy fatty acid found in olive oil and other natural sources. Our research aims to explore how oleic acid can help dairy cows better manage their energy, reduce oxidative stress, and stay healthy during this demanding period.Here's what we've discovered: when cows are fed oleic acid, their bodies don't break down fat as aggressively. This means they conserve more of their energy reserves, lose less weight, and maintain better overall body condition. But there's more to the story—oleic acid also enhances the performance of the cow's mitochondria-the powerhouses of cells. By enhancing mitochondrial efficiency, oleic acid reduces the production of ROS. Lower ROS levels help mitigate oxidative stress, reducing cellular damage and promoting better overall health. It's like giving the cow's cells an energy upgrade and protective shield, enabling them to thrive during lactation without burning out.Why is this important? Nearly 60% of dairy cows experience some sort of health issue after giving birth due to the strain on their bodies. These health problems can have long-lasting consequences for both the cow and farmer. Sick cows produce less milk, leading to decreased productivity, and higher veterinary costs. Incorporating oleic acid into their diets, we could help cows avoid these health risks, produce more milk, and recover quicker after giving birth.Why should we care? Dairy cows are vital to our agricultural system, and their health is essential for their overall well-being. By promoting better energy management and reducing oxidative stress, we can support cows in maintaining their strength and vitality, leading to an improved approach to dairy farming.That's the "why." Healthier cows mean healthier milk, stronger farms, and a more sustainable future for the dairy industry. It all begins with understanding how to help these remarkable animals thrive. By making this small tweak—a little addition to their diet—we can create a positive impact for dairy cows and the people who rely on them.

Notes:

3MT02 - Nanovaccine and probiotic integration: Pioneering a new era in *Salmonella* prevention in poultry

Sara Dolatyabi[1]

[1]The Ohio State University. dolatyabi.1@buckeyemail.osu.edu

Session: 3MT Competition, 2025-01-18, 4:10 - 4:15

Food safety is a global challenge, and one of the biggest culprits behind foodborne illnesses is a bacteria called Salmonella. Poultry products are a significant source of Salmonella infections, leading to millions of cases of food poisoning annually. While we have made progress in developing vaccines to prevent salmonellosis, the current vaccines have limitations. Some can revert to harmful forms, posing a risk of infection, or they may be restricted in use, especially before slaughter. This presents a pressing challenge in food safety—how can we make poultry vaccines safer and more effective?This is where my research comes in. I set out to explore a potential solution by combining two powerful approaches: a cutting-edge nanovaccine and probiotics. Unlike traditional vaccines, nanoparticle-based vaccines avoid the limitations of live vaccines, such as reversion to harmful forms or restrictions on their use in broilers. They use ultra-small particles to deliver the vaccine components directly to targeted areas of the immune system, enhancing the body's ability to recognize and fight off threats like Salmonella. Meanwhile, probiotics, often called "good bacteria," are known for supporting gut health. But what if they could do more? My research investigates whether probiotics can not only improve gut health but also amplify the immune response to an oral Salmonella vaccine, creating a more effective defense against salmonellosis.To test this, we used a nanovaccine designed to target specific immune sites in the intestine, helping the immune system respond more effectively to potential Salmonella exposure. This nanovaccine contains highly immunogenic proteins of Salmonella. It acts as a signal to provoke the immune system, priming it to recognize and fight the pathogen if the chickens are later exposed. In our study, some chickens received both the nanovaccine and the probiotic, while others were given the vaccine alone, and a control group received neither.The results were promising. Chickens receiving the nanovaccine and probiotic had significantly less Salmonella load in their intestines compared to a widely used commercial live vaccine, reducing the risk of disease spread. But the benefits didn't stop there. We also observed a more robust and more sustained immune response in the chickens that received both probiotic and nanovaccine combination, with increased specific activation of B-cells, which produce antibodies, and cytotoxic T-cells, which destroy infected cells. This indicated a better-equipped immune system to combat salmonellosis. Moreover, these chickens had higher levels of secretory IgA antibodies in their intestines, suggesting that the probiotic helped boost their gut immunity, which is vital for fighting foodborne pathogens.What does all of this mean? By combining a probiotic with an oral nanovaccine, we were able to boost the chickens' immune system in a way that not only reduced Salmonella infection but also promoted long-lasting immunity. This has significant implications for food safety. A safer, more effective vaccine means less Salmonella contamination in poultry, leading to fewer cases of foodborne illness in humans. This combination strategy could represent the future of how we protect both animals and people from food poisoning bacterial infections.

Notes:

3MT03 - Uncovering hidden origins: using alternative sequencing methods to map strain-level populations of prevalent liver abscess taxa along the gut-liver axis

Kayla Hazlett[1]

[1]Texas A&M University. kayla.hazlett@tamu.edu

Session: 3MT Competition, 2025-01-18, 4:15 - 4:20

Liver abscesses (LAs) are one of the most common and costly issues in the North American beef industry, raising welfare concerns and resulting in millions of dollars of economic losses each year. The traditionally accepted pathogenesis of liver abscesses emphasizes the disruption of the rumen epithelium and the translocation of Fusobacterium to the liver. However, recent studies indicate that LAs are highly polymicrobial and may be seeded from the hindgut in addition to the rumen. Specifically, bacteria typically associated with the hindgut (i.e., members of Bacteroides) dominate some LA microbial communities. The microbiome of LAs is becoming increasingly characterized using 16S rRNA amplicon sequencing, yet 16S sequencing is not able to detect and quantify prevalent LA microbial taxa in the gut, where they are typically in very low abundance. Even for taxa detectable with 16S sequencing, accurate classification beyond the rank of genus is highly limited. To address these issues, we are investigating the use of alternative sequencing-based approaches to enrich for these low-abundance microbes within sequencing libraries. This will enhance their detection and quantification along the gut-liver axis, while also allowing classification beyond the level of genus. We have shown that target enriched metagenomics (TE) massively enriches for strains in taxa of interest, and we believe its use here will let us quantify the microbes prevalent in LAs and identify their probable source in the bovine gut. TE relies on a bait-capture system to enrich sequences of interest, and we have designed baits for 4 genera of interest that are prevalent in LAs (Fusobacterium, Trueperella, Bacteroides, and Porphyromonas). The bait design process involves identifying divergent lineages independent of existing strain-level taxonomy. For example, based on average nucleotide identity, we designed baits for Trueperella around 7 lineages despite the genus containing only 6 defined species. In addition to TE, we have used rpoB sequencing to characterize strain-level populations of Fusobacterium within liver abscesses themselves. Differences in the diversity and composition of the Fusobacterium population exist between abscesses with different microbial fingerprints. We hope to establish new, more powerful tools for investigating strain-level populations of LA microbial taxa without the need for culture. We anticipate these tools will have applications well beyond the gut-liver axis of cattle. Ultimately, this project will provide a better understanding of the pathogenesis of LAs. In doing so, it will aid researchers in the development of new LA prevention strategies, reducing the use of anti-microbial drugs, and resulting in more efficient, healthier cattle.

Notes:

3MT04 - Inflammatory pathways contribute to pathology of *Clostridioides difficile* infection in a murine model: A spatial transcriptomics study

Niloufar Ghahari[1]

[1]Purdue University. nghahari@purdue.edu

Session: 3MT Competition, 2025-01-18, 4:20 - 4:25

OBJECTIVE: To investigate the differential gene expression in the cecum and colon of symptomatic and asymptomatic mice infected with Clostridioides difficile using spatial transcriptomics analysis.METHODS: After antibiotic pre-treatment and infection with C. difficile, mice were monitored for clinical signs of Clostridioides difficile infection (CDI). Symptomatic mice were euthanized upon showing severe signs, and intestinal tissues were harvested. Formalin-fixed paraffin-embedded (FFPE) intestinal sections were analyzed using NanoStringTMspatial transcriptomics technology. Differential gene expression in the cecum and colon was evaluated and compared between the superficial and deep mucosal layers.RESULTS: The IL-17 pathway, including Lcn2, Cxcl2, and S100a8 genes, was significantly upregulated in symptomatic mice. Activation of the IL-17 signaling pathway triggered downstream signaling through NF-κB and MAPK pathways. Gene expression was markedly altered between the superficial and deep layers of the intestine, revealing layer-specific differences in gene expression patterns between symptomatic and asymptomatic mice.CONCLUSIONS: Spatial gene expression patterns in the enteric mucosa are strongly associated with clinical signs and lesions observed in symptomatic CDI mice. These findings provide insight into the role of inflammatory pathways in the pathology of CDI and highlight the significance of tissue layer-specific responses in the disease progression.

Notes:

3MT05 - Understanding the epidemiology and mortality-associated factors of atypical canine infectious respiratory disease complex: a cross-sectional study based on dog owners' perspectives in the United States

Mahamudul Hasan[1]

[1]Kansas State University, Manhattan, Kansas, USA. mahamudul@vet.k-state.edu

Session: 3MT Competition, 2025-01-18, 4:25 - 4:30

In a world where dogs are not just pets but cherished family members, a new and mysterious respiratory disease has emerged, threatening the happiness these animals bring to our lives. My research focuses on a fast-spreading outbreak of an atypical Canine Infectious Respiratory Disease Complex (aCIRDC) that rose across the United States earlier this year. Unlike typical respiratory infections, this new form tested negative for known pathogens, resistant to typical treatments, and led to several mortalities. Thus, understanding the spread of this disease epidemiology and the factors linked to deaths are essential for protecting dogs and preventing any potential risk to humans.This study is one of the first systematic epidemiological studies on aCIRDC, filling an important knowledge gap in veterinary and infectious disease fields in USA. Most previous studies on canine respiratory disease focused on known pathogens such as Bordetella bronchiseptica or canine influenza. However, this outbreak was different, presenting a rare case where the standard diagnostic tests failed to detect the cause. Thus, a cross-sectional survey was conducted from November 2023 to January 2024. Survey responses were collected from the members of the "2023 Canine Infectious Respiratory Disease Tracking" Facebook group. This research investigated the broader context of aCIRDC and the associated mortality risk factors by analyzing 478 complete responses from dog owners. The data shown alarming trends—high incidence rates were found in states like California, Colorado, and Florida, particularly in dogs exposed at dog parks, boarding facilities, and dog events. Most dogs suffered from cough, vomiting, loss of appetite, lethargy, fever, runny nose, and difficult breathing within 5-10 days of exposure. Doxycycline was the most used antibiotic for treatment. However, 8.58% of dogs were reported to have died. The univariate binary logistic regression analysis showed that clinical symptoms such as lethargy, fever, and difficulty breathing were strongly associated with death, as were treatments like IV fluids and oxygen therapy, indicating that dogs receiving these treatments had a nearly 9.4 time increase in mortality risk. On the other hand, multivariate logistic regression analysis confirmed that dogs exhibiting only these severe symptoms act as an independent factor of mortality with a significant odds ratio (14.11).The next step of this research is to analyze over 700 swab samples from dogs using advanced next-generation sequencing to uncover possible new pathogens connected to aCIRDC. Also, we are focusing on detecting a previously unidentified pathogen, named IOLA, from these samples. IOLA has been found in lung fluid samples from humans and pigs suffering from respiratory infections that could also have a potential cause of respiratory illness in dogs.In summary, the data gathered from this study serves as a first step toward mitigating the impact of the disease and could lead to interventions that save the dogs from that disease. By enhancing public awareness and understanding of aCIRDC, the hope is to mitigate further outbreaks and safeguard the health of dogs nationwide.

Notes:

3MT06 - Integration of automation with 384-well RT-qPCR assay advances high-volume diagnostic testing for Porcine Reproductive and Respiratory Syndrome Virus (PRRSV)

Bala M. Reddi[1]

[1]Iowa State University. mounika9@iastate.edu

Session: 3MT Competition, 2025-01-18, 4:30 - 4:35

Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) continues to be a significant burden on the swine industry, contributing to over $1 billion in annual losses. Effective disease management hinges on early and accurate detection, which can prevent the rapid spread of the infection. Quantitative Real-Time- Polymerase Chain Reaction (RT-qPCR) is a widely used diagnostic technique for identifying the virus in the samples. At Iowa State University's Veterinary Diagnostic Laboratory (ISU-VDL), nearly 400,000 PRRSV samples were tested in 2023, highlighting the necessity for a more efficient and high-volume testing platform. This research evaluates the performance of an automated 384-well RT-qPCR system using the Quant Studio 7 Pro (QS7 Pro), comparing it to the conventional 96-well format on the ABI Fast 7500. The goal is to streamline diagnostic workflows, ensuring greater speed, precision, and accuracy in high-volume testing environments.Workflow optimization begins with the extraction of viral RNA from samples using automated extraction platforms such as the KingFisher Apex. Integrating automated liquid handler (BRAVO) for handling the reagents and extracted samples at lower volumes and transferring them to 384 well plates. The 384 well plates are then sealed, centrifuged, and placed inside the automated incubator maintained at 4°C. Automated plate movers sequentially load the prepared 384-well plates from an incubator to the QS7 Pro platform and unload the plates after the run to load the next plate in the queue.Our study has shown that the automated 384-well system performed similar sensitivity (lowest concentration of the viral RNA) compared to the traditional 96-well assay. By adopting 384-well assays, 384 samples can be processed with a total reaction volume half (10µL) that of the 96-well assay (20µl), which processes only 96 samples at once. This automated system has the potential to process around 4000 samples overnight (11 hours), which would take approximately 42 hours for a conventional 96-well system with manual plate loading.This work not only addresses the needs of high volume testing but also aims to assess the economic feasibility of adopting such systems more broadly. Future research includes testing the automated platform across various viral strains and sample types, ensuring its robustness in diverse clinical scenarios. Ultimately, this research could transform routine surveillance and outbreak response in the swine industry, protecting both animal health and the economic stability of agriculture.

Notes:

3MT07 - The fat of the matter: The role of endocannabinoids in dairy cow health

Madison Myers[1]

[1]Michigan State University. smit2477@msu.edu

Session: 3MT Competition, 2025-01-18, 4:35 - 4:40

Imagine you've just finished running a marathon. Now, instead of resting, you're handed a screaming baby, a grocery list, and a pile of dirty laundry. That's the life of a dairy cow transitioning from pregnancy to lactation! These hardworking animals face intense pressure to produce milk while breaking down their fat reserves for energy—a process called lipolysis. And, while necessary, too much fat breakdown can lead to udder chaos: metabolic stress, inflammation, and a drop in milk production.That's where my research comes in. I'm diving into how bioactive lipids—particularly endocannabinoids—act as the cow's internal peacekeepers, helping balance their energy needs while fighting off inflammation. Endocannabinoids are tiny molecules, best known for their role in the brain where they influence mood and appetite (think "the munchies"). But in cows, they have an even bigger job—regulating fat metabolism and inflammation. Think of them as babysitters, making sure that fat breakdown and immune responses don't wreak havoc and trash the house.My work revealed that activating a key player in the endocannabinoid system, the cannabinoid-1 receptor (CB1), affects fat metabolism differently depending on the cow's stage of lactation. In non-lactating cows, CB1 is like yoga instructor—calmly reducing fat breakdown and promoting fat storage, keeping everything zen. But after cows give birth, CB1 can't work its magic. It's as if early lactation stress causes the system to go haywire, preventing CB1 from controlling fat breakdown.So, what happens when you add inflammation into the mix? I explored how immune challenges like endotoxemia affect endocannabinoid levels. Spoiler: it's a wild ride. During these "immune storms," key endocannabinoids like AEA and OEA surge, stepping in to control the inflammation. Cows exposed to repeated inflammatory events showed even more dramatic changes, suggesting that their endocannabinoid system adapts to constant stress—much like our immune system after repeated colds.To make things more interesting, I compared different fat breakdown pathways—one triggered by stress and the other by inflammation—and how each affects endocannabinoid production. Stress-induced fat breakdown led to a surge in 2-arachidonoylglycerol (2-AG), while inflammation pushed AEA levels through the roof. It's as if the endocannabinoid system is running two different rescue missions depending on whether the crisis is energy demand or an immune challenge.These discoveries highlight a delicate balance: cows need to break down fat for energy, but doing so too quickly can trigger inflammation and metabolic problems. Endocannabinoids seem to play a key role in managing this balance. Understanding how these systems interact could lead to new ways to keep cows healthy and productive during stressful periods. By targeting the endocannabinoid system, we could help cows stay healthy, produce more milk, and lead happier lives—because, after all, a happy cow is a productive cow!

Notes:

3MT08 - From farm to fork: Eugenol nanoemulsion as a safe and effective approach to control *Salmonella Enteritidis* in the poultry industry

Jodie Allen[1]

[1]University of Connecticut. jodie.allen@uconn.edu

Session: 3MT Competition, 2025-01-18, 4:40 - 4:45

Many consumers are not informed that their favorite chicken product may harbor Salmonella bacteria, a major poultry-associated foodborne pathogen causing millions of illnesses every year in the United States, due to consumption of contaminated poultry products. In general, chickens act as a reservoir host, wherein the pathogen colonizes the ceca leading to product dissemination during slaughter. To reduce Salmonella colonization in live birds and dissemination on poultry products, pre-harvest strategies and post-harvest are employed with varied degrees of efficacy. Currently, I am investigating the anti-Salmonella efficacy of a GRAS status plant compound known as Eugenol (EG; obtained from cloves). I have developed nanoemulsions of eugenol (EGNE) using gum arabic and lecithin (GAL) as emulsifiers to overcome the low solubility of EG. The objectives of my thesis are: Investigating the efficacy of EGNE in reducing SE colonization in broiler chickens and investigating the efficacy of EGNE in inactivating SE on poultry carcass.Broiler chickens were supplemented EG or EGNE 0.03% in drinking water for 21 days. On day 14, birds were inoculated orally with SE, and sacrificed on days 21 and 28, followed by SE enumeration in cecal contents. Moreover, chicken skins were inoculated with SE, followed by dipping in EG, or EGNE 0.3, 0.6, 1.25% for 15, 30, 240 or 480 min at 4oC. Post treatment, the surviving SE on chicken skin was enumerated.Results suggest that supplementation of EGNE in chicken drinking water reduced SE colonization by ~1.52 log CFU/g in cecal content as compared to control after 21 days of treatment. We also observed EGNE reduced SE on chicken skin by ~3.26 log CFU/sample after 480 min of treatment. Results from my thesis will present an alternative antimicrobial strategy to reduce SE colonization in broiler chickens as well as reduce SE survival on poultry products, thus producing a wholesome and safe product for human consumption.

Notes:

3MT09 - Defending poultry: Unraveling APEC's virulence to drive new innovations in vaccine strategies against colibacillosis

Klao Runcharoon[1]

[1]University of Georgia. kr71624@uga.edu

Session: 3MT Competition, 2025-01-18, 4:45 - 4:50

Colibacillosis is a serious disease affecting poultry worldwide, primarily caused by Avian Pathogenic E. coli (APEC). This disease is a big threat to poultry production as it can significantly impact one of mankind's cheapest sources of protein. While APEC was once seen as an opportunistic pathogen, it is now recognized as a primary pathogen and a major threat to poultry health. To tell APEC apart from regular fecal E. coli (AFEC), scientists use virulence genotyping. This method detects specific genes, particularly those linked to CoIV plasmids, like cva, iroN, ompT, iss, and hlyF. Traditionally, APEC serogroups O1, O2, and O78 were the main focus, but new serogroups have recently emerged, especially in Georgia. However, because APEC's genetics are constantly changing, genotyping alone isn't enough to fully understand APEC virulence. The virulence of these new APEC serogroups in chickens is still largely unknown. To explore this, we conducted a study using nine APEC serogroups (10 strains) in different chicken age groups, including embryos, 1-day-old chicks, and 3-week-old hens. We carefully monitored mortality rates and disease severity. Our results were significant: some strains were particularly deadly to embryos and chicks, and one strain showed surprising virulence in older birds. Some strains also showed a higher presence in organs like the liver and lungs, indicating a potential for dissemination within the body. These findings highlight the dangerous potential of these new APEC strains, especially in young chickens, emphasizing the need for better health management in hatcheries and breeder operations. The insights gained from these tests are paving the way for new strategies to combat these emerging APEC serogroups. We have also sequenced selected APEC strains and our ongoing analysis aims to discover the genes or mechanisms responsible for their virulence at different stages of bird development. Currently, the three available commercial APEC vaccines may provide limited protection against the wide variety of APEC serogroups and strains especially emerging serogroups that occur. My mission is to identify additional virulence-associated genes to develop new vaccine candidates with broader application. These vaccines will target a broader range of APEC serogroups, addressing the pathogen's genetic diversity. My goal is also to create a vaccine that strengthens passive immunity in chicks, which are particularly vulnerable to APEC infections. By advancing a new vaccine against colibacillosis, we aim to protect poultry health and welfare, ensuring the stability of this vital protein source for human consumption.

Notes:

3MT10 - Emerging disease at the global wildlife-livestock-human interface

Alaina Macdonald[1]

[1]University of Guelph. alaina@uoguelph.ca

Session: 3MT Competition, 2025-01-18, 4:50 - 4:55

Infectious pathogens of pandemic potential are recognized with increasing frequency and often with highest impact in the poorest places. Yet, current surveillance efforts focus on reacting to disease (identifying and controlling) which variably reduces illness and death, usually with marked financial costs. These efforts often miss the earlier events that predisposed the population to disease emergence. Focused on these challenges, we envisioned a system where multispecies health is better understood and upstream signals could be developed to help experts of any kind recognize changes in health and work together to restore balance before harm occurs. We asked ourselves the following: if we could better understand and characterize changes in health where disease emerges, can we create signals which contribute to a health-based early surveillance system? This project seeks to answer the former part of this question; to ask the scientific literature, "When disease emerged, what happened? Why?" Of over 14 000 studies from four databases, 151 studies met the eligibility criteria. Most studies (n=101) involved emerging diseases caused by viral pathogens, followed by bacteria (n=41), helminths (n=3) and protozoa (n=3). Most studies focused on a zoonotic pathogen (n=123), many with significant economic consequences. The most common pathogens studied were highly pathogenic avian influenza virus H5N1 (n=29), followed by Mycobacterium bovis (n=16), and Bacillus anthracis (n=11). Wild birds were the most frequently studied wild species (n=48), followed by wild boar (n=5) and ungulates (n=3). Among livestock species, poultry (n=52), cattle (n=29) and pigs (n=12) were most often represented. Reported drivers of disease emergence included: 1) high wildlife and livestock population densities, 2) contact between livestock and wildlife via migration or trade, 3) changes in temperature and precipitation, 4) anthropogenic land use change, and 5) political instability and poverty. Understanding determinants of changing health status and developing signals of change will help future decision makers respond to cues before disease is detected. Recognizing the unequal distribution of resources for health will be a required step towards sustainable and more equitable actions to promote health of all species and avoid rapid undetected disease spread.

Notes:

3MT11 - Challenging paradigms: how management decisions effect long term health outcomes

Hudson McAllister[1]

[1]Texas A&M University - VERO Program. hmcallister15@tamu.edu

Session: 3MT Competition, 2025-01-18, 4:55 - 5:00

If you have recently attended a conference or talk related to cattle, you have probably heard an expert speak on how respiratory vaccination or marketing strategies impact Bovine Respiratory Disease (BRD). Our understanding of managing BRD with vaccines has expanded from including just bacterial pathogens in the beginning of the twentieth century to including viral pathogens and discussing the next generation of vaccine technologies into commercial operations. Today's commercial BRD vaccines have become the primary method for prevention, but we understand little regarding how those vaccines influence immunity over time or how effective they are in practical application. This leads to us to ask the question: what do we really know about the relationship between respiratory vaccination and titer values, health, and performance outcomes in beef and dairy cattle? First, we conducted a systematic review evaluating this specific question and concluded that, while respiratory vaccination has been heavily studied in the U.S and Canada over the several decades, it is immensely difficult to compare outcomes across published literature due to heterogeneity in reporting outcomes. Determined, we developed a hypothesis that we may use management decisions like vaccination and marketing strategy to identify biomarkers, either a production metric or genomic marker or combination thereof, that can be used to alter management practices related to disease outcomes. Using novel techniques like RNA sequencing, we have discovered that immune system development, antigen presentation cell activity, and Th17 cell immunity is stimulated long after initial attenuated viral vaccination. Moreover, following cattle in a multi-year randomized controlled trial, we are incorporating health and production data, transcriptome, microbiome, and inflammatory cytokine concentrations to dynamically evaluate how these commonly used tactics against BRD influence cattle in ways never evaluated before. Ultimately, we aim to provide novel data that will change how a calf moves through a production system and improve our disease management practices.

Notes:

3MT12 - Coevolutionary protein arms-race between phages and bacteria in beef-processing plant drains

Vignesh Palanisamy[1]

[1]Texas A&M University, College Station, TX. vignesh.bioenv@tamu.edu

Session: 3MT Competition, 2025-01-18, 5:00 - 5:05

Bacteriophages, the most abundant entities on Earth, engage in intricate interactions with bacteria, particularly within environmental biofilms, fueling an ongoing ecological arms race. Biofilms are aggregates of multiple species of microorganisms adhered to surfaces, cooperating for survival. These biofilms foster microbial communities that can result in beneficial and harmful interactions, including the development of antimicrobial resistance. This study investigates the interaction between phages and bacteria in the drains of beef-processing plants using high-throughput sequencing and metagenomic analysis. The intended purpose of this study is to gain a mechanistic understanding of phages and multi-species biofilm interaction and potentially employ bacteriophages as a targeted intervention to control pathogens in biofilm and address the growing challenge of antimicrobial resistance.Beef processing facilities are a man-made built environment; the processing activity shapes the microbiome of the built environment. The microbiome of the floor drains is a collective representation of the processing environment, as they are the focal point of concentration and collection of the rinse water from animal carcasses, equipment, conveyor belts, and food-contact and non-food-contact surfaces. These drains can be hotspots for pathogen persistence and cross-contamination. Microorganisms within these drains can form biofilms as they provide a stable and protective niche. These biofilms may harbor pathogens or spoilage microorganisms, posing a food safety and spoilage risk. As biofilm-associated food-borne pathogen outbreaks and food spoilage are rising, it is critical to develop effective strategies to control biofilm formation. The control strategies are shifting more towards natural alternatives due to the surge in antimicrobial resistance due to the overuse of antimicrobial agents. Bacteriophages offer a promising natural alternative solution, with their inherent ability to target bacteria. Understanding the dynamics between bacteria and phages is an essential step toward leveraging phages as a viable intervention strategy.The metagenomic data from this work revealed a treasure trove of information that unveiled the coexistence of bacteriophages and bacteria in the drains. The subsequent sequence extraction and taxonomic annotation of viral sequences from the complex metagenomic datasets revealed a rich diversity of phages in the drains, including those targeting Pseudomonas, Klebsiella, and Enterococcus. The functional annotation of viral sequences identified phage structural proteins, infective genes, and lysis mechanisms, providing profound insights into the potential impact of phages on bacterial communities. Equally fascinating was the functional annotation of the bacterial sequences, which identified bacterial defense mechanisms against phages, including CRISPR-Cas systems, restriction-modification, and other antiphage systems. The study also unearthed the presence of anti-CRISPR proteins in phages, suggesting a counterattack on the bacterial defense. These findings not only provide evidence for phage attack, bacterial defense, and phage counterattack but also underscore the significance of the ongoing co-evolutionary arms race between phages and bacteria.This study highlights the intricate co-evolutionary battle between bacteriophages and bacteria within biofilms in beef-processing plant drains, offering a path toward harnessing phages as a natural solution to combat biofilm formation and mitigate food safety risks. By leveraging metagenomic insights, the research opens new avenues for targeted, sustainable interventions in the face of rising antimicrobial resistance.

Notes:

3MT13 - Air-liquid cultured porcine-derived primary respiratory epithelial cells (ALI-PRECs) as a model for studying *Mycoplasma hyopneumoniae*

Ana F. Castillo Espinoza[1]

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

Session: 3MT Competition, 2025-01-18, 5:05 - 5:10

Mycoplasma hyopneumoniae (Mhp) has been a focal point of research since its description as the causative agent of Enzootic Pneumonia in pigs and one of the primary agents of Porcine Respiratory Disease Complex. A range of laboratory tests, including bacterial isolation, serology, quantitative Polymerase Chain Reaction (qPCR), and Immunofluorescence (IFA), are currently available for Mhp diagnosis. Despite being the sole method for assessing Mhp viability, the bacterial isolation method faces persistent challenges, including low sensitivity, slow growth rate, and the frequent overgrowth of other mycoplasmas. In this context, in vivo models have been crucial for studying Mhp pathogenicity; however, the shift towards a reductionist approach now favors in vitro and ex vivo alternatives. Conventional submerged cell culture fails to replicate the air-liquid conditions regularly encountered by porcine airways. Conversely, porcine-derived primary respiratory epithelial cells (PRECs) cultured under air-liquid interface (ALI) conditions achieve a differentiation and complexity comparable to in vivo, rendering ALI-PREC a suitable model for investigating fastidious respiratory pathogens, such as Mhp.This study evaluates the suitability of ALI-PREC model for studying Mhp, and Mhp mechanism of inducing ciliostasis and cytopathic damage in ALI-PRECs. ALI-PRECs from three biological replicates were inoculated with Mhp strain 232 at various concentrations (105, 106, 107 CCU/ml) for different durations (2 h vs. 5 h of exposure). Cells were monitored for 24, 48, and 72 h post-inoculation (hpi). Additionally, Mhp infection kinetics were tracked up to 144 hpi following 2-h exposure to 107 CCU/ml of Mhp.By 24 hpi, Mhp-inoculated ALI-PRECs exhibited progressive loss of integrity, clustering, decreased PRECs, and ciliostasis. These results were confirmed by the reduction ($p<0.05$) of the cell number, and the detection of Mhp P46 protein in Mhp-inoculated ALI-PRECs. Mhp DNA was detected by qPCR in all Mhp-infected ALI-PRECs; however, Mhp DNA detection in subnatant indicated that epithelial disruption but also cell number were influenced by the time of exposure to Mhp, and occurred in a dose- and time-dependent manner. Downregulation ($p<0.001$) of genes related to ciliary motility and upregulation ($p<0.05$) of intercellular junction genes in Mhp-inoculated ALI-PRECs was detected at 72 and 120 hpi, respectively. Interestingly, despite a decrease in ciliary beating, ciliary motility persisted over time in two biological replicates.In summary, Mhp adheres to ALI-PRECs, causing cytopathic damage and ciliostasis, with a potential wound healing response in ALI-PRECs that requires further investigation. Our findings underscore variability in ciliary responses, suggesting differences in host susceptibility among pigs.The impact of this research lies in demonstrating that ALI-PRECs is a suitable in vitro model to study Mhp-airway interactions, offering the potential to improve Mhp diagnosis and address the limitations of bacterial isolation methods. Notably, the airway polarity provided by ALI-PREC models allows a more accurate capture of the Mhp-induce effect in a short time frame (by 24 hpi), unlike traditional models.

Notes:

3MT14 - Tracking antibiotic resistance from poultry farms to our plate

Pankaj Gaonkar[1]

[1]Auburn University. ppg0001@auburn.edu

Session: 3MT Competition, 2025-01-18, 5:10 - 5:15

When you are sick, your doctor will prescribe you medicine, called antibiotics. Unfortunately, these antibiotics are not effective these days as they used to be. Because of overuse of antibiotics, bacteria have evolved to protect themselves leading to antibiotic resistance. Antibiotic resistance is a global concern and this silent pandemic is estimated to kill millions of people.As the earth's population grows, there is an increase in demand for protein of animal origin, such as chicken meat - a cheap and easily available source of protein worldwide. This will lead to an increase in antibiotic use to treat and prevent diseases in highly intensive production systems and contribute to development of antibiotic resistance. Antibiotic resistance can spread among poultry, environment and humans, threatening One Health and decreasing antibiotic efficacy to treat diseases in humans and animals. Therefore, to control antibiotic resistance spread, it is important to have comprehensive understanding of the interaction between poultry production, their environment and humans.To tackle this problem, the poultry industry is moving toward reducing antibiotic use, but our research shows that reducing antibiotic use alone is not enough to tackle antibiotic resistance.Our study focuses on examining these interactions, specifically between the inside and outside of the poultry environment and across different stages of production, from start to finish. We assessed antibiotic resistance status in 29 poultry farms from 2 major producers in the United States practicing reduced antibiotic use. These farms used antibiotics only for treating diseases and not for preventing them.We found that despite of reduced antibiotic use, antibiotic resistance increases in the environment as production reaches the broiler stage when birds are ready to be harvested. Additionally, we observed distinct antibiotic resistance profiles between litter and soil, with litter showing a higher resistance load. Antibiotics commonly used were found in higher proportions in litter. However, soil had a diverse and evenly distributed resistance profile. This indicated that litter was more frequently exposed to antibiotics than soil.However, we found two farms where antibiotic resistance profiles were similar for litter and soil, indicating potential transmission of antibiotic resistance between inside and outside poultry houses. Thus, our study showed that antibiotic resistance from poultry can contaminate the outside environment, indicating breaches in biosecurity.Finally, we screened the final stage, the processing plant, and found that antibiotic resistance persists in the chicken meat despite all the efforts taken to reduce meat contamination during processing. This raises concerns about the transmission of antibiotic resistance to consumers.Our research highlights that simply reducing antibiotic use is not enough to combat antibiotic resistance. We must also take into account the role of the environment as a reservoir of antibiotic resistance, which is often neglected. We are getting closer to understanding the pathway by which antibiotic resistance spreads from poultry to their environment and humans. This information will help farmers, veterinarians, and companies to develop antibiotic resistance reduction strategies in poultry and protect human, animal, and environmental health.

Notes:

3MT15 - Host-environment omics and interactions for health prediction and outcomes

Bradly Ramirez[1]

[1]Texas A&M University. ramirez_b@tamu.edu

Session: 3MT Competition, 2025-01-18, 5:15 - 5:20

In a world of multifactorial diseases of complex etiology, multiomic approaches to understand pathogenesis and prognosis are required to properly characterize and mitigate these illnesses. My thesis combines transcriptomic, metagenomic, and metatranscriptomic sequencing to shed insights into the development of bovine respiratory disease and the various ways that the host immune system and microbiome reacts to industry-driven changes in homeostasis.

Notes:

3MT16 - Unraveling the antibiotic-dependent evolutionary changes in globally emerging multidrug-resistant *Salmonella*

Samuel Ajulo[1]

[1]Texas Tech University School of Veterinary Medicine. sajulo@ttu.edu

Session: 3MT Competition, 2025-01-18, 5:20 - 5:25

Antimicrobial resistance claims millions of lives every year globally, and antibiotic use is one of the most important strategies in the fight against pathogenic bacteria. The conventional paradigm is that the use of an antibiotic not only drives bacterial resistance to that antibiotic but may promote co-selection of unrelated AMR traits enhancing bacterial fitness in the presence of these antibiotics. However, bacterial carriage of AMR traits also imposes a fitness disadvantage, negatively impacting its persistence within a specific ecological niche, particularly in the absence of antibiotics. Thus, the evolution of AMR is a delicate balance between depends on balance between the fitness advantages and costs imposed by AMR traits. Presently, what remains unclear is how the various classes of antibiotics differentially impact the evolution of AMR, especially in clinically relevant bacterial strains. This study aims to investigate how different antibiotic classes influence AMR evolution and associated fitness and uncover the molecular mechanisms driving this process.We selected the globally emerging multidrug-resistant Salmonella Kentucky ST198 (MDR-SK198), resistant to over five antibiotic classes, as our model pathogen. This strain is known to colonize chicken gut globally, contributing to its widespread threat. In our study, we exposed the pathogen to five distinct antibiotics over 4000 generations, applying long-term selection pressure to understand antibiotic resistance (AMR) evolution. After 265 days, we observed that the evolved clonal evolutionary lineages (CELs) followed three distinct evolutionary trajectories based on antibiotic exposure. Under ciprofloxacin (CIP) and streptomycin (STR) pressure, the non-CIP and non-STR AMR traits decayed rapidly. In contrast, exposure to sulfamethoxazole-trimethoprim (STrim), amoxicillin (AMOX), and tetracycline (TET) maintained the non-STrim, non-AMOX, and non-TET AMR traits. Notably, the no-antibiotic (NOABX) passage exhibited varied AMR persistence, highlighting complex evolutionary outcomes. The genotype of these lineages confirmed AMR phenotypes, further establishing an antibiotic-dependent evolution of AMR.To investigate the genotype-phenotype association and bacterial fitness impacts, we selected CELs exposed to CIP, STrim, and NOABX conditions as representative of distinct evolutionary pathways. Comparative genomic analysis, using strain PU131 as a reference, revealed 157 gene deletions and 40 single nucleotide polymorphisms (SNPs), with most changes occurring in the CIP lineages. These genetic alterations, including AMR and non-AMR traits, were functionally classified using NCBI BLAST and STRING databases, revealing that over 70% of changes in the CIP lineages likely impacted bacterial fitness, virulence (e.g., SPI-3), metabolism, and stress responses. Non-AMR phenotypic changes were also evaluated through growth assays, biofilm production, motility tests, and high-throughput phenotype microarray (PM) analysis across 380 phenotypes under various metabolic and stress conditions. These phenotypic changes mirrored the genetic shifts observed, reinforcing the connection between genotype and phenotype.Our study demonstrates antibiotic pressure-dependent AMR evolution, alongside non-AMR genetic and phenotypic changes impacting bacterial fitness. These findings enhance our understanding of AMR evolution in MDR-SK198 and may inform future strategies for combating MDR bacterial pathogens, offering critical insights into the interplay between antibiotic pressure, bacterial fitness, and AMR. Our next step is to investigate how these changes may contribute to the pathogenesis and virulence of bacteria in the relevant hosts.

Notes:

3MT17 - Microbial gatekeeping: Understanding and preventing the spillover of zoonotic disease

Kirsten Zwally[1]

[1]Texas A&M University. kirsten.zwally@tamu.edu

Session: 3MT Competition, 2025-01-18, 5:25 - 5:30

My research focuses on zoonotic disease transmission and microbial ecology through a One Health perspective. The nexus of wildlife and livestock interactions, which occur in commonly shared areas (water and feed vessels, rangelands) manifests a prime opportunity for pathogen (re)emergence or spillover events. Approximately 60% of all human infections and numerous global pandemics are of animal origin. In conjunction with global warming, which contributes to warmer temperatures, loss of natural habitats, and more frequent anthropogenic disasters, the cross-border spread of disease is increasing the risk of future zoonotic disease outbreaks. In the United States, shared boundaries contribute to human and/or animal migration. Of the entire U.S.-Mexico border, Texas covers approximately half, stretching over 1,254 miles. Native diseases may be transported with their host to new territories depending on proper regulations and undocumented entry. Therefore, it is of great importance to further understand disease reservoirs and dissemination.To further understand and assist in the prediction of disease evolution and pathogenicity, it is critical to understand the invisible. Microbial ecology and microbiome studies are rapidly gaining popularity in every field and for good reason. Microbes are the foundation of life and impact every creature, resource, and function. While they are ubiquitous, microbes are highly diverse and have an incredible capacity to adapt and constantly evolve. Even within the large state of Texas, there are over ten distinct ecoregions each with unique resources and climates.Coupled with the shared border space and highly diverse ecosystems, Texas was an ideal candidate for my longitudinal research project. Nine research stations from nine different counties were sampled every season (based on the Gregorian calendar) over a year. Environmental samples were collected from communal matrices (i.e. water vessels, water biofilms, soil, and feces). Agnostic, deep shotgun sequencing will be performed to produce comprehensive metadata including microbial taxonomies, functional annotations, and gene predictions. This will allow us to assess similarities and differences between sample type, geographical region, and season. Furthermore, metabolomic assays will demonstrate a clearer picture of how these communities' phenotypes and metabolic responses contribute to the retention and transmission of identified pathogens.These results will be utilized to deepen our understanding of wildlife/livestock environmental microbe communities through temporal and spatial conditions, what role zoonotic pathogens may play in these systems, and what metabolic pathways have been activated. This project will be immensely useful in predicting zoonotic disease hotspots, particularly regarding wildlife and livestock animal operations. Education and awareness are essential yet overlooked prophylactics in the reduction of disease spread. Moreover, this research will contribute to the development of zoonotic disease and pandemic mitigation and prevention strategies as the One Health initiative continues to combat the global surge of infection from the holistic perspective of environmental, animal, and human health.

Notes:

3MT18 - Enhancing poultry afety: Linalool as a one-step solution to combat *Salmonella* and *Campylobacter* in chickens

Leya S. Viju[1]

[1]University of Connecticut. leya.viju@uconn.edu

Session: 3MT Competition, 2025-01-18, 5:30 - 5:35

S. Enteritidis (SE) and C. jejuni (CJ) are major foodborne pathogens transmitted through poultry products, contributing to over 70% of foodborne infections and hospitalizations, and 55% of the deaths related to foodborne illnesses in the United States. Chickens are the reservoir host of SE and CJ, with their intestinal colonization constituting the most significant factor causing faecal shedding and meat contamination. Broiler carcasses are reportedly among the meats most contaminated with these two pathogens from processing facilities. Therefore, reducing SE and CJ populations in chickens could potentially reduce the contamination of poultry meat and products. Linalool, an acyclic monoterpene alcohol, is a major compound found in the essential oils of several plant species, including lavender, basil, neroli oil, coriander, and rosewood. Additionally, linalool's broad-spectrum antibacterial properties have been documented against various foodborne pathogens such as Salmonella sp., Staphylococcus aureus, and Listeria monocytogenes in vitro. Along with the antimicrobial effectiveness, the water-solubility properties and palatability of linalool offer an additional advantage for potential applications within the poultry industry. Further, since linalool is a component in a wide variety of plants, it offers a potential pathway for mass preparation, thereby enhancing its economic significance. As incidents of Salmonella and Campylobacter contamination continue to pose significant threats to both public health and the poultry industry, the exploration of linalool as a pre-harvest in-feed supplementation offers an innovative and potentially effective approach to enhance poultry food safety and sustainable poultry production. Our research aims to explore linalool as a 'single bullet' strategy that targets both SE and CJ in poultry, addressing two major pathogens simultaneously in the poultry industry. Three independent trials with broiler chickens were conducted for both SE and CJ experiments. In each trial involving 212 day-old chicks, 192 birds were randomly assigned to eight groups of 24 chicks each. The remaining 20 birds were used to check SE/CJ colonization efficiency on day 2 post-challenge. Linalool a GRAS-status plant compound, at 1, 1.5 and 1.8% was supplemented infeed for broiler chicks from day 1 through day 34. The cecum was collected on days 14, 24 and 34 and analyzed for SE/CJ colonization. Linalool at 1, 1.5 and 1.8% significantly reduced colonization of SE and CJ in broiler chickens at all three time points. The compound did not adversely affect the growth/ production parameters as well as the gut microbiome of the chickens. Results suggest that in-feed supplementation of linalool is effective in reducing SE/CJ colonization in chickens and can be an effective Pre-harvest control strategy.

Notes:

3MT19 - Beware of goose dropping at the recreational parks: The carriage of beta-lactamase-producing *Escherichia coli* in migratory geese in West Texas, United States

Yamima Tasnim[1]

[1]Texas Tech University School of Veterinary Medicine. ytasnim@ttu.edu

Session: 3MT Competition, 2025-01-18, 5:35 - 5:40

If we picture a neighborhood park on a bright day where families are enjoying picnics, kids are playing, and geese are grazing close by. It's a familiar, peaceful scene. But what will happen if those innocent geese could be carrying a hidden danger in their feces which is also spread in the soil and the water of the lake? A danger that poses a direct threat to our health. Migratory geese are important birds that frequent recreational parks and can serve as reservoirs of important zoonotic pathogens and antimicrobial-resistant bacteria of public health concern. This study aimed 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. A descriptive study was conducted, and we collected geese fecal (n=165), water (n=118), and soil (n=74) from 22 recreational parks in West Texas. We used Chromogenic agar to isolate extended-spectrum beta-lactamase (ESBL)-producing E. coli. The whole genome sequencing (WGS) method was used to determine the genomic characteristics of selected E. coli isolates. Among 357 samples, 12.61% (95%CI: 9.34-16.50) were positive for ESBL-E. coli. From WGS of 20 E. coli isolates, 19 isolates harbored at least 1 beta-lactamase gene including blaCTX-M-1, blaCTX-M-65, blaCTX-M-14, blaCTX-M-15, blaCTX-M-27, blaCTX-M-55, blaCTX-M-32, blaTEM-1A, blaTEM-1B. Most of the isolates carried genes conferring resistance to tetracyclines-(tet(A), tet(B)), aminoglycosides-(aac(3)-IIa, aph(6)-Id, aph(3')-Ia, aadA1), sulfonamides-(sul1,sul2), amphenicol-(floR), trimethoprim-(dfrA1, dfrA14, dfrA17) and streptogramin-B(MLSB) agent-(mph(A)). 13 isolates showed chromosomal mutations in the promoter region G of the ampC beta-lactamase gene. We also detected sixteen incompatibility plasmid groups, and 60 virulence genes identified, which are related to adherence, exotoxin, invasion, and nutrition/metabolic factors. Similar genome analysis showed that all isolates were genetically like human isolates previously reported from China, Japan, and the United Kingdom. This study highlighted the risk of environmental exposure of humans and pets that frequent recreational parks to MDR and ESBL-producing E. coli isolates. The result of the study showed that migratory geese at recreational parks are reservoirs of resistant bacteria with diverse serotypes and sequence types of E. coli isolates that can create a microbial storm. Migratory geese often defecate in areas where humans frequent, near water sources, playgrounds, and picnic spots. So, people can be infected with the resistant bacteria while they are going to recreational parks for jogging or walking through the feces of geese on the ground. People also can be infected with resistant organisms from lake water while they are fishing or handling the fish. Pets can be infected with bacteria through their paws while they are walking around the recreational park and from the pet people can be infected or vice versa. Moreover, people can also get infected by these bacteria after coming back home and not maintaining proper hygiene. Based on our findings, detecting a multidrug-resistant E. coli strain reinforces the importance of adequate hygiene practices for humans and pet animals after returning from the recreational park.

Notes:

3MT20 - Are mice resistant to leptospirosis? I doubt it!

Bryanna Fayne[1]

[1]University of Tennessee. brynfayn@vols.utk.edu

Session: 3MT Competition, 2025-01-18, 5:40 - 5:45

Leptospirosis is a significant, life-threatening, and underrecognized global human and animal health problem caused by the spirochete bacteria Leptospira. Approximately 1 million cases and 50,000 deaths are estimated globally due to leptospirosis. Outbreaks are common during rainy seasons and flooding for people living under poor socioeconomic conditions. Sporadic cases are reported in people participating in outdoor recreation and in agriculture workers. Animals can develop fatal leptospirosis, but at the same time, they can harbor Leptospira asymptomatically in their kidneys. Factors leading to host susceptibility, resulting in asymptomatic infection in some hosts and severe disease in others, are unknown. Therefore, elucidating the enigmatic pathogenic mechanisms of this disease is needed. Rodents are one of the major Leptospira reservoirs. A C3H/HeJ mice model with a tlr4 gene mutation is widely used in Leptospira studies. This mutation may contribute to the inability of this mouse strain to detect the Leptospira lipopolysaccharide, making it less susceptible to endotoxic shock or to an immunocompromised status due to the absence of stream antigen recognition events. We hypothesized Leptospira infection outcome in the wild-type mice strain will be different from the mice strain with mutated tlr4 gene. To test this, we compared the response of the C3H/HeJ strain, and its wild-type, the C3H/HeN strain, to Leptospira infection. We inoculated 8-week-old C3H/HeJ and C3H/HeN strains with a virulent Leptospira strain (6 x 106 Leptospira interrogans serovar Copenhageni strain Fiocruz L1-130) intraperitoneally and observed daily for any clinical signs of leptospirosis. Temperature, weight, and clinical scores were recorded daily. Mice were humanely euthanized at IACUC-approved endpoints. Heart, lung, liver, spleen, kidney, and blood were collected to assess Leptospira colonization and host response via qPCR, bacterial culture, ELISA, and Microscopic Agglutination Test (MAT). Surprisingly, the wild-type mice strain C3H/HeN started showing clinical signs of leptospirosis earlier than the C3H/HeJ strain. They exhibited clinical signs such as conjunctivitis, piloerection, lethargy, and hypothermia as soon as three days after infection. The wild-type mouse, C3H/HeN, had a significantly higher IgM and IgG antibody response compared to C3H/HeJ mice after infection. We confirmed the presence of Leptospira in all infected mice organs by culture and PCR. In conclusion, contradicting common notion, the wildtype C3H/HeN mice model was highly susceptible to clinical leptospirosis. We are currently comparing cytokine and gene expression profiles of both strains to gather a better understanding of the differences in pathogenesis.

Notes:

3MT21 - Diving into the *Salmonella* saga: Linking populations in feedlot holding ponds to those in cattle feces and the overall microbial metropolis

Valeria Lugo-Mesa[1]

[1]Texas A&M University. valerialugo@tamu.edu

Session: 3MT Competition, 2025-01-18, 5:45 - 5:50

Feedlot operators manage large quantities of manure, both in solid and liquid form. Significant rainfall in open feedlots creates liquid runoff that carries manure and solids, which must be properly managed. Feedlots typically use runoff control systems, including holding ponds, to temporarily store this runoff. Holding ponds allow heavier manure solids to settle, which can later be removed and utilized as fertilizer. Given the fecal content of the liquid runoff, it likely harbors enteric pathogens shed by cattle, such as Salmonella. This bacterium can cause both clinical subclinical infections in cattle, with clinical outbreaks typically manifested as diarrhea and fever, especially in calves. These infections not only lead to economic losses in cattle operations but also pose a significant food safety risk to humans. With the rise of wastewater-based epidemiology, this study aims to characterize the diversity and composition of Salmonella populations in holding ponds and feces from nearby cattle pens. Based on selective culture and PCR, Salmonella was detected in nearly half of the sampled feedlot ponds and nearly half of fecal samples collected from pens closest to those lagoons. Further, CRISPR SeroSeq sequencing identified an average of 1.88 serovars across positive samples. Using shotgun metagenomics and metatranscriptomics - neither of which rely on culture - we detected Salmonella across all the ponds and fecal samples, albeit in very low abundance. The abundance of Salmonella within sequencing libraries was higher from culture-positive samples, suggesting agreement between culture-dependent and independent techniques. Correlations in Salmonella populations between ponds and feces could lay the groundwork for exploring trends in community-wide Salmonella disease patterns in beef cattle through wastewater surveillance. Additionally, identifying correlations between the microbial populations present in these holding ponds and the trends observed in Salmonella populations could enhance our understanding of the bacterium's ecology in these aquatic environments.

Notes:

3MT22 - Barking up the right tree: Nutrition lessons from dogs

Janice O'Brien[1]

[1]Virginia-Maryland College of Veterinary Medicine. janiceobrien@vt.edu

Session: 3MT Competition, 2025-01-18, 5:50 - 5:55

Dogs and people have been together for over 36,000 years - sharing our homes and our food. During that time, we have experienced co-evolution as species: because our environments were the same, we evolved similar genetic adaptations. It has been shown that dogs have many more starch digestion genes than their wolf ancestors because they've been sharing our starch-rich diets. Because of this, and because of their shorter lifespans, pet dogs could be a previously unexplored model for human nutrition research that would allow researchers to discover diet-health links in years instead of decades. Because pet dogs still share our homes and lifestyles like their ancestors did, they are exposed to many of the same environmental and lifestyle exposures as their owners. Finding out how diet may be able to exacerbate or modulate certain other environmental exposures by studying dogs could allow us to discover secrets to our own human health and nutrition. In order to do this, we had to validate an epidemiological research tool called a Food Frequency Questionnaire (FFQ), which allows researchers to quantify dietary exposures so that they can be measured and studied. This is the first time an FFQ has been validated for dogs using the same rigorous methods used in human epidemiology. With a validated FFQ, researchers can ask questions like "are dogs who consume raw carrots less likely to develop eye diseases later in life?" One exciting possibility to consider is how previous clinical research in dog nutrition could factor into epidemiology research. Because dogs and their ideal diets have been extensively studied in controlled trials, there are recommended standards for what constitutes a complete and balanced diet. We can use this to our advantage in epidemiology research to study how important a complete diet is in the context of other environmental exposures: something we struggle to do in human nutrition epidemiology. Studying pet dogs living their full lives with their owners is something that will revolutionize not only dog nutrition but human nutrition too.

Notes: