The 106th CRWAD 2026 Conference of Research Workers in Animal Diseases

Proceedings

Conference of Research Workers in Animal Diseases
January 17–20, 2026

Chicago Marriott Downtown Magnificent Mile
Chicago, IL

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CRWAD 2026 Schedule at a Glance

Saturday, January 17, 2026

8:30 am – 4:00 pm	NC1202 Annual Meeting	Chicago AB, 5th Floor
10:00 am – 12:00 pm	ACVM Board of Directors Meeting	Kansas City, 5th Floor
12:30 pm – 3:30 pm	AVEPM Workshop	Chicago FG, 5th Floor
12:00 pm – 3:00 pm	NC1192 Annual Meeting	Chicago C, 5th Floor
4:00 pm – 6:00 pm	3MT Competition	Grand Ballroom Salon II, 7th Floor

Sunday, January 18, 2026

8:30 am – 10:00 am	Animal Vaccinology Research Network Symposium	Grand Ballroom Salon II, 7th Floor
	Featured Speakers • Alan Young BS, PhD • Mahesh Kumar BS, MS, PhD	
8:30 am – 10:00 am	CRWAD Research Abstracts	Chicago Ballrooms, 5th Floor
10:00 am – 10:30 am	Break	Grand Ballroom Foyer, 7th Floor
10:30 am – 12:00 pm	Animal Vaccinology Research Network Symposium (continued)	Grand Ballroom Salon II, 7th Floor
10:30 am – 12:00 pm	CRWAD Research Abstracts	Chicago Ballrooms, 5th Floor
12:00 pm – 1:30 pm	Break (Lunch on own)	Grand Ballroom Foyer, 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	Grand Ballroom Salon II, 7th Floor
	Featured Speaker • Qijing Zhang BVSc, MS, PhD	
2:45 pm – 3:30 pm	CRWAD Special Symposium: The Use of Artificial Intelligence in Animal Health Research	Grand Ballroom Salon II, 7th Floor
	Featured Speaker • Cesar de la Fuente, PhD	
3:30 pm – 4:00 pm	Break	Grand Ballroom Foyer, 7th Floor
4:00 pm – 6:00 pm	CRWAD Special Symposium (continued)	Grand Ballroom Salon II, 7th Floor
	Featured Speakers • Beatriz Martinez Lopez, DVM, MPVM, PhD • Brad White, DVM, MS • Panel Discussion	

Monday, January 19, 2026

8:30 am – 10:00 am	ACVM Distinguished Microbiologist Symposium	Grand Ballroom Salon II, 7th Floor
	Featured Speakers • Yung-Fu Chang, DVM, MS, PhD, ACVM Distinguished Veterinary Microbiologist • Eric R Burrough, DVM, PhD, DACVP	
8:30 am – 10:00 am	CRWAD Research Abstracts	Chicago Ballrooms, 5th Floor
10:00 am – 10:30 am	Break	Grand Ballroom Foyer, 7th Floor

10:30 am – 12:00 pm	ACVM Distinguished Microbiologist Symposium (continued)	Grand Ballroom Salon II, 7th Floor
	Featured Speakers • Sreekumari Rajeev, BVSc & AH, PhD, DACVM, DACVP • Mark Lawrence, DVM, PhD	
10:30 am – 12:00 pm	CRWAD Research Abstracts	Chicago Ballrooms, 5th Floor
12:00 pm – 2:00 pm	Break (Lunch on own)	Grand Ballroom Foyer, 7th Floor
12:00 pm – 2:00 pm	AAVI Luncheon	RSVP Needed
2:00 pm – 3:00 pm	AAVI Distinguished Immunologist Symposium	Grand Ballroom Salon II, 7th Floor
	Featured Speakers • Jodi McGill MS, PhD, AAVI Distinguished Veterinary Immunologist • Bonnie Mallard, MS, PhD	
2:00 pm – 3:00 pm	CRWAD Research Abstracts	Chicago Ballrooms, 5th Floor
3:00 pm – 4:15 pm	CRWAD Business Meeting	Chicago E, 5th Floor
3:30 pm – 4:00 pm	Break	Grand Ballroom Foyer, 7th Floor
4:15 pm – 5:45 pm	AAVI Distinguished Immunologist Symposium (continued)	Grand Ballroom Salon II, 7th Floor
	Featured Speakers • Crystal Loving, PhD • John Driver, PhD	
4:15 pm – 5:45 pm	CRWAD Research Abstracts	Chicago Ballrooms, 5th Floor
6:00 pm – 8:00 pm	CRWAD Poster Reception	Grand Ballroom Salon III, 7th Floor

Tuesday, January 20, 2026

8:30 am – 10:00 am	AVEPM Calvin Schwabe Symposium "Science's Social Contract: Epidemiology in the Real World"	Grand Ballroom Salon II, 7th Floor
	Featured Speakers • Stuart Reid, CBE, BVMS, PhD, DVM, DipECVPH, FRSE, FRCVS, Calvin Schwabe Award • H. Morgan Scott, DVM, PhD	
8:30 am – 10:00 am	CRWAD Research Abstracts	Chicago Ballrooms, 5th Floor
10:00 am – 10:30 am	Break	Grand Ballroom Foyer, 7th Floor
10:30 am – 12:00 pm	AVEPM Calvin Schwabe Symposium (continued)	Grand Ballroom Salon II, 7th Floor
	Featured Speakers • Dan O'Neill, MVB, PGCertVetEd, FHEA, MSc(VetEpi), PhD, FRCVS • Natalia Cernicchiaro, DVM, MS, PhD • **Gearhart Awardee** Alejandra Arevalo-Mayorga	
10:30 am – 12:00 pm	CRWAD Research Abstracts	Chicago Ballrooms, 5th Floor
12:15 pm – 1:30 pm	CRWAD Closing Remarks and Award Presentations	Chicago D, 5th Floor

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National Center for Disease Control and Public Health Georgia

CRWAD President's Message

I am so honoured to serve as the Council President of the Conference for Research Workers in Animal Diseases (CRWAD), the largest professional organization for animal disease researchers with more than 100 years of history. On behalf of the Council, I welcome you to the CRWAD 2026.

I want to congratulate Dr. Scott McVey on serving as the new Executive Director of the CRWAD organization and on his leadership in organizing an excellent conference. I also want to take this opportunity to thank the Executive Council members, the Scientific Program Committee, the CRWAD Fellow Committee, all CRWAD members, the generous sponsors, and especially FASS for their dedication and support in making this organization stronger and this conference better.

The CRWAD 2026 starts on Saturday (Jan. 17, 2026), featuring our dynamic 3MT (Three Minute Thesis) competition, showcasing the Council Keynote Presentation, and highlighting the Conference Theme Special Symposium – The Use of Artificial Intelligence in Animal Health Research, the ACVM Distinfuished Microbiologist Symposium, the AAVI Distinguished Immunologist Symposium, and the AVEPM Calvin Schwabe Symposium – Science's Social Contract: Epidemiology in the Real World. I assure you that you will enjoy the exceptional talent and enthusiasm from our vibrant speakers and presenters.

Like many senior members who joined this great organization during graduate school or early professional careers, I have been an active CRWAD member for over two decades. This organization, particularly its annual conference, provides a unique platform to interact directly with colleagues, make new friends, and receive feedback and suggestions to improve my ongoing research. I have also witnessed firsthand that attending the annual conference enables trainers and junior faculty to network with senior members and enhance or reshape their careers and research programs. I encourage our students, postdoctoral fellows, and even junior faculty members to be more actively engaged in discussion and networking to improve visibility and seek guidance and collaboration with members to advance their research in animal diseases.

This over 100-year-old organization has survived and thrived through major economic downturns and social turmoil, including the Great Depression, the Recession, and, more recently, COVID-19. I firmly believe that, to stay together, we can overcome adversity and make this organization better and stronger. I appreciate members reaching out to district and state leaders and further advertising the critical roles of animal disease research in food animal production, US and global food security, animal health and wellbeing, environmental health, and human health.

Finally, I enjoy serving as the President of this great organization. I highly value thoughts and ideas from current and new members to further strengthen CRWAD and the annual conference. I am looking forward to seeing you at CRWAD 2026 in the magnificent city of Chicago.

Weiping Zhang, PhD,
H. J. Detmers Chair in Veterinary Microbiology, Professor of Bacteriology/Vaccinology,
University of Illinois Urbana-Champaign

CRWAD 2025 Keynote Speaker

The Dark Alliance: Co-evolution of Antimicrobial Resistance and Bacterial Virulence

Qijing Zhang BVSc, MS, PhD
Iowa State University

Sunday, January 18, 2026, 1:30 pm – 2:45 pm

CRWAD Special Symposium

Accelerating Antibiotic Discovery Using AI

Cesar De La Fuente, PhD
University of Pennsylvania

Sunday, January 18, 2026, 2:45 pm – 3:30 pm

Advances in Artificial Intelligence for Population Medicine and Disease Dynamics: Current Progress and Future Opportunities

Beatriz Martinez Lopez, DVM, MPVM, PhD
University of California, Davis

Sunday, January 18, 2026, 4:00 pm – 6:00 pm

Integrating AI in Animal Health Research and Graduate Education: Challenges and Opportunities

Brad White, DVM, MS
Kansas State University

Sunday, January 18, 2026, 4:00 pm – 6:00 pm

CRWAD 2026 – Distinguished Career Awards

Molecular Characterization of a Virulence Factor, Lig Protein

Yung-Fu Chang, DVM, MS, PhD
Cornell University, AAVI Distinguished Veterinary Immunologist

Monday, January 19, 2026, 8:30 am – 10:00 am

Optimizing Innate Immunity to Protect Against Bovine Respiratory Disease

Jodi McGill, MS, PhD
Iowa State University, ACVM Distinguished Veterinary Immunologist

Monday, January 19, 2026, 2:00 pm – 3:00 pm

Science's Social Contract: Epidemiology in the Real World

Stuart W. J. Reid, CBE, BVMS, PhD, DVM(hons), DipECVPH, FRSB, FRSE, FRCVS
Royal Veterinary College, University of London, AVEPM Calvin Schwabe Awardee

Tuesday, January 20, 2026, 8:30 am – 10:00 am

CRWAD 2026 Featured Speakers

It's All Academic Until You Need a Checkbook—Commercial Applications of Immunology

Alan Young, BS, PhD
Medgene

Sunday, January 18, 2026, 8:00 am – 10:00 am

Opportunities and Challenges in the Development of Vaccines for Emerging Diseases of Livestock

Mahesh Kumar, BS, MS, PhD
Zoetis

Sunday, January 18, 2026, 8:00 am – 10:00 am

Bridging the Translation Gap: How to Develop Tools for Animal Disease Control by Getting the Best of Both Worlds

Meghan May, MS, PhD
IDEXX

Sunday, January 18, 2026, 10:30 am – 12:00 pm

Brachyspira spp. Infections in Animals

Eric R Burrough, DVM, PhD, DACVP
Iowa State University

Monday, January 19, 2026, 8:30 am – 10:00 am

Leptospira Infection: Conventional Wisdom vs. Current Perception

Sreekumari Rajeev, BVSc & AH, PhD, DACVM, DACVP
University of Tennessee

Monday, January 19, 2026, 10:30 am – 12:00 pm

Edwardsiella Ictaluri Attachment Mechanisms: A Novel Approach to Prevent Enteric Septicemia of Catfish

Mark Lawrence, DVM, PhD
Mississippi State University

Monday, January 19, 2026, 10:30 am – 12:00 pm

From Concept to Commercialization: Using Immunogenetics and Immunoceuticals to Naturally Improve Animal Health

Bonnie Mallard, MS, PhD
University of Guelph

Monday, January 19, 2026, 2:00 pm – 3:00 pm

Lifting the Veil: Modern Approaches to Addressing *Salmonella* Persistence in Porcine Macrophages

Crystal Loving, PhD
USDA ARS

Monday, January 19, 2026, 4:15 pm – 5:45 pm

A Single-Cell Level Exploration of the Pig's Immune System in Health and Disease

John Driver, PhD
University of Missouri

Monday, January 19, 2026, 4:15 pm – 5:45 pm

Through the Looking Glass: Epidemiology, Policy, and Practice in Reverse

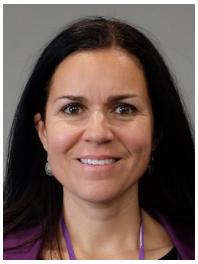
H. Morgan Scott, DVM, PhD
Texas A&M University

Tuesday, January 20, 2026, 8:30 am – 10:00 am

Science Reshaping the Social License to Operate on Dogs with Extreme Conformation—the VetCompass Story

Dan O'Neill, MVB, PGCertVetEd, FHEA, MSc(VetEpi), PhD, FRCVS
Royal Veterinary College, University of London

Tuesday, January 20, 2026, 10:30 am – 12:00 pm



Evidence with a Purpose: Stakeholder-driven Research and Preparedness for Japanese Encephalitis and Other Emerging Threats

Natalia Cernicchiaro, DVM, MS, PhD
College of Veterinary Medicine, Kansas State University

Tuesday, January 20, 2026, 10:30 am – 12:00 pm

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.

2026 CRWAD Fellow Inductee

Paul S. Morley, DVM, PhD, DACVIM
College of Veterinary Medicine and Biomedical Sciences,
Texas A&M University

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Three Minute Thesis: CRWAD Three Minute Thesis Competition

3MT01 - Targeted sequencing is a powerful tool for study of *Salmonella enterica*.

B. O. Hunt*[1], E. Doster[1], K. E. Belk[2], B. A. Burgess[3], R. Valeris-Chacin[1], C. A. Wolfe[1], L. Pinnell[1], and P. S. Morley[1],

[1]Texas A&M University, College Station, TX, [2]Colorado State University, Fort Collins, CO, [3]University of Georgia, Athens, GA

Salmonella enterica is a major pathogen of humans and animals. Failure to detect the organism can lead to widespread death and disease. Traditional attempts to detect *Salmonella* such as enriched culture or PCR can be slow, inaccurate, or fail to detect the organism at all. Animals shed the pathogen at low concentrations, increasing difficulty, particularly in samples containing genomes from many different organisms, such as fecal samples. Fortunately, targeted sequencing is a powerful tool by which we can select genes of interest rather than trying to grow the bacteria on a plate or risk losing it to background noise in PCR. The principle of targeted sequencing is based around using “lures” to “fish” out target DNA from complex environments. We can employ the use of probes, short sequencings of RNA, to bind to the regions of interest we wish to study. Those probes can be pulled off to the side by a magnet, away from the off-target genomes. We can then purify and amplify the target DNA for study. The probes are so specific that we can hunt for *Salmonella* genomes that would otherwise be very hard to culture or detect by PCR. The data that targeted sequencing generates enables us to track strains of *Salmonella enterica* across infected individuals with unparalleled sensitivity. Current diagnostic methods can sometimes only identify *Salmonella* down to the genus level. Targeted sequencing can go much deeper, targeting the minute differences in the genomes allowing us to see exactly which serovars are spreading, which ones are more prevalent, and which ones have resistance to antimicrobials. Targeted sequencing is a powerful tool that will significantly increase our ability to study low-abundance organisms within complex environments. The more we study and learn about targeted sequencing, the more optimized it will become and the more information we will have to better tackle problems in human and animal health.

Financial Support: Texas A&M University

Key Words: *Salmonella*, targeted sequencing, veterinary

3MT02 - Genome to vaccine: A shortcut to protection.

S. Madesh* and S. Rajeev,

University of Tennessee, Knoxville, TN

Tick-borne diseases are emerging as one of the most serious threats to both human and animal health worldwide. Among these pathogens, *Ehrlichia canis* and *Anaplasma platys* are obligate intracellular bacteria that cause canine monocytic ehrlichiosis and canine cyclic thrombocytopenia and are transmitted by the common tick vector. They primarily affect dogs but have also been reported in humans, underscoring their relevance to both veterinary and public health. Infected dogs often develop fever, anemia, and bleeding disorders, and in many cases the disease progresses to a chronic stage that persists even after the antibiotic treatment with doxycycline. Despite this widespread impact, no vaccine is available, leaving prevention dependent on tick control and treatment reliant on antibiotics, which is insufficient and unsustainable. Our research aims to address this critical gap by identifying and characterizing protective antigens from these pathogens. Traditional approaches have been limited because these bacteria are obligately intracellular, encodes many uncharacterized proteins, and are difficult to culture. To overcome these challenges, we combined computational analyses with experimental screening to systematically identify protective antigens. We performed a genome-wide search for immunogenic proteins using a peptide microarray designed from the complete genomes of these pathogens. The array contained 5,000 predicted B-cell epitopes and was tested against sera from naturally infected dogs, allowing us to identify which bacterial proteins were recognized by the host immune system during infection. This approach revealed several candidate antigens that were strongly recognized by host antibodies. Overlapping peptide microarray spanning multiple protein regions further highlighted targets with potential for vaccine or diagnostic development. Our work demonstrates a systematic pipeline for antigen discovery in intracellular pathogens that are difficult to study. Integrating bioinformatics with functional immunoassays provides an approach that can also be applied to related organisms where similar gaps in vaccine and diagnostic development remain. A protective antigen-based vaccine could reduce the burden of canine tick-borne infections and improve animal health, while antigen-based diagnostics would enable earlier detection and better treatment. Together, these advances support stronger strategies for controlling tick-borne diseases.

Financial Support: American Kennel Club

Key Words: *Ehrlichia*, *Anaplasma*, vaccine, peptide microarray

3MT03 - Sequencing the unseen: A deep dive into liver abscess microbial communities.

K. H. Hazlett*, C. A. Wolfe, P. S. Morley, E. Doster, R. Valeria-Chacin, M. A. Scott, and L. J. Pinnell,

VERO Program, College of Veterinary Medicine and Biomedical Sciences, Texas A&M, Canyon, TX

Liver abscesses (LAs) pose a significant challenge 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 necrophorum* 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 has effectively been characterized using 16S rRNA amplicon sequencing, yet 16S sequencing is unable 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 refine our characterization and 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 used shotgun metagenomic sequencing to characterize species level microbial populations within liver abscess, further confirming the polymicrobial nature of liver abscesses, allowing for functional profiling, and informing the design of target enrichment (TE) tools. Additionally, 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. Target enrichment relies on a bait-capture system to enrich sequences of interest, and we have designed baits for 10 species of interest that are prevalent in LAs. 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.

Financial Support: This work was supported by Texas A&M University and Texas A&M AgriLife Research. Portions of this research were conducted with the advanced computing resources provided by Texas A&M High Performance Research Computing.

Key Words: liver abscess, beef cattle health, shotgun metagenomics, target-enriched metagenomics

3MT04 - Using artificial intelligence to reduce cellulitis rates in broiler chicken farms.

A. Diler*, M. Boulianne, and P. Valdes-Donoso,

Université de Montréal, Montréal, QC, Canada

In 2024, 11 million broiler chicken carcasses were rejected at the slaughterhouse due to pathological defects in Canada. Over 70% of these condemnations are due to cellulitis, a bacterial infection, resulting in more than 2 million Canadian dollars in lost revenue. Cellulitis is caused by *Escherichia coli*, which enters the skin through wounds or scratches. Several risk factors, such as environment, production, and health can affect its development in chicken barns. However, detecting and correcting these various factors to minimize the rate of cellulitis is complex, and the very short production cycle (about 30 d) leaves little time for adjustments. We hypothesize that the pattern-recognition capacity of Artificial intelligence (AI) can effectively analyze risk factors to predict cellulite-related condemnations. Our main objective is to identify AI models to predict the cellulitis rate for each production cycle based on chicken rearing conditions. We will collect environmental data (i.e., temperature, humidity and CO_2) using sensors installed in chicken barns. These data will be supplemented with condemnation rates at slaughterhouses, mortality records and stocking density data from the Éleveurs de volailles du Québec. We will first conduct a preliminary analysis that includes data cleaning, integration of data from multiple sources in a single database, and visualization, to identify trends and patterns in the data set. Then, we will select, train, and evaluate AI models based on their ability to predict the cellulitis rate in chicken barns under specific conditions. If the model performs well, we will use it to provide recommendations for early interventions in poultry production, which will in turn improve animal welfare and profitability.

Key Words: artificial intelligence, precision agriculture, animal welfare, revenue loss reduction, cellulitis reduction, broiler chicken

3MT05 - Turning viruses into friends: Phage therapy as an antibiotic alternative to fight salmonellosis.

N. Abdelkader* and Y. Helmy,

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Salmonella is a major foodborne pathogen transmitted to humans primarily through the food chain and traditionally treated with antibiotics. The escalating crisis of antimicrobial resistance, however, has undermined this approach, highlighting the urgent need for alternatives. Bacteriophages (phages) represent a promising option because they specifically infect and kill bacteria. Their precision enables targeted elimination of pathogens, and several FDA-approved phage products are already in use to control foodborne *Salmonella* and reduce antibiotic dependence in livestock. Our work focuses on developing optimized phage cocktails designed to overcome resistance and penetrate biofilms. In this study, effluent water samples were enriched with *Salmonella* isolates, incubated in tryptone soya broth, and screened for phages using the double agar overlay method. Candidate phages were purified through plaque assays to obtain a single phage population. The isolated phages displayed broad activity against multidrug-resistant and extensively drug-resistant *Salmonella* from food, equine, cattle, human, and environmental sources, covering serotypes *S.* Typhimurium, *S.* Anatum, *S.* Enteritidis, *S.* Agbeni, *S.* Saintpaul, *S.* Thompson, *S.* Hartford, *S.* Bovismorbificans, *S.* Mbandaka, and *S.* Dublin. Time-kill assays over 210 min at MOIs of 1, 10, and 100 showed that Sal-Φ1 was the most effective, reducing bacterial growth by 93.1%, 95.5%, and 96.5%, respectively. Sal-Φ29 achieved 90.5%, 93.2%, and 93.7% inhibition, while Sal-Φ8 reduced growth by 87.2%, 86.0%, and 82.8%. In conclusion, these results demonstrate the strong potential of phages for controlling *Salmonella* infections. Advancing phage-based products brings us closer to practical solutions that protect animal health, strengthen the food chain, and reduce risks to public health within a One Health framework.

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Key Words: antimicrobial resistance, bacteriophage, One Health, phage therapy

3MT06 - On the frontlines of bird flu: Wild birds, mammals, and outbreak response.

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Imagine a virus that starts in wild ducks, jumps into chickens and turkeys, and wipes out millions of birds—and occasionally infects people. That virus is highly pathogenic avian influenza H5N1, or bird flu. Wild aquatic birds are the natural reservoirs of influenza viruses, carrying almost all known influenza subtypes. Most of the time, these viruses cause little harm. But, certain strains, such as H5N1, or bird flu, can be deadly. Only in the US, during the 2014 to 2015 outbreaks, over 50 million poultry died or depopulated. And since 2021, a new wave of outbreaks, carried by migratory birds, has spread widely, affecting wildlife, livestock, and even humans. Now, the risk to people is still low. But, every time the virus infects a mammal, it gets another chance to adapt. Another chance to spread among us. And, that is why many scientists worry: Could bird flu cause the next pandemic? My research tackles this threat from 3 angles. First, we considered wild birds. We studied the virus in wild aquatic birds. From 2023 to 2025, we collected over 3,500 samples from wild birds across the Mississippi migratory flyway in the United States. About 16% tested positive for influenza, and sequencing revealed a mix of avian influenza viruses—including the same H5N1 lineage that has caused global concern, with mutations that may help it survive in mammals and resist antiviral drugs. Second, we considered wild mammals. We assessed the risk of spillover of the virus from avian species into wild mammals. In Ohio, USA, we tested raccoons, foxes, and other species that primarily live alongside birds. We did not find active infections, but almost half had antibodies. That means that they have already been exposed to bird flu, raising alarms about cross-species spread. Third, we considered outbreak response. When bird flu strikes a bird farm, the main defense is mass depopulation—the grim but necessary strategy to stop outbreaks. To learn challenges and find gaps in this practice, we surveyed gamebird producers across North America. Many reported the same struggles: slow response times, lack of equipment, and difficulties handling both live birds and carcasses. These weaknesses threaten not just farms, but our broader ability to contain outbreaks. Together, these findings give us a clearer picture of how influenza viruses circulate in birds and mammals, and where our outbreak response systems are breaking down. This knowledge is essential not just for protecting poultry and wildlife, but also for reducing the risk of the next pandemic.

Key Words: bird flu, wild bird, mammal, outbreak response

3MT07 - Cattle traceability: The ABCs of foot-and-mouth disease and electronic identification.

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When disease outbreaks occur in livestock, diseased and at-risk animals need to be traced and identified faster than the disease can spread to gain control. The transboundary disease of cattle we are most concerned about in the United States (US) is foot-and-mouth disease (FMD). FMD infected animals can become infectious in as short as 2 or 3 d which makes rapid tracing vital. The objective of my thesis was to use simulation models to assess the use and effect of electronic identification (EID) and digital tracing in US cattle. Animal disease traceability systems have existed in the US for almost 80 years but have historically been designed for diseases that spread much slower than FMD. To achieve the rapid tracing needed for FMD, EIDs and digital tracing are likely required. The current US system does not require EIDs in all cattle nor is there a national database of all animal movements. This leaves gaps in the movement record, impedes control and surveillance efforts, and requires more time and human labor to complete traces. My first project modeled outbreaks of FMD in the US comparing current and potential EID tracing speeds and accuracy. The ideal EID scenario could trace 99% of contact farms within 24 h. EID tracing markedly decreased the likelihood of the largest and most impactful outbreaks even when ideal EID tracing was only partially achieved. Not only did the number of farms infected decrease, but also the farms that had to be quarantined and surveilled. My second project created a simulated 30-d network of all US cattle movements. To assess EIDs we assigned various farm-level tagging and movement reporting rates to create a series of networks containing traceable movements. We then compared the traceability of selected farms across the networks. Our results suggest that implementing a useful EID tracing system in the US will require substantial tagging rates and reporting to a central database accessible to the animal health official conducting the trace. The results of my work improve the ability for producers and animal health officials to make informed decisions about EIDs in cattle. The use of EIDs in an FMD outbreak could greatly reduce the number of infected and affected cattle and improve control effort resource efficiency. However, for these benefits to be realized, marked increases in EID usage and movement reporting need to occur.

Key Words: cattle, traceability, foot-and-mouth disease, electronic identification

3MT08 - Lactulose metabolism in *Salmonella*: Transcriptomic insights and nutrient-based modulation of biofilm and colonization potential.

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Lactulose is a synthetic disaccharide commonly used as a therapeutic agent in human and veterinary medicine, particularly for the treatment of hepatic encephalopathy and constipation. It is also used as a prebiotic in different dairy foods to promote gut microbiota. Lactulose has traditionally been considered fermentable only by beneficial gut microbes; thus, lactulose utilization by pathogenic bacteria such as *Salmonella* has largely remained unexplored. Recently, we reported that non-typhoidal *Salmonella* (NTS), including *Salmonella* Kentucky, can metabolize lactulose as their sole energy source for growth in a serotype-independent manner. However, the molecular pathways regulating lactulose catabolism and the potential role in modulating biofilm in NTS remain uncharacterized. In this study, we used *S.* Kentucky as a model to identify lactulose catabolic pathways and explore the potential impact on biofilm and colonization of *Salmonella* in vitro. Seven epidemiologically distinct *S.* Kentucky strains were grown in lactulose or glucose (control) supplemented media, followed by RNA sequencing to identify differentially expressed genes in response to lactulose. The modulation of biofilm phenotype under lactulose induction was evaluated using Congo Red/Coomassie Brilliant Blue agar, Calcofluor agar, and microtiter plate assays. Among 4,499 protein-coding genes, 846 were upregulated and 733 were downregulated in response to lactulose, highlighting extensive lactulose-induced transcriptional remodeling. Transcriptomic analysis and functional profiling revealed differentially regulated genes with a functional upper pathway for lactulose uptake and hydrolysis, involving several novel transporters and hydrolases. In addition, downstream catabolic pathways of galactose and fructose showed evidence of both canonical and non-canonical routes, underscoring the metabolic flexibility of *Salmonella*. Phenotypic assays further demonstrated that lactulose impairs biofilm formation across multiple NTS serovars. This study provides the first transcriptomic characterization of lactulose metabolism in NTS and establishes a novel link between nutrient utilization and biofilm modulation. Current investigations are focused on whether lactulose also modulates motility and adhesion, with implications for colonization potential. These findings lay the foundation for developing nutrient-based strategies to control *Salmonella* persistence and colonization in diverse ecological niches.

Financial Support: This study was funded in part by the Texas Tech University School of Veterinary Medicine (TTU-SVM).

Key Words: *Salmonella*, lactulose, metabolism, transcriptomics, biofilm

3MT09 - Breaking the bacterial conversation: AI-2 inhibition to control *Salmonella* virulence.

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Salmonella remains one of the major causes of foodborne gastroenteritis in both humans and animals. *Salmonella* infection is transmitted through the consumption of contaminated food and water, with poultry and poultry products being the predominant sources of human transmission. Although antibiotics are traditionally used to manage *Salmonella* infections, the growing prevalence of antimicrobial resistance underscores the urgent need for antibiotic alternatives. Quorum sensing (QS) is bacterial cell-to-cell communication mediated by the synthesis, release, and uptake of signaling molecules called autoinducers (AI-2). Quorum sensing is known to regulate the expression of bacterial virulence factors. In this study, we screened approximately 2,500 small molecules (SMs) for their ability to inhibit QS/AI-2 without affecting bacterial growth. The cell-free supernatants from *Salmonella* treated with 10 µ*M* of each SM were subsequently subjected to *Vibrio harveyi* BB170 bioluminescence assay to quantify AI-2 activity. We identified 10 SMs that inhibited QS/AI-2 by more than 95% without inhibiting growth. The 6 compounds that were found to be non-toxic to human colorectal carcinoma cells at 20 µ*M* concentrations were then selected for further development. The top 6 non-toxic compounds exhibited potent anti-biofilm activity, achieving 95% to 100% inhibition. Similarly, all 6 SMs demonstrated a significant reduction ($P < 0.05$) in survival of *Salmonella* inside human intestinal (HT-29) cells, with SM8 and SM9 demonstrating complete clearance. Gene expression analysis revealed the downregulation of QS- and virulence-associated genes linked to biofilm, invasion, and motility. Our future work will focus on evaluating the top 2 compounds on the *Salmonella* transcriptome. We will also perform in vivo validation to inhibit *Salmonella* colonization in chickens and advance them as safe and promising antibiotic alternatives.

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Key Words: *Salmonella*, quorum sensing, autoinducer, small molecule, antibiotic alternative

3MT10 - Transcriptomic profiling of equine myometrium identifies immune networks underlying placentitis-induced activation.

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Equine ascending placentitis is a leading cause of abortion, stillbirth, and perinatal mortality in horses. A key event of placentitis-induced preterm labor is loss of myometrial quiescence which triggers the onset of labor. However, the molecular drivers of myometrial activation in horses are not well understood. To address this, we examined the myometrial transcriptome in mares with induced ascending placentitis (n = 6) at ~290 d gestation compared with gestationally age matched controls (n = 6). RNA sequencing was performed using Illumina NovaSeq6000, with raw reads processed using TrimGalore 0.4.3 before mapping to EquCab3.0 via STAR 2.4.3a. Mapped reads were quantified using Cufflinks 2.2.1 with NCBI annotation. Differentially expressed genes (DEGs) were identified with Cuffdiff 2.2.1 with a false discovery rate (FDR) <0.05. We identified 263 DEGs, including 7 genes (*APOBEC3Z1B*, *PLA2G2D*, *CXCL17*, *MMP8*, *MMP13*, *S100A8*, *S100A9*) uniquely upregulated during placentitis. Functional groups of DEGs pointed to key pathways of myometrial activation: connexins (*GJC2*), matrix metalloproteinases (*MMP1*, *MMP8*, *MMP13*, and *MMP25*), prostaglandin signaling (*PTGS2* and *PTGER3*), and calcium regulation (*TRPM2*, *RAMP2*, *CACNA1D*, and *PRKCB*) point to a clear pathway for equine placentitis induced myometrial activation. Several chemokines (*CCL2*, *CCL3*, *CCL4*, *CCL5*, *CXCL1*, *CXCL3*, *CXCL16*, and *CXCL17*) were also enriched, correlating with pronounced leukocytic infiltration—a hallmark of myometrial activation. To identify potential upstream regulators of myometrial activation, we performed upstream regulator analysis using Ingenuity pathway analysis, which revealed 12 potential regulators: *TLR2*, *STAT1*, *IRF7*, *SPI1*, *PTGS2*, *S100A8*, *S100A9*, *ICAM1*, *NRG1*, *HIF1A*, *PRKCB*, and *TYROBP*. Immunolabeling confirmed protein-level upregulation of TLR2, PTGS2, S100A8, S100A9, and HIF1A. Overall, this study enhances our understanding of immune networks and molecular mechanisms involved in myometrial activation during equine placentitis, which could inform therapeutic development to prevent preterm labor and treat placentitis. In conclusion, these data highlight TLR2 as a central initiator of inflammatory signaling in the equine myometrium, promoting contraction-associated transcripts such as PTGS2, PTGER3, MMP1, MMP8, and GJC2. Therefore, targeting TLR2 and its associated pathways, such as through TLR antagonists, could offer potential therapeutic strategies to prevent placentitis-induced preterm birth.

Financial Support: Boehringer Ingelheim Equine Research Award

Key Words: equine, placentitis, immune network, myometrium, transcriptomics, abortion

3MT11 - From farm to table: How bacteria can protect your chicken dinner.

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The excessive usage of antibiotics in human health and agricultural production has resulted in the development of multidrug-resistant bacterial strains, creating an urgent global health threat. Necrotic enteritis (NE), caused by *Clostridium perfringens*, is an economically significant gastrointestinal inflammatory disease leading to weight loss, diarrhea, and up to 50% mortality in severe cases. To address the worldwide antibiotic resistance issue, the US restricted the use of in-feed antibiotics in 2017. As a consequence, there has since been an increased incidence of NE, costing the global poultry industry $6 billion each year. Therefore, identifying alternative treatment and prevention solutions for NE is crucial to improving poultry health. This study aimed to investigate the potential of leveraging commensal bacteria to prevent *C. perfringens* colonization and confer protection against NE. Twelve bacterial isolates were chosen for investigation from a library of bacteria isolated from the intestinal tract of healthy feral chickens. Through an in vitro competitive inhibition assay, one commensal was identified with the ability to inhibit the growth of *C. perfringens* by 62%. To our excitement, oral administration of this top-performing bacterium in a chicken NE model provided robust protection to chickens leading to an increase in survival rate and a reduction in intestinal tissue damage. Overall, our findings demonstrate the potential of a beneficial commensal bacterium to protect chickens against NE. This finding could provide poultry producers with a cost-effective antibiotic alternative to protect flocks from NE and other disease, enhance animal welfare, and decrease production losses.

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3MT12 - Good bugs versus superbugs: Next-generation probiotics to fight staphylococcal infections.

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Staphylococcus aureus is an opportunistic pathogen that can cause severe infections in both humans and animals. Its rapid acquisition of antibiotic resistance poses a threat to treatment efficacy across clinical, agricultural, and environmental settings, highlighting the need for new therapeutics. Among these, novel probiotics, also known as next-generation probiotics (NGPs), represent a promising category of beneficial bacteria designed for use in both food and dietary supplementation, as well as specific therapeutic applications. Unlike traditional probiotics, these strains are specifically selected and optimized for their ability to target specific pathogens, regulate host immunity, and inhibit microbial virulence. In this study, 45 probiotic strains were screened for their ability to inhibit *S. aureus* using agar well diffusion assays. Several strains showed clear inhibitory activity, with inhibition zones ranging from 7.0 ± 0.7 mm to 23.5 ± 0.0 mm (mean ± SD). The most effective strains were P15 (23.5 ± 0.0 mm), P24 (22.2 ± 0.4 mm), P11 (18.2 ± 1.1 mm), and P31 (18.2 ± 0.4 mm), whereas others showed moderate or no inhibition. These findings highlight the promise of NGPs as viable alternatives to conventional antibiotics for controlling *S. aureus*. Future work will focus on evaluating the efficacy of the selected best probiotic strains in vitro and therapeutic applications within a One Health framework to control infections in animals and humans.

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Key Words: *Staphylococcus aureus*, next-generation probiotics, antibiotic resistance, animal, human

3MT13 - Spatio-temporal patterns and space-time cluster analysis of foot-and-mouth-disease outbreaks in Punjab, Pakistan, from 2021 to 2022.

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Foot-and-mouth disease (FMD) is a highly contagious transboundary animal disease with widespread occurrence across Asia, Europe, and Africa, causing significant economic losses, particularly in low- and middle-income countries like Pakistan, where livestock plays a critical role in the national economy and household food security. This study aimed to investigate the spatiotemporal trends of FMD outbreaks in the Punjab province, Pakistan, and to identify the space-time disease clusters. The data of 881 FMD outbreaks reported in the Punjab province during 2021 to 2022 was analyzed to test the hypothesis of complete spatial randomness. A retrospective space-time permutation method was used to identify the space-time clusters using SaTScan. The FMD virus serotype O was the most widely distributed, accounting for more than 89% (n = 789) of the outbreaks, followed by serotype A (6%, n = 54) and Asia-1 (2.5%, n = 22), whereas in 2% (n = 16) of the outbreaks serotype A and O co-existed. In these 881 FMD outbreaks during 2021 to 2022, a total of 4,707 animals were affected. The majority of cases occurred in mixed cattle and buffalo herds (47%, n = 2,207), followed by cattle-only herds (45.4%, n = 2,138) and buffalo-only herds (6.4%, n = 297). Small ruminants were rarely affected, representing only 1% of cases (n = 45), whereas a few outbreaks involved all species (n = 32) cattle and goats (n = 6). In 2021, the highest number of outbreaks was recorded in March (n = 287), whereas in 2022, outbreak numbers were lower overall, peaking in January (n = 23). Across both years, the majority of outbreaks occurred between January and April, suggesting a potential seasonal trend. SaTScan identified 14 significant space-time clusters during 2021 to 2022, with a maximum cluster radius of 60.35 km ($P < 0.05$). Most of the clusters (n = 13) occurred in 2021, whereas only one cluster was detected in 2022. In conclusion, findings about significant spatial autocorrelation and space-time clustering of FMD outbreaks will help prioritize resource allocation and targeted surveillance and control for FMD in Punjab, Pakistan.

Key Words: foot-and-mouth disease, disease outbreak, spatio-temporal analysis, disease surveillance, space-time clusters

3MT14 - Assessing the potential of liquid-liquid phase separation body formation via intrinsic protein disorder within the hepatitis E virus ORF1 polyprotein.

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Hepatitis E virus (HEV) is a zoonotic pathogen, with an asymptomatic primary reservoir in swine, that causes viral hepatitis in the human host. Despite its prevalence, the molecular mechanisms of HEV replication remain poorly understood, hindering the development of effective antiviral treatments. Recent advances in cellular biology have revealed that biophysical processes, such as liquid-liquid phase separation (LLPS), are pivotal in regulating RNA replication. Liquid-liquid phase separation occurs when biomolecules, such as viral proteins and nucleic acids, form membraneless condensates, creating microenvironments that facilitate cellular biochemical reactions. Notably, intrinsic disorder within protein structures plays a crucial role in mediating LLPS. No studies have explored the role of LLPS in HEV replication, leaving a significant gap in our understanding of the virus's interaction with the host cellular environment. This study investigates whether HEV ORF1 polyprotein (ORF1p), containing the HEV replication machinery, forms phase-separated condensates due to its intrinsic disorder, enhancing viral replication. We used 2 computational tools, PICNIC and IUPred2A, to predict the condensation propensity of ORF1p. These tools analyzed ORF1p from 2 genotype 3 HEV strains, Kernow C1 P1, originally isolated from a chronically infected patient, and its cell culture-adapted derivative, Kernow C1 P6. The predicted condensate scores were 0.25 and 0.26, respectively, indicating a moderate likelihood of phase separation. This prediction was further supported by the presence of extensive intrinsically disordered regions within the hypervariable region of ORF1p. In vitro assays and immunofluorescence staining revealed the formation of LLPS droplets, consistent with condensate behavior, and confocal microscopy further confirmed their dynamic formation within cells, providing direct visual evidence of phase separation. To further elucidate the functional relevance of ORF1p condensation, flow cytometry will be employed to assess cellular changes during infection, and qRT-PCR will quantify viral genome copy numbers. This approach will enable us to determine the correlation between phase separation dynamics and HEV replication efficiency. By integrating computational predictions with biochemical and cell-based assays, this study will enhance our understanding of HEV replication and may uncover novel therapeutic targets to disrupt phase-separated viral structures.

Key Words: hepatitis E, liquid-liquid phase separation, intrinsically disordered protein, hepatitis E virus replication

3MT15 - Molecular epidemiology of *Staphylococcus saprophyticus* in a One Health context.

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Staphylococcus saprophyticus is a gram-positive bacterium that is associated with disease in humans and animals. In dairy cattle, this species is one of the understudied organisms associated with intramammary infections and has been isolated from milk, body sites, and the dairy environment. In humans, *S. saprophyticus* is the second most prevalent organism associated with urinary tract infections. Previous studies have identified a possible foodborne origin of some *S. saprophyticus* isolates identified in humans. Little is known about the molecular epidemiology of this organism in dairy farms and the role of dairy animals and their environment in transmission to humans. Therefore, this study aimed to characterize *S. saprophyticus* isolates from dairy farm origin and compare their phenotypic and genomic traits with previously characterized isolates from human origin. This study will provide a great insight into the relationship between *S. saprophyticus isolates* originating from humans, dairy farms, and the dairy environment, and will increase our understanding of *S. saprophyticus* in dairy farms and help determine if this is a public health concern.

Financial Support: This study was funded by a University of Missouri Research Council Grant.

Key Words: dairy origin, intramammary infection, One Health, *Staphylococcus saprophyticus*, urinary tract infection

3MT16 - Do dog diets really matter?

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There are a lot of opinions about the best nutrition for companion dogs. Aside from research on dogs already diagnosed with specific disease processes, there is surprisingly little epidemiological research on how nutrition affects the health of dogs. Are dogs who consume grain-free diets more likely to develop cardiac conditions? Are dogs who consume commercially processed diets more likely to develop clinical signs consistent with canine cognitive dysfunction? In this thesis work, in a series of studies using different large data sets of dogs with diet and health data collected prospectively, we demonstrate the effect of various types of diets on predispositions to future diseases, using causal frameworks. Grain-free diets are not associated with increases in cardiac disease. Kibble diets, in general, appear to have the lowest risk of future disease diagnoses when looking at cardiac disease and cancer. From this thesis, pet owners and veterinarians can make scientifically informed diet choices for their otherwise healthy dogs to reduce the risk of future disease.

Key Words: canine, dog, diet, nutrition, epidemiology

3MT17 - Evaluation of a mucosally delivered subunit vaccine against bovine tuberculosis in a calf vaccination-challenge model.

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Bovine tuberculosis (bTB), caused by *Mycobacterium bovis*, remains one of the most damaging livestock diseases globally, leading to substantial economic losses and posing a significant zoonotic threat—particularly due to persistent wildlife reservoirs. Control efforts are hampered by limited vaccine options. Although the Bacillus Calmette–Guérin (BCG) vaccine has been used, a recent meta-analysis of ~1,300 studies estimated its direct protective efficacy in cattle at only ~25%, showing notable reductions in lesion severity but minimal effects on infection prevention or clearance. To address this gap, we evaluated a mucosal subunit vaccine utilizing *Bacillus subtilis* spores engineered to display conserved *M. bovis* antigens (Ag85B, CFP10, and ESAT-6). Holstein calves were vaccinated via both oral and intranasal routes and then challenged with *M. bovis* embedded in Matrigel, allowing localized assessment of bacterial growth. Longitudinal blood samples were collected to monitor systemic immune responses, and Matrigel was recovered at necropsy for microbiological culture, histological analysis and transcriptomic analysis. Systemic responses will be further characterized through the transcriptional profiling of Matrigel and peripheral blood mononuclear cells (PBMCs), along with serum cytokine analyses. Bacterial burden analysis revealed that spore-vaccinated calves harbored ~10^5 cfu at the challenge site, compared with ~10^6 to 10^7 cfu in both unvaccinated and BCG-vaccinated calves—a 1.5-log reduction ($P < 0.001$ vs. unvaccinated; $P < 0.01$ vs. BCG). Notably, BCG did not reduce bacterial burden relative to controls. Additional analyses—including histopathology (H&E, acid-fast staining), multiplex cytokine profiling, and RNA-Seq of Matrigel and PBMCs—are underway to elucidate immune correlates of protection. These findings demonstrate that the mucosal *B. subtilis* spore subunit vaccine conferred superior protection compared with BCG under the tested conditions. Ongoing studies aim to define the immune mechanisms driving this enhanced efficacy.

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Key Words: bovine tuberculosis, *Bacillus subtilis*, spore subunit vaccine, Matrigel, *Mycobacterium bovis*, RNA-Seq

3MT18 - Antibody testing against *Coxiella burnetii* in beef cattle using 2 different antibody ELISA kits.

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Coxiella burnetii is a select agent that causes Q fever in human and abortions in ruminants. Antibody tests are more commonly used as a standard diagnostic method. We evaluated 2 commercially available Q-fever antibody testing kits with relatively a large number of serum samples collected from beef cattle operations. A collection of 1,129 serum samples were tested with a commercial *Coxiella burnetii* antibody ELISA kit (kit 1). All positive samples identified by kit 1 were tested with a different commercial ELISA kit (kit 2). Animal age and pregnancy status were recorded at sample collections. Bovine leukemia virus (BLV) was tested with a qPCR assay as a potential immunodeficiency condition indicator. Test kit 1 identified 147 antibody-positive animals. Among these 147 samples, 73 samples were also tested positive with the test kit 2. The remaining results by kit 2 included 17 suspected positive (borderline between positive and negative) and 57 negative samples. There were 137 of the 147 (93.2%) Q-fever antibody positive animals by test kit 1 were pregnant animals. Similar results were obtained by test kit 2, from which 69 of the 73 (94.5%) antibody-positive animals were pregnant. The proportion of BLV positive samples also remain consistent across 2 assays. There were 58 animals out of 147 (39.5%) Q-fever antibody positives were also positive to BLV by test kit 1; and there were 27 animals from the 73 (36.9%) Q-fever positives were also BLV positive. Despite the significant different results observed between the 2 Q-fever antibody testing kits (13.0% by kit 1; 6.5% by kit 2), Q-fever antibody was clearly detected in beef cattle operations. Our results demonstrated that *Coxiella burnetii* has been circulating not only in dairy cattle (as reported by others), but in beef cattle as well. Its effects on public health, though, are not yet clear and need more investigations. It appears that more pregnant animals were Q-fever antibody positive. However, 1,013 of the 1,129 animals we collected samples from were pregnant. Thus, there is no clear relationship between pregnancy and Q-fever status. From the 1,129 animals that we collected serum samples, whole blood samples from 567 were also collected and test by a *Coxiella burnetii* qPCR assay. None of the 567 blood samples were positive, indicating the infection stage at sample collection may be critical to qPCR testing.

Financial Support: CDC - Association of Public Health Laboratories Fellowship, Kansas Veterinary Diagnostic Laboratory

Key Words: *Coxiella burnetii*, Q fever, ELISA, beef cattle, diagnosis, public health

3MT19 - Exploring the role of inflammatory microRNAs in calf health.

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Colostrum is a bioactive secretion produced by the mammary gland, rich in immunoglobulins, growth factors, and cytokines, which are essential for neonatal metabolic and immune development. Recent studies have identified microRNAs (miRNAs) in colostrum, highlighting their key role in gene regulation, immune modulation, cell differentiation, and apoptosis. This study aimed to evaluate the presence of inflammatory related miRNAs in the bovine colostrum. Colostrum samples (50 mL) were collected from multiparous Holstein cows (n = 10) within 4 h after calving for total RNA extraction, miRNA enrichment, and miRNA quantification using RT-qPCR with specific miRNA primers. Among the miRNAs detected, let-7a-5p was the most abundant across samples (mean CT = 22), followed by miR-21-5p, miR-101-3p, and miR-29c-3p (mean CT = 25). However, miR-142, miR-222-3p, and miR-340-3p had low expression (mean CT = 30). MicroRNAs let-7a-5p and miR-21-5p are associated with immune regulation during intramammary infections, while the miR-29 family is involved in different lactation pathways. Additionally, miR-101-3p may play a role in calf development, potentially influencing neonatal immunity and metabolic programming. In contrast, miR-142 is a potential biomarker of mammary gland health. Further studies are required to elucidate the regulatory pathways of colostrum-derived miRNAs and their implications for early-life programming, immune development, and dairy production. Understanding these mechanisms could contribute to optimizing milk quality in commercial dairy systems and improving neonatal and mammary gland health.

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.

Key Words: calf, colostrum, immunity

3MT20 - Characterizing the antiviral role of ZFP36L1 in suppressing RNA viruses associated with gastroenteritis.

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Viral gastroenteritis in pigs causes significant damage to swine worldwide, including the United States. The primary etiological agents are porcine rotaviruses and swine enteric coronaviruses, including transmissible gastroenteritis virus, porcine epidemic diarrhea virus, and porcine deltacoronavirus. Identifying host proteins that broadly suppress replication of these viruses could inform antiviral strategies. The protein ZFP36L1, a CCCH-type zinc finger RNA-binding protein involved in mRNA turnover, has demonstrated antiviral properties across RNA viruses. This study investigates the mechanism by which ZFP36L1 suppresses replication using human coronavirus OC43 (HCoV-OC43) and human rotavirus A strain Wa (HRV-Wa) as model systems, which share similarities with swine enteric coronaviruses and porcine rotaviruses, and evaluated sodium butyrate as a potential inducer of ZFP36L1. Wild-type, ZFP36L1-overexpressing (OE), and knockdown (KD) HCT-8 cells were infected and analyzed for viral titers and transcripts via qPCR. OE in HCT-8 (HCoV-OC43) and MA104 clone 1 (HRV-Wa) cells significantly reduced titers, while KD increased titers versus wild-type ($P < 0.05$). Statistical significance was assessed using paired *t*-test, one-way ANOVA, and 2-way ANOVA ($P < 0.05$). To test poly(A) deadenylation, CNOT1 knockdown was performed in OE cells; titers were not rescued, indicating suppression independent of poly(A) deadenylation. Computational analyses (RPISeq, docking) predicted strongest binding of ZFP36L1 to HCoV nucleocapsid RNA, confirmed by RNA immunoprecipitation and validated by luciferase assays, likely contributing to reduced replication. PyMOL modeling identified residues critical for this interaction. Extension to HRV studies showed interaction with segment 6 (VP6), which may contribute to reduced titers and under study. Sodium butyrate treatment upregulated ZFP36L1 expression and suppressed HCoV-OC43 titers ($P < 0.05$). Together, these findings demonstrate that ZFP36L1 restricts RNA virus replication through a poly(A)-independent mechanism involving viral RNA interactions, highlighting its capability as a broad-acting antiviral protein against enteric RNA viruses and suggesting relevance for controlling gastroenteritis in pigs. Future studies will confirm roles of predicted residues through mutagenesis and luciferase assays, investigate non-deadenylation pathways such as PARN and DCP1/2, and evaluate effects of exogenous nucleocapsid supplementation on titers.

Financial Support: Gillespie College of Veterinary Medicine at Lincoln Memorial University

Key Words: ZFP36L1, HCoV-OC43, rotavirus, poly(A) deadenylation, nucleocapsid, sodium butyrate

General Abstracts: Session 1—Animal Vaccinology Research Network Symposium

1 - It is all academic until you need a checkbook—Commercial applications of immunology.

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“If you build a better mousetrap, the world will beat a path to your door” is a frequently repeated adage that, unfortunately, does not always hold in the practical world. Academia and industry face different challenges to meet the needs of producers. Both share a common goal to care for the health of animals and assist veterinarians and farmers in supporting food security. Over the past few years, a growing movement demonstrating a basic disagreement regarding the roles of vaccines and technology in general has created greater challenges for both groups. It is therefore critical to understand and appreciate the challenges of transitioning technology from the bench to commercial product availability. Academia traditionally focuses on pursuit of basic science questions and advancing knowledge in general, but industry must remain focused on providing these tools to end-users within a complex regulatory environment while generating a reasonable return for their investors. The traditional, academic-based small-scale demonstration of efficacy represents only the first in a long pathway to product availability. New technologies represent opportunity, but they must be viewed by industry in terms of the regulatory environment, need for new investment, and likelihood of commercial success to warrant years of investment to gain approval. Unfortunately, the types of studies most likely to be supported by competitive grant funding to academics frequently are those least likely to generate a significant revenue stream for commercialization. It is critical to minimize these challenges in an era of scientific cynicism and challenging grant funding.

2 - Opportunities and challenges in the development of vaccines for emerging diseases of livestock.

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New and emerging diseases continue to have a significant effect in the livestock industry. New diseases can be transient with minor consequences or highly pathogenic that cause significant mortality and economic losses. Re-emerging diseases such as highly pathogenic avian influenza continues to affect chickens and turkeys. We witnessed the spread of highly pathogenic avian influenza in dairy cattle that affected many herds in the United States. Although we have successfully developed a vaccine for both avian and dairy, they are yet to be used in the control of the disease. Although emerging diseases become endemic and vaccines have been successfully developed and employed to contain the problem, many are of limited use and commercially not viable. The presentation will review the unique opportunities and challenges associated with developing vaccines for animals against emerging infectious diseases. Examples of recent successes and failures in this area and their effects will be discussed.

General Abstracts: Session 2—Bacteriology: Cattle

3 - Tet44: A new ribosomal protection protein conferring tetracycline resistance in clinical isolates of *Mycoplasma bovis*.

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Mycoplasma bovis is a ruminant pathogen that can result in substantial economic losses in affected herds. There are increasing global reports of antimicrobial resistance in *M. bovis*. Tet44 confers resistance to tetracycline via ribosomal binding and was first described in *Campylobacter fetus* in 2010. Here we report the detection, spread, and genetic diversity of the *tet44* gene in *M. bovis*. Whole genome sequencing (WGS, Illumina) was performed on 49 clinical isolates of *M. bovis* obtained from dairy calves in the United States since 2020, and the *tet44* gene was identified during in silico screening. The predicted protein structure was obtained with Alphafold and contained a predicted tetracycline binding site. To assess the historic incidence of *tet44* in *M. bovis*, we performed a BLAST nucleotide and protein search of >280 genome assembly and annotation reports in GenBank. Our existing collection of 134 *M. bovis* clinical isolates obtained over the past 35 years was screened by PCR to detect the presence of *tet44*. Clinical isolates were obtained from the lung, milk, ear, heart, trachea, lymph node as well as unknown body sites of both dairy and beef cattle across all regions of the United States. The *tet44* gene was not detected in any of the GenBank genomes. In contrast, we found that 87.5% (28/32) of our clinical isolates from 2021 to 2024 contained the *tet44* gene; *Tet44* was detected in all clinical isolates (n = 17) in 2025. Before 2021, we detected the *tet44* gene in 62% (18/29, 2015–2020), 11% (5/45, 2000–2014), and 5% (1/21, 1986–1987) of clinical isolates, with the first detection seen in 1987. Our data suggest that the acquisition of the *tet44* gene is a relatively new occurrence in *M. bovis* but has spread rapidly. The encoded Tet44 protein (417 aa) was conserved across all *M. bovis* isolates. There were 8 amino acids that differed from the Tet44 protein found in *Campylobacter*, but the same changes seen in the *M. bovis* Tet44 protein also were found in Tet44 proteins from other bacterial species. The *tet44* gene is typically associated with a transposable element. WGS (combined Illumina and nanopore) of selected *M. bovis* isolates are in progress to identify potential mobile elements associated with *tet44.* Unlike many mycoplasmas, *M. bovis* can persist in the environment for more extended times and could potential contribute to the increased antimicrobial resistance in *M. bovis* as well as provided an environmental source for other bacteria.

Key Words: *Mycoplasma bovis*, antimicrobial resistance, *tet44*

4 - Comparative evaluation of *lpd* mutant and wild-type *Mycoplasma mycoides* ssp. *mycoides* via aerosol challenge in cattle.

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Contagious bovine pleuropneumonia, caused by *Mycoplasma mycoides* ssp. *mycoides Small Colony* (*MmmSC*), persists as a major concern in endemic regions due to high morbidity and its highly infectious nature. It constitutes a critical transboundary threat to countries such as the United States, where eradication in 1892 has left modern cattle populations immunologically naïve and highly susceptible to reintroduction. Genetic determinants of virulence in this pathogen remain poorly defined. Previous studies in other mycoplasma species, including *Mycoplasma gallisepticum*, demonstrated that disruption of the *lpd* gene encoding dihydrolipoamide dehydrogenase led to significantly reduced virulence in the chicken infection model, with mutants showing diminished ability to colonize and survive within the host and less severe histopathologic lesions compared with wild-type strains. To determine the contribution of *lpd* to the virulence of *MmmSCAfadé*, we evaluated a Δ*lpd* mutant in vivo using a bovine aerosol challenge model. Groups of cattle were individually challenged by nebulization with 5 mL of 1×10^8 cfu/mL of either wild-type *MmmSCAfadé* or the Δ*lpd* mutant (n = 4 control, n = 10 wild-type, n = 10 mutant). Animals were observed daily for clinical progression, with parameters including onset and severity of coughing, rectal temperature, inappetence, and incidences of recumbency. Animals developing severe disease and moderate to severe pain were euthanized via intravenous injection of an overdose of sodium pentobarbitone. Specific criteria requiring euthanasia included = 39.5°C for 6 consecutive days (a day of lower temperature counted as fever if preceding and following days were = 39.5°C), >3 consecutive days of fever = 40°C, lateral recumbency for more than one day, or no feeding for 3 consecutive days. All challenged animals, regardless of strain, developed classical signs and gross pathology associated with contagious bovine pleuropneumonia. The onset of endpoint clinical signs requiring humane euthanasia was slightly delayed in the Δ*lpd* group compared with the wild-type group. However, statistical analysis of clinical endpoints revealed no significant differences between the wild-type and mutant. In contrast to the attenuation seen in *M. gallisepticum*, the pathogenesis of *MmmSC* remains driven by determinants beyond *lpd*, reinforcing the need for continued research into novel targets and strategies to improve the welfare of affected cattle herds.

Financial Support: USDA-ARS, Non-cooperative Agreement

Key Words: contagious bovine pleuropneumonia, virulence factor, dihydrolipoamide dehydrogenase, aerosol challenge

5 - Microbial ecology of bovine mastitis: Contrasting genomic landscapes in organic and conventional dairy systems.

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Bovine mastitis, an inflammatory disease of the mammary glands often caused by bacterial infection, significantly affects milk production, animal health, and dairy farm economics. Despite its high financial cost, microbial ecology underlying mastitis remains poorly understood. Diagnostic data showed a ~10-fold increase in gram-negative bacteria on conventional versus organic dairies, leading to our hypothesis that antimicrobial use influences colonization and maintenance of resistance and pathogenic elements. We analyzed 1,140 clinical isolate genomes from milk and 92 environmental metagenomes across NH, VT, and NY. Milk cultures yielded 35 bacterial taxa. We used scoary to identify accessory genes associated with the organic phenotype in 3 taxa with sufficient sample sizes: *Staphylococcus aureus* (74 organic, 50 conventional), *Streptococcus uberis* (51, 37), and *Streptococcus dysgalactiae* (30, 158). Notably, IS6 family transposase was linked to organic *S. aureus* ($P = 0.003$). Conventional isolates had higher frequencies of β-lactam, efflux, glycopeptide, lincosamide/streptogramin, phenicol, quinolone, rifamycin, and sulfonamide genes ($P \leq 0.021$), whereas organic isolates had more macrolide and tetracycline genes ($P = 0.000$). MDR and XDR predictions were higher in conventional isolates, whereas organic milk isolates had more virulence genes. Environmental samples were compared at the k-mer level, independent of gene annotation. A random forest model trained on normalized 9-mer counts achieved the best performance. Shapley values showed that higher counts of key k-mers improved prediction of the organic phenotype. These findings reveal genetic differences in microbial ecology between farm types, with implications for pathogen evolution and mastitis management.

Financial Support: USDA NIFA award number: 2021-67016-34573

Key Words: mastitis, antimicrobial resistance, microbial ecology, machine learning, genomics

6 - The effect of heat stress and disease on the postpartum uterine microbiome of dairy cattle.

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As environmental temperatures increase globally, it is imperative to determine the effects 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. The prevalence of uterine disease among dairy cattle is increased during the summer months or when cows are subjected to heat stress. We hypothesized that environmental heat stress increases the susceptibility of cows to uterine disease by altering innate immune function which is independent of changes to the uterine microbiome. For 47 d before caving, cows were housed under fans, water soakers and shade (Cooled; n = 10) or shade alone (Heat stressed; n = 10). After calving all cows were housed under cooling conditions. Heat stress increased rectal temperature and reduced gestation length. The uterine content of each cow was sampled using a double guarded swab on d 7, 14, and 21 postpartum. A total of 5 cows were diagnosed with uterine disease that included metritis or endometritis during the first 21 d postpartum. The DNA of each swab was extracted and 16S metagenomic sequencing was performed using the Illumina MiSeq platform and processed using QIIME2. Alpha (amplicon sequence variant counts, Shannon index, and Chao1 index) and β diversity (weighted UniFrac distance) of the uterine microbiome were compared between cooled and heat stress cows, or cows with and without uterine disease. Regardless of the sampling day, the Shannon index was reduced (P = 0.008) and β diversity was different (P = 0.003) in cows with uterine disease. These changes mirror previous reports of decreased bacterial diversity in cows with metritis compared with healthy cows. The difference in diversity could be accounted for by an overabundance of *Fusobacterium* genus bacteria in cows with uterine disease. Amplicon sequence variant counts and Chao1 index were similar between uterine disease and healthy cows. There was no difference in amplicon sequence variant counts, Shannon index, Chao1 index, or weighted UniFrac distance in the uterine microbiome of cooled versus heat stress cows. Collectively, these data suggest that prepartum heat stress does not alter the uterine microbiome of dairy cows. The absence of dysbiosis in heat stressed cows suggests that increased uterine disease after heat stress involves an altered innate immune response to pathogens.

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Key Words: uterine disease, heat stress, microbiome, uterus

7 - Identifying *Listeria monocytogenes* colonization factors in the bovine gastrointestinal tract.

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Listeria monocytogenes (*Lm*) is an invasive pathogen in humans and ruminants. Although acquired orally, *Lm* can breach the intestinal barrier to systemically infect multiple organs, causing highly lethal clinical listeriosis, but many animals carry *Lm* without overt symptoms. Increasing evidence indicates that gastrointestinal (GI) infection determines listeriosis outcome, but very little is known about *Lm* pathogenesis in the bovine GI tract, and there is no suitable bovine infection model to address this knowledge gap. We developed a bovine intestinal infection model for *Lm*, using calf ligated ileal loops. Using this model, we determined *Lm* replication and invasion kinetics, and queried *Lm* genes required for bovine intestinal colonization. Neonatal calves at 3 to 6 weeks were placed under general anesthesia, and abdominal surgeries were performed to ligate the ileum into ~38 loops, with spacers between adjacent loops. The loops were injected with phosphate-buffered saline (PBS) containing *Lm* or PBS alone as mock controls. The ileum was returned to the abdomen and the calf maintained under general anesthesia. Preliminary histopathology revealed no overt mucosal inflammation in response to *Lm* at 8 hpi. However, *Lm* established consistent colonization in both the intestinal lumen and tissue. In 6 animals, strain 10403S (serotype 1/2b) reached median burdens of ~ cfu/g at 5 hpi and 10^6 cfu/g at 8 hpi in the lumen. Intestinal tissue burdens were consistent, with medians of 10^2 cfu/g at 5 hpi and 5 × cfu/g at 8 hpi. *Lm* mutants that are defective for general stress response (s^B) or systemic virulence factors (actA and hly) had no infection defect, suggesting that GI colonization is mediated by novel genes. We performed TnSeq to identify *Lm* colonization determinants in the GI tract, by infecting 16 loops in 2 animals with *Lm* transposon mutants, at 10^8 cfu/loop for 8 h. The genome-transposon junctions were sequenced by Illumina, mapped to the *Lm* genome using bowtie, and data were analyzed using TnSeq Explorer. TnSeq identified many genes important for intestinal colonization, including phosphotransferase systems, ornithine metabolism, and anaerobic respiration. Preliminary infections with strain F2365 (serotype 4b) in 2 animals suggest that it was more infective than strain 10403S. Together, these data reveal that *Lm* can replicate in the bovine GI tract within 8 h of infection, and it relies on novel stress response and metabolic factors.

Financial Support: USDA 2023-67015-39098

Key Words: *Listeria monocytogenes*, gastrointestinal tract, bovine

8 - Gastrointestinal microbiome alterations in dairy cows with hemorrhagic bowel syndrome.

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Hemorrhagic bowel syndrome (HBS) is an acute and often fatal gastrointestinal condition of adult dairy cattle characterized by intraluminal hemorrhage and obstruction. Despite its clinical significance, little is known about the microbial factors associated with this disease, and research on the bovine gut microbiome in HBS remains scarce. The objective of this study was to characterize the gastrointestinal microbiome of HBS cases to identify microbial community shifts that may be associated with disease status. We enrolled 5 pairs of dairy cows, each consisting of a HBS case and a healthy control matched from the same herd. We collected rumen and fecal samples from controls; from HBS cases, we also sampled the lesion site and an orad site. Bacterial communities were characterized by sequencing the V3-V4 region of the 16S rRNA gene, and reads were processed using QIIME2. Statistical analysis was performed in R to calculate and test α diversity, β diversity, and differential abundance differences between HBS and control cases. Alpha diversity showed a trend of decreased diversity in fecal samples of HBS cases, but while there were no significant differences between groups, only HBS cases had significantly lower richness and Shannon's diversity in their feces, compared with their rumen (P = 0.02 for both). Rumen microbiome composition was significantly different between cases and controls (P = 0.018), but this was not observed in the feces. Fecal samples and lesion-associated sites showed greater divergence, possibly indicating localized microbial shifts associated with disease. Across all samples, communities were dominated by the phylum *Bacillota*, with differences between sites appearing to be driven by shifts in *Pseudomonadota*. This study provides evidence that the microbiome differs between healthy cattle and those affected by HBS, particularly in rumen and lesion-associated GI sites. Rumen communities appear to shift in HBS cases, with associated alterations in downstream gut regions, especially involving *Pseudomonadota*, Further research is needed to distinguish the causality of these trends, but these findings highlight the potential role of site-specific microbial dysbiosis in HBS pathogenesis and establish a foundation for studies aimed at identifying microbial risk factors and mechanisms underlying this poorly understood disease.

Key Words: hemorrhagic bowel syndrome, dysbiosis, microbiome, dairy, cow

General Abstracts: Session 3—Foot-and-Mouth Disease

9 - Leveraging intelligent adversarial games to evaluate the effects of artificial intelligence technologies on competition and deterrence dynamics against agro-bioterrorism.

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Theoretical frameworks for adversarial risk analysis have been developed to evaluate the effects of social, political, environmental, and technological disruptions on competition and deterrence dynamics, e.g., simultaneous defend-attack models, sequential defend-attack-defend models, and sequential defend-attack models with private information. Recent developments in artificial intelligence (AI)-enabled human computer interactive games provide new opportunities to discover counter agro-bioterrorism strategies and build deterrence through biosecurity in cattle production supply chains. We hypothesize that *accelerating diffusion of digital data and AI technologies will have a disruptive effect on competition and deterrence dynamics through provision of AI-enabled foresight about competitor's perceptions of the net benefits of aggression relative to restraint*. A pilot attacker-defender gaming experiment, originally designed to control the accidental or deliberate introduction of "Transboundary Animal Diseases" (e.g., African swine fever and foot-and-mouth disease) in the US food supply chains was played by 100 human attacker players, sampled from Amazon Turkers. Static machine agents adopted biosecurity to mitigate their losses from human attackers. From the pilot experiment, we discovered that human players acting as attackers quickly learned to inflict damage in the absence of strong deterrence from a static defender machine agent. Data from this experiment is being used to train a deep reinforcement learning based AI defender agent in phase 2 of this game. Our preliminary results confirm our hypothesis that human attacker players learn to exploit the static aggression to constraint derived rules of defender machine players. In the next phase, gaming experiments will test AI-enabled dynamic defender agents versus human attackers, and conversely human defenders against static and dynamic AI attackers. Data from both phases of the game will enable us to fully test the hypothesis and derive implications for building deterrence against agro-bioterrorism in the age of AI.

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Key Words: artificial intelligence, bioterrorism, biosecurity, human computer interaction, adversarial game, deterrence

10 - Evaluating electronic identification for cattle traceability through analysis of simulated movement networks.

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Control of a foot-and-mouth-disease outbreak in the United States (US) will require animal health officials to trace the diseased and at-risk animals faster than the disease can spread. Infected animals can shed virus in as few as 2 to 3 d which means electronic identification (EID) and digital tracing are likely needed. The current US animal traceability system does not require all cattle to have EID nor report all movements to a database. This project's objective was to evaluate EIDs through the creation of tagged networks. InterSpread Plus generated a complete 30-d national network of cattle movements. In the network, farms are nodes, and animal movements are edges. A random sample of 10 farms were chosen as farms of interest (FOI) for tracing. To create tagged networks, nodes were randomly assigned to tag or not to tag at the following rates: 25% (low EID use), 50% (medium EID use), or 75% likelihood (high EID use). Nodes were also 50% or 90% likely to report the movement to the database. For a movement to be included in the tagged network, the source node must tag, and the destination node must report. Multiple iterations of each scenario were completed. The complete network had approximately 58,000 daily movements and a mean degree was 6.2. All FOIs had at least one contact in the complete network. The tagged networks had approximately 7,200 daily movements with low EID use and reporting and increased gradually to 36,700 with high EID and reporting. The mean degree in all tagged networks was approximately 1.5. With low EID and reporting, 30% of tagged networks had zero traceable contacts for all 10 FOIs. With high reporting, the low EID networks had at least 1 farm partially traceable in all networks. Medium EID networks improved slightly from low EID use. Under high EID and low reporting up to 7 farms were partially traced across the networks. With high EID and reporting, all 10 farms were partially traceable in 2 iterations. All FOIs had at least one iteration where no contacts were found. These results show the need for increased EID tag usage and movement reporting for effective tracing. The untraceable contacts may cause delays in tracing the at-risk farms, which will ultimately delay and undermine the efficacy of control efforts. Ongoing efforts in this project focus on farm-type specific tag and report rates and a larger number of farms to be traced.

Financial Support: USDA:APHIS National Animal Disease Preparedness and Response Program Assessing effect of current and increased EID adoption on cattle traceability success with a focus on beef calves, stockers and feeder cattle. Grant # AP24VSSP0000C051

Key Words: foot-and-mouth disease, cattle, traceability, electronic identification, network analysis

11 - Transmission of FMD through auction market cattle movements during an outbreak.

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Foot-and-mouth disease (FMD) is a major economic threat to the US cattle industry, and a primary route of disease spread is through the movement of livestock. The Secure Beef Supply (SBS) project offers guidelines to control the spread of infection, but there are no quantitative estimates of FMD transmission risk from cattle movements. While movement controls are necessary for disease control, overly restrictive control measures could severely disrupt business continuity. Estimates of the transmission risk of cattle movements during an FMD outbreak are needed to balance control with business continuity. Using InterSpread Plus (ISP), a spatially explicit and stochastic model of FMD transmission, simulations were performed to estimate daily infection incidence risks for cattle farms with risk stratified by proximity to an infected premises (IP). For infected farms, within-herd disease spread dynamics and active observational surveillance were used to estimate the probability of disease detection before shipment. Infected, but not detected, farms in the auction market catchment area were eligible to ship to the market. Risk of an infected shipment to a market and the number of infected sale lots was estimated from consignment lot sorting, mixing, and transmission within the auction market. Local transmission was the predominant risk for farms within 3 km of infected premises, with infection risk decreasing sharply as distance increased. Catchment area infection intensity and effectiveness of producer active observational surveillance were important risks for transmission to, and through, the auction market. The number of infected sale lots was influenced by the number of infected animals in the consignment shipment and the degree of sorting or mixing at the market. Transmission of FMD within the auction market resulting in new infections was low due to the short time of contact. In the event of an outbreak of FMD, prompt and focused disease control measures, balanced with the need to maintain business continuity, will be essential. Quantitative data to assess cattle movement risk can help to estimate the risk of specific cattle movements and allow those that are low risk to facilitate business continuity. The results from this study can improve our ability to make safe, informed cattle movement decisions, supporting both effective disease control and business continuity within the cattle industry.

Financial Support: USDA:APHIS NADPRP grant #AP22VSSP0000C020

Key Words: foot-and-mouth disease, modeling, infection risk, cattle movement

12 - Integration of sensor data and lesion history for predictive modeling of dairy cow lameness.

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Timely identification of individual lameness is critical for dairy herd health and productivity. While traditional methods rely on subjective visual scoring, the adoption of precision dairy technologies provides an opportunity for objective, continuous monitoring. The study's objective is to develop and validate a predictive model for locomotion score using behavioral data collected from neck-mounted sensors and historical foot lesion occurrence. Sensor data were collected from 2 commercial dairy farms in Minnesota between January and July 2025. The dataset included daily totals for eating time, rumination time, and inactive time, measured by collar sensors, alongside corresponding visual locomotion scores (0–3) provided by experts (score 0 is a non-lame and score 3 is a severely lame cow). After data cleaning, a total of 6,561 cow-days from 846 unique cows were available for analysis. The locomotion scores were categorized as lame (score 0 and 1; n = 176) and non-lame (score 2 and 3; n = 670) cows. To account for repeated measures on individual cows, the dataset was split into training and testing sets by individual cow, ensuring no overlap between sets. A random forest model was developed using average eating, rumination, and inactive time from the 2 weeks before the locomotion score date, their standard deviations, days in milk, lactation number, and lesion history as predictors. Model performance was evaluated across 30 randomized iterations. While the predictive model achieved high average accuracy (76%), its performance was driven by high specificity (84%) at the expense of low sensitivity (46%), indicating the model was proficient at identifying non-lame cows but struggled to detect lame cows. Lower sensitivity might be caused by unbalanced classes in the dataset. The initial results indicate that behavioral measures can explain a substantial portion of the variation in locomotion scores.

Financial Support: This work is partially supported by a grant form "Animal Health Capacity Grant"

Key Words: lameness, dairy cattle, wearable sensor, machine learning, animal behavior, precision dairy

13 - Evaluating regional zoning for foot-and-mouth disease control in the New England milkshed using InterSpread Plus.

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Foot-and-mouth disease (FMD) poses a major threat to the US dairy industry, with potential for extensive economic losses due to trade restrictions and control interventions. This study assessed the efficacy of regional zoning in mitigating FMD dissemination, with a focus on the New England milkshed, employing the InterSpread Plus (ISP+) spatial stochastic simulation 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, were used to create a farm file encompassing 69,481 dairy cattle premises. The model simulated high-risk live animal transfers based on frequency, distance, and transmission probabilities. Indirect movements were categorized into 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. We modified a baseline ISP+ setup, excluding regional zoning, and integrating milk plants, dealers, and cattle slaughterhouses, using 2022 data derived from state agricultural reports and Federal Milk Marketing Orders (FMMOs). We posited that New England regional zoning would diminish national FMD impact by constraining cross-zone movements amid outbreaks. Simulations comprised 100 iterations spanning 70 d, contrasting the regional zoning scenario against the baseline. Outcome metrics included infected premises counts and epidemic duration. In the baseline, the epidemic failed to peak by d 70, attaining a median of ~55 infected dairy premises daily, with a cumulative median of ~1,598 infected premises (IQR: 1,150–2,178) across iterations. Under New England regional zoning, the epidemic peaked near d 25, with a median of 8 infected premises daily, and a cumulative median of ~389 infected premises (IQR: 272–505). Kruskal-Wallis analysis revealed significant reductions in infected premises ($P < 0.001$). These results indicate that zoning may bolster FMD containment in dense dairy locales like New England. Policy implications advocate for preemptive regional zoning frameworks to alleviate economic burdens. Limitations encompass model assumptions on heightened local spread probabilities for dairy premises, stemming from absent empirical data from US FMD outbreaks, though consistent across scenarios. Future investigations should evaluate the cost-effectiveness of regional zoning.

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Key Words: biosecurity, dairy industry, foot-and-mouth disease (FMD), InterSpread Plus (ISP+), regional zoning, simulation modeling

14 - eBeam-driven killed vaccines: A novel approach to preserve antigenic integrity and control bacterial lameness in broilers.

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Poultry is one of the primary sources of foodborne pathogens such as *Salmonella*, *Campylobacter*, *Clostridium perfringens*, and *Staphylococcus*, which not only cause diseases in birds but also pose risks to human health and the economy. Bacterial chondronecrosis with osteomyelitis (BCO), a debilitating lameness condition affecting broiler chickens, is associated with multiple pathogens, including *Staphylococcus*, *Enterococcus*, and *Escherichia coli*, resulting in substantial economic losses and animal welfare concerns. Traditional killed vaccines rely on chemical inactivation, such as formalin, which can damage protein epitopes, critical targets for immune recognition, and potentially compromise vaccine efficacy. In contrast, electron beam (eBeam) technology inactivates pathogens by inducing double-stranded DNA breaks, preventing replication without altering surface proteins. This preserves native epitopes, enhancing immunogenicity. In controlled studies, broiler chickens were vaccinated with an eBeam-inactivated multistrain *Staphylococcus* vaccine, compared with formalin-killed, combination, and sham groups. Birds were exposed to aerosolized, natural BCO challenge for 56 d. The eBeam-vaccinated group showed a 50% reduction in lameness and a complete absence of *Staphylococcus* in BCO lesion sites, indicating substantial protection. These results demonstrate that eBeam technology enables the development of highly effective, epitope-preserving killed vaccines. This approach holds promise not only for BCO control but also for broader applications in preventing zoonotic and poultry diseases. The vaccine is protected by provisional patent #2534205, and several animal vaccines are currently in clinical trials.

Key Words: eBeam-killed vaccine, electron beam, inactivation, vaccine, *Staphylococcus*, bacterial-induced lameness

General Abstracts: Session 4—Epidemiology 1

15 - A targeted field survey of parasites on oyster aquaculture farms in the context of the natural environment.

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Oyster aquaculture is a growing industry both nationally and internationally. While there are benefits of growing oysters in the natural environment, production can also be negatively impacted by the environment. The overarching goal of this research was to evaluate potential drivers of parasitic infections on Maryland oyster aquaculture farms in an unprecedented level of detail to guide farm management decisions for the Eastern oyster, *Crassostrea virginica*. A total of 400 oysters were sampled from 8 farms in June and August. We diagnosed infection of adult, market-sized oysters with 2 tissue parasites that have caused mortality events, using microscope-based diagnosis for dermo (caused by *Perkinsus marinus*) and qPCR diagnosis for MSX (caused by *Haplosporidium nelsoni*). We quantified shell bioerosion by *Poldyora* spp. mud blister worms and *Cliona* spp. boring sponges. For each parasite, we used generalized linear models (GLMs) and maximum likelihood estimation to evaluate whether the farm and time point predicted infection. We then compared these drivers to oyster size and co-infection status. Dermo prevalence differed significantly across farms but was best predicted by increasing *Polydora* mud blisters. The MSX results are pending and will be discussed. *Cliona* spp. sponge prevalence differed significantly across farms and depended on *Polydora* mud blisters, decreasing as blister intensity increased. *Polydora* mud blisters varied significantly across farms and were significantly worse when *Cliona* spp. sponge infections were absent. Oyster body condition was best predicted by the farm rather than infection. Our findings suggest that interactions among parasites play an important role in parasitic infection patterns and could have serious implications for oyster mortality events caused by dermo. We highlight the importance of interactions between shell and tissue parasites, which are seldom considered. The greater importance of oyster farm for body condition compared with tributary or co-infection suggests that farm practices or small-scale environmental conditions are key players for oyster health. Using future October 2025 data combined with annual state disease monitoring surveys, this project will also test the proximity to wild oyster populations as a driver of parasite prevalence and intensity. Disseminating timely results to farmers has provided the opportunity for discussion and hypotheses about the complex disease patterns in this system.

Financial Support: This project was supported by Agriculture and Food Research Initiative Competitive Grant no. 2024-67015-42418 from the USDA National Institute of Food and Agriculture.

Key Words: oyster, aquaculture, parasite, coinfection, environment, *Crassostrea virginica*

16 - Effects of varying temperatures on host-pathogen coevolution across populations.

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As the global climate warms, we are seeing a variety of ecological responses to temperature 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. However, little is known about the evolutionary and coevolutionary effects of a warming climate on host-pathogen interactions and transmission. 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 regimens using 2 separate populations. Fall armyworms were reared at below optimum, optimum, and above optimum temperatures given their thermal performance curve. Since populations did not survive at above optimum temperatures, we only examined populations at below optimum and optimum temperatures. To examine disease transmission over multiple generations, we constantly exposed hosts that had been coevolved with the virus to varying viral doses. These dose-response experiments were analyzed using a binomial model to calculate summary statistics. Trends in the summary statistics were analyzed using a linear model. At lower temperatures, below the optimum of the thermal performance curve, the virulence of the virus did not change in terms of the lethal dose at which 50% of infected individuals would die (LD50). At optimum temperatures, virulence declines but to a differing extent when comparing between the 2 populations. At the LD95, a dose at which the vast majority of individuals would die, there was no change in virulence. Increases in temperature impact disease transmission by decreasing the virulence of the virus in this tightly linked host-pathogen system. However, at colder temperatures, there is no change in virulence, perhaps due to the host experiencing physiological stress. These patterns hold regardless of population origin. 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 pathogens. Thus, this research can improve our ability to determine how best to use these pathogens as bioinsecticides under different ranges of temperatures as both host and pathogen coevolve.

Financial Support: This work was funded by USDA grant 20196701429919 as part of the joint NSF-NIH-USDA Ecology and Evolution of Infectious Diseases program.

Key Words: host-pathogen interaction, baculovirus, dose-response, insect pest

17 - Grain-free diets and cardiac diagnoses in companion dogs.

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Grain-free diets have been the subject of much scientific scrutiny since their meteoric rise in sales in the 2010s, and their potential association with dilated cardiomyopathy (DCM) in dogs first reported in 2018. Since the announcement of the FDA's investigation into grain-free diets, many veterinarians have been recommending moving away from grain-free diets for dogs with any cardiac disease (not just DCM), or any dog predisposed to cardiac disease, out of an abundance of caution. However, there have not been any studies regarding the effect of grain-free diets on all cardiac diseases. This study used data collected from over 47,000 dogs enrolled in the Dog Aging Project and their owner-reported grain-free status of their diet and new cardiac diagnoses to determine whether grain-free diets had an effect on incident cardiac diagnoses over 2.5 years in the cohort. By using directed acyclic graphs to identify confounding factors, prospectively collected diet and diagnosis data, general linear mixed effect models, and general linear models, we approach this analysis using methods consistent with causality. There was no observed effect of grain-free diet status on incident cardiac diagnoses (OR 1.05, 95% CI: 0.91–1.21). In addition, we performed an analysis to determine the misclassification bias present in the dataset, and simulated what effect this bias would have on any computed results. The particular type of misclassification bias would attenuate computed associations, but only minimally. Together, these findings suggest that there is a minimal attenuated, or no effect of grain-free diets on new cardiac diagnoses. This is preliminarily reassuring information for the pet owners who prefer grain-free diets who have been awaiting evidence for these diets on cardiac diseases other than DCM.

Key Words: cardiac, grain-free, canine, diet

18 - Diet type and cardiac and cancer diagnoses in the Dog Aging Project cohort.

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Diet types (i.e., kibble vs. canned vs. home-prepared, etc.) are believed to be associated with health outcomes in companion animals. Preliminary research in home-prepared diets revealed that approximately 95% of these are deficient in micronutrients and amino acids according to recommended feeding guidelines. Canned diets are higher in moisture content, but also have higher by-products of cooking secondary to the retort process necessary for food safety, and preliminary research has demonstrated that high levels of these by-products can be associated with cellular damage. To determine the effect of diet type over time in the Dog Aging Project cohort, we used general estimating effects (GEE) regression models with directed acyclic graph-identified confounders to examine population-level associations of diet type on diagnoses of cardiac disease and cancer over 4 years of data collected from over 47,000 dogs. Home-prepared (both cooked and raw) and commercially prepared raw diets were the only ones statistically significantly associated with an increase in cardiac disease when compared with kibble diets as the referent group in this prospective analysis (OR 1.14, 95% CI 1.01–1.3 and 1.23, 95% CI 1.05–1.45, respectively). Only commercial fresh cooked diets were associated with an increase in cancer (OR 1.33, 95% CI 1.08–1.64) when compared with kibble as the referent group. Kibble diets had the least risk of cardiac or cancer outcomes.

Key Words: diet, canine, dog, cardiac, cancer

19 - Development of a novel survey instrument to assess the impact of companionship loss on physical and behavioral health outcomes in household-living dogs.

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Affiliative bonds are an important currency for group-living mammals in the context of access to social status, protection, and other resources. But beyond the transactional benefits associated with bond formation, companionship is increasingly understood as an exposure implicated in broader quality of life and physical and emotional health outcomes in multiple species. With dogs assuming significant roles in the realm of allospecific companionship for many humans, it is important to understand how these animals are affected by gain and loss of social partners, and how companionship as a risk factor relates to overall disease burden for household dogs. Preliminary study results from descriptive analyses of data sourced from more than 40,000 dogs enrolled in the Dog Aging Project (DAP) revealed that 93% of owners who reported grief-like responses in their dogs also reported concurrent physical and/or behavioral changes in the dog (e.g., sundowning, anxiety, etc.), with 90% attributing these changes directly to loss. Among those reporting family additions, nearly half of changes reported were considered positive. These findings prompted the present study wherein we develop the Companionship-Loss and Outcomes Survey (C-LOS), a novel instrument designed to investigate the short- and long-term effects of permanent change in familial social structures (i.e., changes to the household that alter the number and/or types of stable relationships a dog has) on dogs' physical, cognitive, and behavioral health. We recruited 15 owners who had previously opted to share information about their dogs' response to loss of household companions to participate in 1/2-h-long, semi-structured qualitative interviews. Interview data were coded and analyzed using Atlas.ti. Patterns, themes, and categories of risk and response that emerged during this analysis informed C-LOS construction. The resulting instrument presents 3 survey streams (Loss, Gain, and Multiple Incidents) comprising 5 questionnaire sections, Household Composition, Characterization of Companionship Change, Observed Behaviors & Health Conditions, Human Disposition, and Supportive Interventions. In deploying the instrument, we will examine the impacts of loss on dog health, with an aim to quantify "for the first time" the exhibition and associated effects of grief-like behaviors in dogs, with important implications for understanding companionship as a social determinant of health in non-human animals.

Financial Support: Morris Animal Foundation [MAF: D25CA-041]

Key Words: small animal, exposome, shared risk, qualitative method, social determinant of health, canine health

20 - Demographic factors of dog owners and dogs with pet health insurance.

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In the United States, only about 4% of pet owners have a pet insurance policy even though such policies mitigate the financial risk of veterinary expenses. While current pet insurance research has found that pet insurance policyholders spend more on medical expenses and wellness plan subscribers visit veterinary hospitals more frequently, it is not known if dogs with pet insurance have better health outcomes. In this cross-sectional exploratory study, dog owners' responses from the Dog Aging Project (DAP) initial enrollment survey, Health and Life Experience Survey (HLES), were evaluated. The HLES was completed between the dates of January 1, 2020, and December 31, 2023. Dog and dog owner demographic data were collected and compared using descriptive statistics. Perceived health status of the dogs, reported by their owners, was compared between insured and uninsured dogs using a chi-squared test of significance. Based on descriptive analysis, dog owners with pet insurance tend to be racially diverse, between the ages of 25 and 44, college educated, and in the highest earning income bracket. Dogs with pet insurance tended to be a single breed, mature adults, intact, acquired from a breeder, and obtained for a purpose other than companionship. A higher proportion of insured dogs, deemed to be statistically significant using chi-squared analysis, were perceived to have excellent health. Demographic variables of insured dogs and their owners are meaningfully different from the overall DAP population and previous pet owner demographic peer-reviewed studies. The health status of dogs with pet insurance is statistically different compared with those without pet insurance. The results of this study can help guide veterinary discussions with pet owners about pet insurance; however, further investigation of the impact of pet insurance on pet health is warranted.

Key Words: pet insurance, dog, veterinary, demographics

General Abstracts: Session 5—Porcine Reproductive and Respiratory Syndrome

21 - Contemporary United States PRRSV 1-4-4 L1C.5 causes severe infection similar to historic highly pathogenic PRRSV.

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Porcine reproductive and respiratory syndrome virus (PRRSV) with a 1-4-4 RFLP pattern and L1C.5 sublineage is the most detected PRRSV strain in the United States (US). Swine producers report variable disease from PRRSV 1-4-4 L1C.5 outbreaks, with some reporting extreme morbidity and mortality. We compare disease caused by a contemporary 1-4-4 L1C.5 isolate (previously reported more pathogenic than other 1-4-4 L1C strains) that was confirmed to be genetically distinct from historic examples of moderately and highly pathogenic PRRSV (HP-PRRSV), including HP-PRRSV JXwn06 that emerged in China in 2006 and moderately pathogenic PRRSV MN184 that emerged in the US in the early 2000s. Four-week-old pigs intranasally inoculated with 2 mL 3×10^5 $TCID_{50}$/mL mock, MN184, L1C.5, or JXwn06 inoculum (n = 20/group) were necropsied at 2, 6, and 10 d post-infection (dpi). Clinical disease, viral loads, cytokines, antibody, and pathology were assessed and data integrated via multidimensional reduction to gain an overview of disease dynamics. Whole-transcriptome sequencing of whole blood and thymus was performed and integrated via multidimensional reduction to gain overviews of transcriptomic relationships. Differential gene expression (DGE) analysis was also performed. Dimensionality reductions indicated divergence of L1C.5 and JXwn06 from mock and MN184 samples as dpi progressed, with L1C.5 and JXwn06 samples maintaining high degrees of similarity to one another throughout. L1C.5 and JXwn06 caused severe disease in animals, including elevated temperatures, weight loss, coughing, lethargy, high viral loads, heightened cytokine/antibody levels, lung pathology, and distinct gene expression. The DGE analysis identified large numbers of differentially expressed genes between L1C.5 or JXwn06 versus mock or MN184 but minimal differentially expressed genes between L1C.5 versus JXwn06. Overall, a contemporary PRRSV 1-4-4 L1C.5 isolate and HP-PRRSV JXwn06 caused highly similar disease based on assessed parameters. Mechanisms by which historic Asian HP-PRRSVs caused severe disease may be similar to pathogenic mechanisms utilized by currently circulating PRRSVs causing severe disease in the US, despite unique viral genetics, geographic origins, and dates of emergence. Further research is needed to understand genetic and phenotypic characteristics of HP-PRRSV strains that will be informative for developing vaccine and therapeutic strategies.

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Key Words: porcine reproductive and respiratory syndrome virus (PRRSV), L1C.5, 1-4-4, JXwn06, highly pathogenic porcine reproductive and respiratory syndrome virus (HP-PRRSV), RNA-sequencing (RNA-seq)

22 - Vehicle movements and risk of PRRS and PED transmission: What do we know?

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Vehicle movements play a critical role in the spread of porcine reproductive and respiratory syndrome (PRRS) and porcine epidemic diarrhea (PED) among swine farms. Although recent studies have highlighted their importance, key risk factors remain insufficiently quantified. This study aimed to identify and describe vehicle cab- and trailer-related factors associated with PRRS and PED status in US swine farms. We integrated farm identification, barn-based geolocation, and GPS data from vehicle cabs (including truck cabs and worker transport vans) and trailers transporting pigs, feed, and personnel, stored in the RABapp database. Data were obtained from 4 production systems from January 1, 2024, to July 30, 2025, and outbreak data were sourced from RABapp and MSHMP. Vehicle visits to farms were identified and used to reconstruct contact networks that accounted for pathogen decay and cleaning and disinfection (C&D) events and effectiveness. We also estimated farm reachability, defined as the proportion of farms that could be infected through time-respecting vehicle contacts. Associations between vehicle visit frequency, network metrics, and infection status were evaluated using univariate and multivariate logistic regression models. Over the study period, vehicle cabs connected 2,758–2,770 farms, whereas trailers connected 1,484–1,491 farms. Pig transport cabs were the most influential vehicles in the network, representing 79% of cab movements and linking the largest number of farms, allowing 60% farm reachability; trailer movements allowed 82% reachability. Accounting for C&D reduced 3%–5% of cab and 14%–15% of trailer network edges. Recent exposure of vehicles to positive farms increased the risk of PRRS and PED by 299%–489% per additional weekly visit. In contrast, greater vehicle loyalty (repeated visits of the same vehicles or trailers to the same farms) and C&D events were protective, reducing infection risk by up to 85% and 6%, respectively. Our findings show that recent exposure of vehicles to infected farms markedly increases infection risk, whereas consistent vehicle-farm pairings and effective C&D mitigate transmission. These insights can help swine companies optimize C&D protocols, prioritize vehicle monitoring, and reduce the likelihood of costly PRRS and PED outbreaks.

Financial Support: USDA-NADPRP

Key Words: disease surveillance, sampler, pig, response plan, outbreak management

23 - Comparative transcriptomics reveals novel and differential circular RNA responses underlying interferon-mediated antiviral regulation in porcine alveolar macrophages.

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Porcine reproductive and respiratory syndrome (PRRS) continues to impose major economic losses on the swine industry, yet the non-coding RNA mechanisms underlying host antiviral responses remain poorly understood. In this study, we profiled the global circular RNA (circRNA) landscape in porcine alveolar macrophages (PAMs) following treatment with a modified live virus (MLV) vaccine or 2 type I interferon (IFN) subtypes, IFN-α1 and IFN-ω5, which differ in antiviral potency. We performed whole-transcriptome sequencing on PAMs from each treatment, followed by circRNA annotation, normalization with the TPM method, and statistical testing. We further analyzed circRNA–miRNA networks using miRanda, predicted open reading frames (ORFs), internal ribosome entry sites (IRESs), and N6-methyladenosine (m6A) sites with deep learning-based tools (DeepIRES, DeepCIP, deepSRAMP), and examined Gene Ontology (GO) and pathway enrichment (KEGG) of circRNA-associated genes. We identified over 1,000 differentially expressed circRNAs across treatment groups, with IFN-ω5 eliciting the most extensive upregulation. Most circRNAs were exon-derived, and their length and chromosomal distributions were broadly conserved across conditions. Hierarchical clustering revealed 5 distinct expression clusters, with IFN-α1 and IFN-ω5 inducing partially overlapping but quantitatively distinct circRNA profiles, whereas the MLV vaccine triggered unique patterns. IFN-ω5 strongly upregulated circRNAs from immune-related genes such as JAK2, AKIRIN2, SMARCA5, and TRPM7, which are implicated in immune cell activation, NF-κB coactivation, mRNA stability, and exosomal targeting. Gene Ontology and KEGG analyses showed enrichment for immune-related and metabolic processes, including cytokine signaling, leukocyte transendothelial migration, NF-κB and TNF signaling, and methylation pathways—particularly prominent under IFN-ω5 stimulation. Collectively, our results reveal both conserved and treatment-specific circRNA responses during early antiviral regulation in PAMs and highlight IFN-ω5 as a potent stimulator of circRNA expression and immune activation. These findings also align with recent in vivo studies, which demonstrated that IFN-ω5 plays a critical role in the early phase of immune activation, supporting its promise as a target for immunomodulatory and therapeutic strategies.

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Key Words: porcine reproductive and respiratory syndrome virus (PRRSV), porcine alveolar macrophage, small RNA-seq, microRNA regulation, interferon subtype, antiviral response

24 - PRRSView: Expanded analytical capabilities to empower swine producers.

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Porcine reproductive and respiratory syndrome virus (PPRSV) continues to be a major swine pathogen in the United States. PRRSView is a website that was created as an analytical resource to help swine producers compare the PRRSV strains present in their production system to those detected at the Iowa State University Veterinary Diagnostic Laboratory (ISU VDL). Due to recent developments in PRRSV research, the capabilities of PRRSView have been expanded to better support user needs. PRRSView has adopted the recently developed variant extension to the PRRSV lineage nomenclature system, enabling finer classification within sublineages. These variants have been incorporated into the BLAST databases, allowing for identification and assigning the variant designation based on sequence similarity. A new visualization that tracks the top 30 variants detected at the ISU VDL has been developed, providing current perspective of variant prevalence. To supplement information regarding the genetic diversity of contemporary PRRSV, PRRSView generates a monthly report summarizing all sublineages with more than 10 detections in the previous month. In response to user requests, PRRSView now offers a basic homology tool that accepts FASTA-formatted sequences and generates a downloadable homology table. This tool is designed to pairwise align sequences using a Needleman-Wunsch algorithm without affine gap penalty and can produce slightly different results compared with other bioinformatics tools. This tool is optimized to run online, with 60 ORF5 sequences as the recommended upper limit. Although this tool is hosted on PRRSView, it is pathogen agnostic and can report percent nucleotide homology of other virus comparisons. A new tree building tool has also been added to visualize phylogenetic relationships between PRRSV sequences and to supplement requests for dendrograms. This system uses sequences in FASTA format as an input, aligns the sequences using the Needleman-Wunsch method, and infers a neighbor-joining phylogenetic tree for visualization. These bioinformatics tools are designed to empower producers and PRRSView users with timely, actionable insights. By providing access to analytical capabilities, PRRSView supports informed decision making for the ongoing effort to manage PRRSV.

Key Words: porcine reproductive and respiratory syndrome virus (PRRSV), PRRSView, phylogenetics, tool

25 - Construction of arterivirus infectious clones using circular polymerase extension reaction (CPER).

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While various reverse genetic approaches have been used to generate infectious cDNA clones of positive-sense, single-stranded RNA viruses, circular polymerase extension reaction (CPER) has emerged as a high-fidelity and rapid tool for this purpose. CPER relies on the amplification and assembly of overlapping cDNA fragments of the viral genome, with a linker fragment connecting the 5′ and 3′ untranslated regions and containing functional mammalian expression elements. This generates a circular product ready to be transfect into permissive cells, initiating viral replication and production. The goal of this study was to generate reporter infectious clones of animal arteriviruses (equine arteritis virus KY84 [EAV KY84] and porcine reproductive and respiratory syndrome virus VR2332 [PRRSV VR2332]) using CPER. Viral genomic RNA was reverse transcribed into cDNA. Overlapping fragments spanning the entire genome were amplified and circularized with a linker sequence containing a human cytomegalovirus promoter, hepatitis delta virus ribozyme, and bovine growth hormone polyadenylation signal using CPER. The product was then transfected into BHK-21 cells. Infectious recombinant EAV KY84 (rEAV KY84) was successfully rescued 48 h post-transfection and confirmed by immunofluorescence (IFA). The successful rescue of recombinant PRRSV VR2332 (rPRRSV VR2332) was confirmed by passaging the CPER-derived P0 virus stock in MARC-145 cells and IFA at 4 d post-infection. Similarly, we generated reporter viruses by incorporating the mCherry gene downstream of the nsp1/nsp2 cleavage junction in EAV KY84 ORF1a and the GFP gene downstream of ORF1b of PRRSV VR2332, respectively. Recombinant viruses expressing reporter genes were recovered. All CPER-generated viruses showed high identity to their wild-type strains by next-generation sequencing. Growth kinetics of CPER-generated viruses were compared with the wild-type strains via one-step growth curves. rEAV KY84 and rPRRSV VR2332 growth kinetics were comparable to wild-type viruses, while reporter viruses showed significantly lower titers (3 independent replicates; 2-way ANOVA). The stability of the reporter genes was analyzed over 15 passages. While GFP expression was stable for 15 passages, the mCherry signal was negligible at passage 15 and associated with the deletion of the reporter gene. Overall, CPER can be used as a rapid tool to generate infectious clones of arteriviruses, allowing the study of viral gene functions and their interactions with the host.

Key Words: porcine reproductive and respiratory syndrome virus (PRRSV), equine arteritis virus (EAV), circular polymerase extension reaction (CPER), reverse genetics, reporter gene

26 - Shedding patterns of PRRSV vaccine viruses in multiple specimens from weaned pigs vaccinated with six MLV vaccines under experimental conditions.

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Porcine reproductive and respiratory syndrome virus (PRRSV) remains a major challenge for the swine industry, yet data on viral shedding after modified live virus (MLV) vaccination are limited. Seventy PRRSV-naïve weaned pigs were randomly assigned to 7 groups (10 pigs/group). At 0 d post-vaccination (DPV), group 1 (G1) received virus-negative medium while groups 2–7 received one of the following vaccines: Prime Pac (G2), Ingelvac ATP (G3), Fostera (G4), Ingelvac MLV (G5), Prevacent (G6), and PRRSGard (G7). At 35 DPV, all pigs were challenged with a wild-type L1C.5 strain. Individual serum, nasal swab (NS), oral swab (OS), and fecal swab (FS) samples were collected at 0, 2, 4, 7, 10, 14, 21, 28, 35, 37, 39, and 49 DPV. Samples collected from 0 to 35 DPV were tested using PRRSV screening PCR, whereas those from 37 to 49 DPV were tested using the corresponding PRRSV vaccine-specific PCR. C_T < 40 was considered positive. All G1 specimens remained negative for vaccine viruses. The onset of viremia and viral shedding in NS, OS, and FS varied among G2–G7, with the earliest detections at 2 DPV in serum, NS, and OS, and at 4 DPV in FS. No significant difference was found in the duration of vaccine viremia among groups, although some pigs in G2–G4 and G6–G7 remained positive at 39 DPV while all pigs in G5 were negative by that time. Similarly, vaccine viral shedding duration in NS did not differ significantly among groups, with all pigs testing negative by 39 DPV except for 2 samples in G6. In OS, G6 had a significantly shorter vaccine viral shedding duration compared with G4 and G7, and all pigs were negative by 49 DPV. For FS, G2 and G4 had significantly shorter vaccine viral shedding durations than G7, which still had positive detections at 39 DPV, while other groups tested negative after 37 DPV. From 2 to 39 DPV, vaccine virus loads in serum had significant differences among some vaccinated groups, with G4 higher than G2, G5, and G6. In G2–G7, the overall vaccine viral loads in serum were consistently higher than those in NS, OS, and FS. This study demonstrates vaccine virus shedding and viral load dynamics after 6 PRRSV-2 vaccinations under experimental conditions. The onset and duration of vaccine virus shedding varied among groups. These findings provide useful insights regarding the detection timeline of vaccine viruses, aiding in vaccine performance evaluation and selection of appropriate diagnostic strategies to differentiate vaccine from wild-type virus.

Key Words: porcine reproductive and respiratory syndrome virus (PRRSV), polymerase chain reaction (PCR), modified live virus (MLV) shedding pattern

General Abstracts: Session 6—Pathogenesis and Immunity

27 - Herd-level risk factors and management practices associated with *Mycoplasma bovis* in Brazilian dairy farms.

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My*coplasma bovis* is an emerging pathogen associated with mastitis, pneumonia, arthritis, and otitis in dairy cattle, leading to major economic and welfare losses. Despite its global impact, epidemiological information from South America remains scarce. This study aimed to estimate herd-level prevalence of *M. bovis* in Brazilian dairy systems and to identify herd-level risk factors related to biosecurity and management. A total of 190 dairy herds were evaluated through bulk tank milk PCR testing, nasal swabs from calves, and structured farm-level questionnaires. Prevalence estimates with 95% confidence intervals (CI) were calculated, and associations between herd status and management variables were assessed using Mann–Whitney and Fisher's exact tests. Cluster analysis (k-means, k = 4) was conducted to characterize herds according to productive and sanitary profiles. The national herd-level prevalence was 9.5% (18/190; 95% CI: 6.1–14.5%). Positive herds had significantly larger herd sizes than negative herds (median: 400 vs. 280 lactating cows; $P = 0.0067$). Although not statistically significant, positive herds showed a higher proportion of calf feeding with discarded milk (72% vs. 49%; Fisher's $P = 0.116$). Cluster analysis revealed 4 distinct herd profiles: (1) medium herds with high bacterial counts, (2) large-scale herds with low somatic cell count and excellent milk hygiene, (3) small herds with low productivity and high somatic cell count, and (4) medium-to-large herds with good milk quality and productivity. The distribution of *M. bovis* positivity was numerically higher in cluster 3 (15.9%) compared with other profiles (0–9.1%), though not statistically significant (Fisher's $P = 0.223$). These findings suggest that *M. bovis* is present at moderate prevalence in Brazilian dairy herds and is not restricted to a single production profile, affecting farms of varying sizes and productivity levels. Larger herd size was confirmed as a risk factor, while the trend observed in cluster 3 highlights the potential role of udder health and management practices in pathogen persistence. In conclusion, the presence of *M. bovis* in approximately one in 10 herds underscores the importance of strengthening biosecurity, with special attention to calf feeding strategies and milk hygiene, to prevent dissemination in diverse dairy production systems.

Financial Support: Zoetis and Research Foundation (FAPESP) 2025/10491-9

Key Words: *Mycoplasma bovis*, dairy cattle, biosecurity, risk perception, Brazil

28 - Microbiota's role in inflammatory responses in transitional dairy cows.

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The postpartum period in dairy cows involves inflammatory-mediated processes such as placental expulsion and uterine involution. However, an exacerbated acute-phase response can be detrimental to health and productivity and is associated with disorders such as mastitis, metritis, and ketosis. The bovine gut microbiota is known to influence host physiology, but its association with postpartum inflammation is poorly understood. We hypothesized that alterations in the fecal microbiota during early lactation are associated with systemic inflammation, and that these microbial patterns could serve as early indicators of inflammatory risk. We analyzed fecal microbiota from 71 Holstein cows collected on 1 and 3 d in milk (DIM) in relation to systemic inflammation, measured by serum haptoglobin and fibrinogen. Microbiota profiles were generated from 16S rRNA gene sequences, processed to remove contaminants and adjust for batch effects. Alpha- and β-diversity metrics were evaluated with models accounting technical and biological covariates (extraction date, cow ID, parity, BCS, and DIM). Differentially abundant genera were identified using ANCOM-BC2. Inflammation status was not significantly associated with any α diversity measure (all FDR $P > 0.05$; $R^2 = 0.20–0.31$). However, β-diversity analyses revealed compositional differences linked to elevated inflammation. Elevated fibrinogen revealed significant (FDR $P < 0.05$) shifts based on Aitchison ($R^2 = 0.0073$), weighted ($R^2 = 0.0081$), and unweighted ($R^2 = 0.0074$) UniFrac distances, but not Bray–Curtis. Elevated haptoglobin was associated with Bray–Curtis ($R^2 = 0.0078$) and Aitchison ($R^2 = 0.0081$) distances, but not UniFrac. The BCS ($R^2 = 0.0134–0.0158$) and DIM ($R^2 = 0.0072–0.0109$) also significantly influenced β-diversity. No differentially abundant taxa were found for fibrinogen status, whereas 16 genera differed by haptoglobin status (FDR $P < 0.05$, $\log_2 FC > 1$). Cows with elevated haptoglobin exhibited enrichment of *Succinivibrio*, *Bifidobacterium*, and [*Eubacterium*] *ventriosum* taxa linked to carbohydrate metabolism, alongside depletion of *Akkermansia* and *Clostridium*, which are associated with mucosal integrity and anti-inflammatory roles. These findings suggest that periparturient systemic inflammation is linked to an altered fecal microbiome, with specific taxa potentially serving as indicators of inflammation.

Financial Support: Dr. Neves's discretionary funds. No grant agency sponsored the work.

Key Words: inflammation, transition period, microbiota, dairy cow, bacterial abundance

29 - Interrogating two overlooked ETEC virulence proteins for their impact on pathogenesis.

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Enterotoxigenic *Escherichia coli* (ETEC) causes diarrhea and no intestinal lesions in a multitude of species (pigs, cattle, humans). For over 5 decades, the scientific community has assumed ETEC does not interact with the immune system and focused primarily on studying its plasmid-encoded classical virulence factors. However, vaccine strategies exploiting these targets have failed. Approximately 10%–30% of ETEC's chromosome encodes conserved hypothetical proteins with no known function. Recent work on the single gene dynamics of ETEC in humans and rhesus macaques has identified well-conserved, chromosomal genes that are likely important to the pathogenesis of ETEC. In this study, we explore the role 2 well-conserved, chromosomally encoded, and anaerobically expressed proteins play in ETEC pathogenesis. *In silico* analysis predicted them to be an inverse autotransporter (IAT) and a deubiquitinating (DUB) enzyme. By engineering a library of mutant ETEC and K12 strains, we have confirmed phenotypes important in the virulence of ETEC. When assessed via crystal violet quantification under anaerobic conditions, the IAT demonstrated biofilm formation ($P < 0.001$). Anaerobic J774 macrophage infection assays revealed the IAT to have an intracellular replication phenotype ($P < 0.05$), whereas the DUB displayed a cell death phenotype ($P < 0.05$). These findings indicate that ETEC is capable of subverting macrophages under anaerobic conditions and challenge the existing dogma on ETEC pathogenesis. Studies are ongoing to determine (1) additional pathogenesis traits, (2) host cell species differences, (3) the impact of the anaerobic macrophage microenvironment on ETEC's global transcriptome, (4) the impact of ETEC on anaerobically maintained macrophages and their surface proteins, cytokine expression, and global transcriptome.

Key Words: One Health, pathogenesis, macrophage, *Escherichia coli*, enterotoxigenic

30 - Do animal models overestimate the pathogenesis of recent H5N1 clade 2.3.4.4b?

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To better understand the differences in disease severity observed between ferrets and humans following exposure to recent H5N1 viruses, we investigated the infectivity of several H5N1 virus strains from the clade 2.3.4.4b in an in vitro model of the human respiratory system. We utilized an air-liquid interface (ALI) model with fully differentiated immortalized BCi-NS1.1 human airway epithelial (HAE) cells. Furthermore, to specifically examine airborne transmission, we employed an aerosol settling chamber system designed to assess the aerosol infectivity of influenza viruses in both in vivo and in vitro settings. Our in vivo ferret studies showed that A/Texas/37/2024 (H5N1) (A/Texas) caused severe systemic infection and mortality regardless of whether the virus was administered via the liquid or aerosol inoculation route, consistent with previous studies. However, the results from our HAE/ALI model presented a different picture. While liquid inoculation of HAE cells resulted in widespread infection at both multiplicities of infection (MOI) of 0.1 and 1 at 72 h post-inoculation, aerosol inoculation showed a reduced infection rate. Specifically, for A/Texas, at a MOI of 1, all aerosol-exposed HAE wells were positive for viral infection at 72 h post-inoculation, but at a lower MOI of 0.1, only 33% of the wells were positive. For A/British Columbia/PHL-2032/2024 (H5N1) (A/BC), at a MOI of 0.1, no wells were positive for infectious virus by aerosol inoculation while 50% of wells were positive by liquid inoculation at 72 h post-inoculation. For comparison, the control influenza virus A/California/04/2009 (H1N1) (Ca04) efficiently infected both HAE/ALI and MDCK cells under all tested conditions. Additionally, A/Texas and A/BC readily infected MDCK cells via both liquid and aerosol routes. These in vitro findings suggest that human airway epithelial cells exhibit decreased susceptibility to infection from the H5N1 HPAIV strains, particularly when the virus is introduced via aerosol at a lower dose. This reduced susceptibility in human airway cells may contribute to the milder, conjunctivitis-focused infections observed in humans compared with the severe systemic disease seen in ferrets.

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Key Words: influenza, air-liquid interface (ALI), human airway epithelial cell, aerosol infection, species-specific susceptibility

31 - Assessing *in vitro* protection from antibodies derived from adhesins against *Campylobacter*.

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Enteric diseases of food animals caused by bacterial pathogens represent a significant threat to both animal health and food safety. *Campylobacter* spp., especially *Campylobacter jejuni* and *Campylobacter coli*, are gram-negative gastrointestinal pathogens responsible for an estimated 96 million annual infections worldwide. Once ingested, *Campylobacter* colonizes the mucosal surface of the intestinal crypts, where it elicits marked inflammation, intestinal damage, and clinical symptoms. Successful colonization of *Campylobacter* is critically dependent on surface-exposed proteins known as adhesins, which mediate bacterial binding to intestinal epithelial cells and facilitate subsequent invasion. Well-characterized adhesins include JlpA, PEB1, CadF, and CapA, each of which contributes to host colonization and persistence. Despite their established role in pathogenesis of *Campylobacter*, the relative immunogenicity of adhesins and the protective function of adhesin-specific antibodies remain poorly understood. In this study, we systematically evaluated the immune responses elicited by different adhesins and compared their capacity to block *Campylobacter* adherence. Groups of BALB/c mice were immunized with individual recombinant adhesins formulated with adjuvant dmLT (a double mutant of heat-labile toxin of enterotoxigenic *E. coli*), and serum samples were collected. Antigen-specific IgG titers were measured by ELISA to assess humoral immunogenicity. Functional assays were performed to determine whether antibodies could prevent bacterial adhesion to epithelial cells, reduce invasion, and impair colonization-associated phenotypes. Our results demonstrated that mouse antibodies exhibited functional activity against the in vitro adherence of *Campylobacter jejuni* and *Campylobacter coli* strains. Moreover, among the 4 adhesins included in this study, antibodies derived from JlpA conferred significantly greater protection against *Campylobacter* adherence to host cells ($P < 0.01$). These findings highlighted adhesins as critical targets for disrupting *Campylobacter* colonization and underscored their relevance to enteric diseases. Insights from this comparative analysis informed the selection of the most immunogenic and functionally relevant adhesins, enabling the design of a polyvalent multi-epitope fusion antigen incorporating key protective epitopes against *Campylobacter*.

Key Words: *Campylobacter*, adhesin, enteric disease

32 - *Megasphaera stantonii*, a commensal bacterium, confers colonization resistance against necrotic enteritis in chickens.

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Necrotic enteritis (NE), caused by *Clostridium perfringens*, costs the global poultry industry approximately $6 billion annually. Since the withdrawal of in-feed antibiotics in the United States in 2017, the incidence of NE has increased, highlighting the urgent need for alternative mitigation strategies. This study aimed to investigate the potential of microbiome-mediated colonization resistance to NE in chickens. Twelve fast-growing, phylogenetically diverse commensal bacterial strains were isolated from the ceca of healthy feral chickens and screened for their ability to inhibit *C. perfringens* growth using an in vitro coculture competition assay. Additionally, cell-free supernatants from these bacteria were assessed for their capacity to induce host defense peptide (HDP) expression using a cell-based high-throughput luciferase reporter assay. A chicken NE model was further employed to evaluate the protective efficacy of a selected bacterium. Birds (n = 40/group) received oral administration of the bacterium on d 0 and 1, with or without sequential challenges with *Eimeria maxima* and *C. perfringens* on d 10 and 14, respectively. Cecal and ileal digesta were collected on d 4, 9, and 17 for 16S rRNA gene sequencing, and intestinal lesions were scored post-necropsy on d 17. Our results indicated that 7 of 12 commensal strains inhibited *C. perfringens* growth in vitro, with *Megasphaera stantonii* showing the greatest inhibition (~62%). Its supernatant also strongly induced HDP gene expression. In the NE model, untreated birds exhibited 52% mortality, whereas *M. stantonii* administration improved survival to 98% and significantly reduced intestinal lesion severity ($P < 0.05$). Microbiome analysis further revealed that *M. stantonii* promoted restoration of the intestinal microbiota post-infection by enriching selected short-chain fatty acid– and lactic acid–producing taxa while suppressing pathobionts. These findings demonstrate the potential of *M. stantonii* to mitigate NE through direct pathogen inhibition, enhancement of host innate immunity, and modulation of the gut microbiome.

Financial Support: This research was supported by the USDA National Institute of Food and Agriculture grant (2024-67011-42944) and the Ralph F. and Leila W. Boulware Endowment Fund.

Key Words: colonization resistance, antibiotic alternatives, necrotic enteritis, probiotics

Featured Speaker: Session 7—Animal Vaccinology Research Network Symposium

33 - Bridging the Translation Gap: How to Develop Tools for Animal Disease Control by Getting the Best of Both Worlds

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The divide between foundational disease research and clinical product development, often called the “Translation Gap” or “Valley of Death”, can be difficult to traverse. Priorities and incentive structures are often misaligned between academic and industry research, and at times are in direct opposition to each other. The highest potential for efficiently developing innovative solutions to animal health challenges is for academic and commercial researchers to work synergistically and leverage each others’ strengths. For example, mechanisms of pathogenesis and features of protective immunity are most often described by academic researchers, whereas optimization of product development, distribution, and licensure based on known microbial targets is most efficiently carried out in an industry setting. Mechanistic discovery is often not financially sustainable for industry researchers, but it is highly prioritized in academic settings and often supported by public funds. Conversely, therapeutic, diagnostic, or vaccination targets discovered in academic settings often fall into the Translation Gap without an industry partner due to a lack of regulatory, manufacturing, and distribution infrastructure. Finally, there can be misalignment on ideal designs that are scientifically optimized but prohibitively expensive to produce, or designs that are easy to develop and produce while having unseen potential for complicating results. This talk will provide perspective from both the academic research and industry research sides, and will focus on ways to facilitate partnerships that will allow us to narrow the Translation Gap for animal health products.

General Abstracts: Session 8—Bacteriology—Avian

35 - Identifying *Mycoplasma gallisepticum* primary adhesins, GapA and CrmA, receptor protein(s).

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My*coplasma gallisepticum* is an atypical bacterium that causes chronic respiratory disease in chickens. Tight adhesion to ciliated tracheal epithelium via its primary adhesins, gallisepticum adherence protein A (GapA) and cytadherence related protein A (CrmA), facilitates persistence and are major virulence factors in *M. gallisepticum*. However, the host receptor for these adhesins remains unconfirmed. Adherence to the host is the first and most crucial stage of infection; therefore, identifying the host receptor(s) is important as it provides insight into pathogenesis and tissue tropism while potentially revealing targets for intervention. We utilized protein crosslinking chemistry and mass spectrometry to identify fibronectin as a potential receptor for GapA/CrmA. We confirmed binding to fibronectin via a solid-phase binding inhibition immunoassay using GapA and CrmA specific antibodies as well as a GapA/CrmA deficient mutant strain (ΔGapA/CrmA). Previous studies have indicated the ability of *M. gallisepticum* to bind sialic acids, and we are currently investigating whether this interaction is based on specific sialic acid residues on the fibronectin glycoprotein. We also used the protein-crosslinking approach in a cell culture–based infection with wild type (WT) and the ΔGapA/CrmA *M. gallisepticum* to investigate other host protein targets for the adhesion complex. The results revealed potential involvement with other apical membrane proteins and their associated internal structural proteins, indicating complexity of the *M. gallisepticum* adhesin complex. Our future work will focus on further elucidating these interactions and their downstream effects on host responses using transcriptomic approaches.

Financial Support: USDA Award # 2022-67016-37222 and The Center of Excellence for Vaccine Research

Key Words: *Mycoplasma*, avian, attachment, molecular biology

36 - Evaluating *Campylobacter jejuni* Tn5 single mutants responding to acidity.

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C*ampylobacter jejuni* is a major foodborne pathogen worldwide. Bile acids are weak acids and influence *C. jejuni* infection, but the underlying mechanism remains unclear. This study aimed to investigate *C. jejuni* genes responding to acidic conditions and bile acids. *Campylobacter jejuni* wild type (WT) strain 81176 and its Tn5 single mutations of ΔM10, ΔM27, and ΔM60 were cultured in Brain Heart Infusion (BHI) at various acidic pH. To identify the genotype of the single Tn5 mutants, bioinformatics was applied to identify regions for designing the most unique PCR primers in the Tn5 Kanamycin. The random primers were from previous reports of other laboratories. The first round PCR products visible from mutants but not WT were purified and used for second-round PCR. The second-round PCR products with the mutant-unique DNA bands were extracted and will be Sanger-sequenced for genotyping mutations on acidity response. Differences between groups were analyzed using one-way ANOVA followed by Tukey correction using Prism 7.0 software. Differences were statistically significant if *P*-values were <0.05. *Campylobacter jejuni* WT grew to 9.3 $\log_{10}$ cfu/mL in BHI (pH 7.5), while its growth was significantly reduced in acidic BHI of 6.5, 6.0, 5.75, and 5.5 (9.0, 5.5, 0, and 0 $\log_{10}$ cfu/mL, respectively). Notably, ΔM10 mutant grew significantly better than WT in acidity BHI of pH 6.5, 6.0, and 5.75 (9.1 vs. 9.0, 7.0 vs. 5.5, and 3.9 vs. 0 $\log_{10}$ cfu/mL, respectively). Similarly, ΔM27 mutant grew significantly better than WT in increasing acidity BHI of pH 6.5, 6.0, and 5.75 (9.1 vs. 8.6, 9.0 vs. 4.8, and 7.8 vs. 0 $\log_{10}$ cfu/mL, respectively). Interestingly, ΔM60 mutant grew significantly less than WT in acidic BHI of pH 6.0 (0 vs. 2.5 $\log_{10}$ cfu/mL), but not at pH6.5 (8.9 vs. 8.3 $\log_{10}$ cfu/mL). The first round PCR genotyping showed that ΔM10 mutant PCR had unique bands of 0.7 and 1.5 kb while ΔM27 mutant had unique bands around 1.0 and 1.6 kb. After gel purification of the PCR unique bands of the mutants and the corresponding WT. Unique bands in second-round PCR were identified around 0.4 and 0.6 kb for the ΔM10 mutant while the ΔM27 mutant had a band around 0.6 bp. As contrast, the WT had no visible bands of those sizes in the first- and second-round PCR. In conclusion, Tn5 single mutants were resistant or susceptible to acidic conditions and will be genotyped on acidity response mutations. These mutants could be evaluated for their response to bile acids and for their animal infectivity.

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Key Words: single Tn5 mutant, acidity resistant and susceptible, growth, polymerase chain reaction (PCR) genotyping

37 - Chicken bile metabolites by *Lactobacillus johnsonii* attenuate *Clostridium perfringens* growth.

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Necrotic enteritis (NE) is a prevalent disease in the poultry industry caused by *Clostridium perfringens*. Although bile acid deoxycholic acid (DCA) was effective against *C. perfringens* and NE, it remains largely unclear what the mechanism was. This study aimed to investigate the effect of chicken bile (ch_bile) and *Lactobacillus johnsonii*, a bile acid metabolizing bacteria, on *C. perfringens* growth. *Clostridium perfringens* was cultured with 0.125, 0.37, and 0.75 m*M* ch_bile in BHI or with 0.37 m*M* ch_bile or ox bile (tauro- and glyco-CA) plus *L. johnsonii*. After 24 h, *C. perfringens* growth was measured by serial dilution and plated on *C. perfringens* agar plates (tryptic soy agar [TSA] with sodium thioglycolate, D-cycloserine, and sheep blood). *Clostridium perfringens* virulence of H_2S production was measured by lead acetate disc assay. All the experiments with triplicates were repeated 3 times and the representative results were presented. Differences between groups were analyzed using one-way ANOVA followed by Tukey correction using Prism 7.0 software. Differences were statistically significant if *P*-values were <0.05. In a BHI control broth, *C. perfringens* grew to 7.54 $\log_{10}$ cfu/mL after 24 h culture, while ch_bile at 0.125 m*M*, 0.37 m*M*, and 0.75 m*M* slightly decreased *C. perfringens* growth to 7.47, 7.22 ($P < 0.05$), and 7.02 ($P < 0.05$) $\log_{10}$ cfu/mL, respectively, compared with the control. In separate experiments, *C. perfringens* growth reached 7.40 $\log_{10}$ cfu/mL, while it grew to 7.49 $\log_{10}$ cfu/mL in ch_ bile, 7.40 $\log_{10}$ cfu/mL with *L. johnsonii* (Lj), and 0 $\log_{10}$ cfu/mL in ch_bile +Lj. Notably, the Lj+ch_bile group significantly inhibited *C. perfringens* growth compared with either ch_bile or Lj alone group. In addition, ch_bile +Lj group reduced *C. perfringens* H_2S production compared with strong H_2S production in the control group. Interestingly, *C. perfringens* growth reached 7.53 $\log_{10}$ cfu/mL, while *C. perfringens* grew to 7.83, 7.65, and 7.66 $\log_{10}$ cfu/mL in ox_bile, *L. johnsonii* (Lj), and ox_bile +Lj, respectively. In conclusion, we found that chicken bile, but not ox bile, metabolized by *L. johnsonii* suppressed *C. perfringens* growth and toxic H_2S production. These findings highlight the potential of metabolizing chicken bile as an alternative to control *C. perfringens* infection and chicken NE.

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Key Words: microbial metabolite, necrotic enteritis, bile acid, H_2S production

38 - Comparative transcriptomics of *Campylobacter jejuni* and *Campylobacter coli* in aerobic conditions.

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C*ampylobacter* spp. are major causes of foodborne illness worldwide. Despite a sensitivity to atmospheric oxygen levels, *Campylobacter* spp. often survive exposure to oxygen during meat processing. The genetic mechanisms behind *Campylobacter*'s aerotolerance are not well understood. The objective of this study was to conduct transcriptomic analysis using RNA-Seq Illumina technology for survival under atmospheric oxygen aerobic conditions of 2 species, *Campylobacter jejuni* (S2–20) and *Campylobacter coli* (WA333). Strains were exposed to aerobic conditions in Mueller Hinton Broth medium and triplicate samples were taken at various time points from 15 min through 15 h for RNA isolation. Ribosomal RNA depletion and RNA-Seq libraries for Illumina were prepared with NEBNext rRNA Depletion kit (Bacteria) and NEBNext Ultra II Directional RNA Library Preparation Kit for Illumina and sequenced on a NovaSeq 6000 platform. Differential gene expression under aerobic conditions at time points 15 min through 15 h was compared with microaerobic incubation at time 0. Both species showed significant changes in gene regulation under aerobic conditions; however, the *C. coli* strain differentially regulated a greater number of genes than *C. jejuni* in aerobic conditions. In both species, many genes involved in ribosome formation and flagella formation were downregulated. Genes associated with energy production and conversion were downregulated in both species during the first hour, and genes involved in oxidative phosphorylation were downregulated in both species at most time points. Upregulation of genes associated with iron transport and acquisition was noted in *C. coli* but not *C. jejuni*, suggesting species-specific differences in response to oxidative stress. In conclusion, these findings suggest that *C. jejuni* and *C. coli* utilize different strategies to cope with survival under aerobic conditions. While flagella formation was suppressed in both species, regulation of iron acquisition was enhanced in *C. coli*. To our knowledge, this is the first study to use RNA-Seq to investigate *C. coli* aerotolerancy.

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Key Words: *Campylobacter*, foodborne pathogen, aerotolerance, gene, bacteria, transcriptomics

39 - The potential of a host-specific gut bacterium in modulating chicken lung immunity.

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The poultry industry in the US stands as a vital component of the economic and dietary markets, supplying a large fraction of the global egg and meat production. Extra-intestinal diseases are serious threats to this industry and necessitate implementation of science-based solutions. We previously characterized the effects of a host-specific gut microbe, segmented filamentous bacteria (SFB), that is vital to early immune maturation and not sufficiently acquired in commercial hatching settings. Additionally, prior work has shown potential systemic immune benefits of SFB. Here, we investigated the ability of SFB to bolster the innate immune capability of the lung environment in chickens through scRNA sequencing and qPCR. Day-old Dekalb White layers were inoculated with either PBS or an SFB-enriched inoculum of ~10^5 gene copies. At 3 weeks post-inoculation, 6 birds per group were euthanized, their hearts perfused with RPMI-1640, and lung tissue was excised. The tissue was then dissociated into single cells. Cells were enumerated and pooled in groups of 3 birds per pool and processed through Chromium 10x scRNA sequencing. Data were subsequently parsed by the ISU Core Facility. Cells were further categorized into clusters at a resolution of 1.0, with genes identified from the literature employed to categorize clusters into cell types and identify differentially expressed genes (DEGs) between groups. The DEGs were then further investigated by qPCR of lung tissues collected and frozen from SFB birds at various time points. While cell populations did not significantly vary between groups, 2 cell clusters identified as expressing many interferon-activated antiviral response genes were underrepresented in SFB birds. However, in these 2 clusters, DEGs identified between groups revealed a greater innate immune capability in the SFB birds, including greater expression of BG1, PPR136, BTN3A3L2, and JAKMIP1, and lower expression of SLC7A7. These significant transcriptional alterations in the chicken lung treated indicate a lung-axis immune modulation by SFB. This extends beyond the previously emphasized increases in IgA and Th17 cells. These specific DEGs are known for their implication in resistance to viruses and promotion of autophagy, driving T-cells to an effector memory phenotype, or inducing heightened localized expression of IL-1β and TNFa. Further study will include in vitro or ex vivo virucidal assays justifying eventual in vivo challenge.

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Key Words: gut-lung axis, segmented filamentous bacteria, chicken, single-cell sequencing

40 - Impact of a segmented filamentous bacteria treatment on gut microbiota and function.

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We have demonstrated that providing newly hatched chicks with avian-specific segmented filamentous bacteria (SFB) enhances gut maturation and increases resistance to pathogens relevant to human and poultry. Our treatment involved SFB spores derived from Dekalb White Leghorn (D-SFB), which was tested in both layers and broilers. The D-SFB treatment when provided orally induced gut immune maturation. However, its effect on gut microbiota remains unknown. This study evaluated the D-SFB on gut microbiota composition and its function in Lohmann Select Leghorn (LSL), a widely used genetic line in commercial farms in the US. Day-old commercial LSL chicks were treated with either D-SFB and/or PBS (CON). Chicks were euthanized weekly (n = 6/group/time point) for a month to collect ileal mucosal scrapings and contents (ileum, cecum, and colon). DNA was extracted from these samples and was subjected to 16S Single Stage Mi-seq Illumina sequencing. The changes in the microbiota composition were assessed via PCOA plots, taxonomic bar plots, and microbial enriched and depletion plots. Additionally, functional pathways were analyzed via predicted pathway heatmaps. The PCOA plot indicated high Bray–Curtis dissimilarity among the bacterial community present in the groups. Specifically, short-chain fatty acid (SCFA) (butyrate)-producing species were enriched in the SFB group. Bacteria from the genus *Agathobaculum butyriciproducens* was increased based on relative frequency. Functional pathways tied to anti-inflammatory protocatechuate acid (Protocatechuarte-Ortho-Clevage-Pwy), amino acid biosynthesis (Complete-Aro-Pwy), antioxidant defense (Nonoxipent-Pwy), tight junction induction (Metsyn-Pwy), and SCFA production (Fermentation-Pwy) were enriched in D-SFB-treated birds. Overall, the D-SFB treatment altered the bacterial species present in the microbiota by enhancing the relative frequency of SCFA-producing bacteria and induced functional homeostatic associated pathways. By establishing a healthy microbiota, the treatment has potential to increase health and productivity, which will benefit the poultry industry.

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Key Words: segmented filamentous bacteria, microbiota, poultry, short-chain fatty acid

General Abstracts: Session 9—Antimicrobial Use

41 - Impact of exposing bees to antibiotic residues in pollen.

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The direct exposure of honey bees to antimicrobials used to treat and prevent diseases in orchards and other crops impacts honey bee behavior and elevates mortality. However, the impact of antimicrobial pollution from pollen on pollinator health is less studied. Honey and pollen are reliable bioindicators of environmental AMR. Naturally occurring antimicrobial concentrations in pollen are far below the recommended treatment dose for bees (450,000 ppb tetracycline). Our study aims to infer the effect of directly exposing bees to tetracycline concentrations reported in pollen in nature. Results will allow to model impact when bees are exposed to realistic antimicrobial levels found in the environment. In this controlled cage experiment, *Apis mellifera* honey bees (referred to as bees) were fed pollen containing a low dose (11 ppb) and a high dose (280 ppb) of tetracycline previously reported in pollen collected from nature. For comparison, a control group was included in which pollen without antibiotics was offered to the bees. Bee samples were collected every week for a period of 28 d. Bee gut samples were processed, and the relative concentrations of antimicrobial resistance genes (ARGs) and mobile genetic elements (MGEs) were determined using quantitative PCR. Bees were screened for the presence of pathogenic and opportunistic organisms using culture methods. The mortality rates in all groups were recorded. Gut samples from both live and dead bees from all time points were processed, and metagenomic sequencing was performed to compare the microbiome and resistome of bees in the 3 different treatment groups. Preliminary analysis revealed that bees exposed to antibiotics in pollen under controlled conditions have a significantly lower survival rate compared with the bees in the control group. Moreover, bees exposed to the highest dose of antibiotics had a higher relative quantification of ARGs than bees exposed to the lowest dose over time. The results from the metagenomic data will be presented at the conference. The use of antimicrobials in crops or agricultural settings may lead to the presence of antimicrobial residues in pollen. When bees were exposed to doses of antibiotic residues reported in pollen in nature under controlled conditions, it led to higher mortality rates. This result demonstrates that even low, environmentally realistic doses of antimicrobials present in pollen are sufficient to impact bee health.

Financial Support: This research is funded by the Animal Health & Disease Research Funds, Research and Graduate Studies, Auburn University

Key Words: honey bee, antibiotic residue, antimicrobial resistance

42 - Using the *AMR Visualizer* tool to track antimicrobial resistance (AMR) trends in small animal veterinary practice.

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Antimicrobial resistance (AMR) is a growing concern in both veterinary and human medicine, with significant public health implications. Cumulative antibiograms, when used effectively, help combat AMR by guiding empirical treatments and monitoring resistance patterns within populations. However, generating cumulative antibiograms is often time-consuming for laboratories, emphasizing the need for veterinary-specific, efficient, and accurate tools. This study aims to use *AMR Visualizer*, an online platform under development, to efficiently generate accurate cumulative antibiograms and track resistance trends among feline and canine skin and uropathogens collected over a decade (2015–2024) at Purdue Small Animal Veterinary Hospital, West Lafayette, Indiana. Following limited data preparation and tool customization training, the platform's features were assessed using Docker Desktop and 2024 antimicrobial susceptibility data. Isolates originated from urine (via free catch, catheter, or cystocentesis), uroliths, and swab sites (skin, abscess, wound, lesion, incision). The dataset included 6,259 samples—5,186 from dogs (with *Staphylococcus pseudintermedius* as the most common bacterium, n = 1,074) and 1,073 from cats (with *Escherichia coli* as the most common, n = 306). Among canine samples, about 16% of test results showed resistance to at least one antibiotic, including amikacin, ampicillin, doxycycline, minocycline, penicillin, tetracycline, trimethoprim/sulfamethoxazole, amoxicillin/clavulanic acid, cefazolin, cefovecin, cefpodoxime, cephalothin, clindamycin, enrofloxacin, erythromycin, marbofloxacin, gentamicin, bacitracin, chloramphenicol, rifampin, moxifloxacin, ofloxacin, and oxytetracycline. About 10% of feline samples were resistant to at least one antibiotic tested (ampicillin, tetracycline, cephalexin, trimethoprim/sulfamethoxazole, gentamicin). Data from 2015 to 2024 are being analyzed to identify trends in resistance over the decade, and preliminary results will be presented in this conference poster. With minimal data processing, *AMR Visualizer* allowed the interdisciplinary team to identify improvements for data reporting and management that could be adopted by animal hospitals, clinics, and diagnostic laboratories. These results may guide AMR control efforts, provide valuable surveillance data for public health professionals, and help evaluate the platform's utility as a quick, practical clinical guidance aid.

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Key Words: antimicrobial resistance (AMR), One Health, cumulative antibiogram, resistance trend, susceptibility testing

43 - Genomic insights into the association of antimicrobial resistance and mobile genetic elements in coagulase-negative staphylococci across hosts.

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Coagulase-negative *Staphylococcus* species (CoNS) are ubiquitous, opportunistic, zoonotic pathogens with extensive horizontal gene transfer (HGT) which is the primary mechanism driving AMR spread via mobile genetic elements (MGE). Understanding AMR dynamics in CoNS is essential for developing strategies to mitigate its spread. Our objective is to genetically characterize the abundance, diversity, and distribution of MGE and associated AMR in CoNS isolated from human and animal hosts, and to predict their mobility based on HGT-associated genes. To this end, we used a total of 1,582 publicly available CoNS genomes, 32% of the genomes analyzed were from animal host and 68% from human hosts. These genomes represent 43 *Staphylococcus* species with 51% isolated from both hosts. We used Prokka for genome annotations, ABRicate with the CARD database to identify AMG, MobileElementFinder to identify insertion sequences (IS), composite transposons (cnTn), and unit transposons (Tn), SCCmecFinder to identify SCCmec genomic islands, and Plasforest for plasmid predictions. Preliminary co-localization analysis was determined by the coordinates of the AMR being within the coordinate range of the MGE. We used Spearman correlation to calculate the association between host, AMR class, MGE type, and CoNS. No clear trend was observed among species or hosts when grouped by resistance class. Plasmids were the most prevalent MGE (92%), followed by insertion sequences (56%), cnTn (28%), and Tn (15%). Nearly all genomes (99%) carried an SCCmec element. *Staphylococcus simulans*, *S. warneri*, *S. capitis*, *S. hominis*, *S. epidermidis*, and *S. haemolyticus* were the top species represented in genomes isolated from both hosts. Preliminary analysis showed penam, aminoglycoside, lincosamide, and nucleoside resistance co-localized with plasmids, IS, and Tn. Tetracycline, macrolide, fluoroquinolone, diaminopyrimidine, and mupirocin resistance co-localized with plasmids and IS. Phenicol resistance co-localized with plasmids and Tn. Although SCCmec was identified in 99% of genomes, 75% of these elements were reported as unidentified types, and only 4.4% contained the ccrABC genes required for mobility. These findings indicate that plasmids and IS have the most diverse AMR associations in CoNS across human and animal reservoirs. Further analysis is underway to characterize plasmid types and associated AMR genes as well as predict mobility potential of these MGE to understand the dynamics of AMR dissemination in CoNS.

Key Words: mobile genetic element (MGE), antimicrobial resistance (AMR), coagulase-negative staphylococci, zoonotic

44 - Identification of a novel telithromycin resistance mechanism in *Campylobacter*.

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C*ampylobacter* is a significant foodborne pathogen and is increasingly resistant to medically important antibiotics. As an indicator for its importance, the Centers for Disease Control and Prevention (CDC) lists antibiotic-resistant *Campylobacter* as a serious public health threat. Over the years, whole-genome sequencing (WGS) has become a powerful tool for the detection and surveillance of antibiotic-resistant *Campylobacter*. Previous studies demonstrated a near 100% consistency between WGS data and phenotypical resistance for several antibiotics such as quinolones, macrolides, and tetracyclines. However, the predictive power of WGS was significantly reduced with telithromycin (a ketolide antibiotic), for which several *Campylobacter* isolates exhibited phenotypical resistance but lacked known resistance determinants, suggesting previously unknown genetic mechanisms. We designated these isolates as phenotypically positive but genotypically negative (PhPGN) for telithromycin resistance. The objective of this study was to determine the genetic basis underlying the PhPGN phenotype. Genomic DNA purified from 8 PhPGN isolates was individually transferred into *C. jejuni* NCTC 11168 recipient strain via natural transformation, followed by selection on telithromycin plates. Nine telithromycin-resistant transformants (MIC ≥ 8 µg/mL) were then subjected to WGS and comparative analysis with the recipient genome. This comparison identified single nucleotide polymorphisms (SNPs) in a genomic region spanning 9 ribosomal protein genes were transferred from the PhPGN isolates to the NCTC 11168 transformants. Further transformation experiments revealed that only the G257A mutation in the *RplV*/L22 gene was consistently present in the telithromycin-resistant transformants. To verify the role of this specific mutation in telithromycin resistance, we employed CRISPR/CjCas9-mediated gene editing to introduce the G257A mutation into NCTC 11168, which resulted in an 8- to 16-fold increase in the telithromycin MIC. Together, these results identify the G257A mutation in the *RplV* gene as a novel mechanism for telithromycin resistance, and this finding will facilitate WGS-based surveillance of antibiotic-resistant *Campylobacter*.

Key Words: *Campylobacter*, food safety, telithromycin resistance, antimicrobial resistance, gene editing

45 - Removal of bacterial biofilm matrix using novel anti-biofilm compounds to enhance the antimicrobial susceptibility of bacteria causing chronic infections.

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Bovine respiratory disease, commonly known as “shipping fever” disease, is endemic across the Midwest and West Coast. The infectious agents responsible for this disease are *Histophilus somni*, *Pasteurella multocida*, and *Mannheimia haemolytica*, which create a polymicrobial biofilm extracellular matrix that cannot be penetrated by standard antimicrobials. Our objective was to identify compounds that can remove the biofilm extracellular matrix (ECM) produced by these bacteria, so removal of the ECM may lower the antibiotic MIC and be highly desirable. Initially, 5,000 compounds (0–100 µ*M*) from the Virginia Tech Center for Drug Discovery Screening Laboratory were screened to remove the *Histophilus somni* biofilm. Biofilms formed in 96-well plates were stained with 0.1% crystal violet, the ECM solubilized with acetic acid, and the absorption at 590 nm determined; sodium hypochlorite was used as a control. Cytotoxicity of the compounds for bovine epithelial cells, pulmonary endothelial cells, or endometrial cells was determined by visual rounding up of the cells. The MIC of appropriate antibiotics was established for planktonic bacteria. The minimum biofilm eradication concentration (MBEC) assay was used to determine the MIC of antibiotics for bacteria in a biofilm. The MBEC was repeated with the anti-biofilm compound included in the medium with antibiotics. Statistical significance was determined for triplicate values by Student’s *t*-test using InStat Prism10. Two compounds, A and B, were effective at removing more than 50% of the *H. somni* ECM. The compounds were not toxic for any of the bovine cell lines tested at 100 µ*M*. Compound B was most effective at removing the *H. somni* biofilm, but compound A was more effective at removing the ECM of *Pasteurella multocida* and *Mannheimia haemolytica.* The MIC and MBEC assays confirmed that the bacteria were more resistant to antibiotics when in a biofilm. Compound A is currently being tested for lowering the antibiotic MIC for *P. multocida* in a biofilm. Two compounds were identified that can remove the biofilm ECM and reduce the antibiotic MIC of several pathogens. However, each compound was somewhat species-specific, indicating ECM structural differences. Future work will focus on determining if biofilms in cell culture can be removed by treatment with anti-biofilm compounds, and the mechanism of ECM removal.

Financial Support: Foundation for Food & Agriculture and Morris Animal Foundation

Key Words: bovine respiratory disease, *Mannheimia haemolytica*, *Pasteurella multocida*, *Histophilus somni*, minimum inhibition concentration, minimum biofilm eradication

46 - Copper sulfate treatment and effects on biofiltration in saltwater aquaria.

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Copper sulfate ($CuSO_4$) is a widely used quarantine treatment for saltwater aquarium fishes due to its antibacterial, antifungal, and antiparasitic properties. However, it may negatively impact microbes essential for nitrification and biofiltration, processes critical to water quality and animal health. Despite its broad application, the susceptibility of these microbes to $CuSO_4$, and whether its impact differs between established and naïve systems, remains unclear. Water from 2 saltwater aquarium systems was sampled longitudinally before, during, and after completion of $CuSO_4$ treatments. One system had never been treated with $CuSO_4$ (naïve), whereas the other had been running for over a decade with periodic $CuSO_4$ treatments (established). The DNA was isolated using the DNeasy PowerSoil Pro Kit (Qiagen). Shotgun sequencing libraries (n = 243) were prepared using the Illumina DNA Prep Kit and sequenced on an Illumina NovaSeq 6000 platform. Raw sequencing data underwent adapter removal and quality filtering using Trimmomatic. High-quality reads were classified with Kraken2 using NCBI's 'core_nt' database. Statistical analyses were carried out in RStudio (v4.5.1). The relationship between Shannon diversity and copper concentration in water (mg/L) was explored using partial Spearman's rank correlation, controlling for sampling date. Microbial community composition differences were tested with PERMANOVA. Overall microbial diversity declined with increasing copper levels in both systems (PCOR: R = −0.3, R = −0.27, $P = 0.01$). Notably, the diversity of nitrifying taxa was particularly negatively correlated with copper concentration in the naïve system (PCOR: R = −0.233, $P = 0.04$). The effect of $CuSO_4$ on community composition did not differ between systems (PERMANOVA: $R^2 = 0.9\%$, $P = 0.78$), but copper concentration did impact overall composition (PERMANOVA: $R^2 = 4.1\%$, $P < 0.01$). Interestingly, the interaction between system type and copper concentration had a significant effect on the composition of the nitrifying community (PERMANOVA: $R^2 = 7.3\%$, $P < 0.01$). These findings suggest that while both copper concentration in water and system type affect overall microbial communities, the effect of copper on nitrifying taxa may be more pronounced in a naïve system. Future research will quantify the impact of $CuSO_4$ on key nitrifying taxa. These results offer insight into balancing parasite management while maintaining microbial communities essential to aquatic ecosystem health.

Financial Support: Funding for this project was provided by Texas A&M University and John G. Shedd Aquarium. Portions of this research were conducted with the advanced computing resources provided by Texas A&M High Performance Research Computing.

Key Words: copper sulfate, saltwater aquaria, microbiome, shotgun metagenomics

General Abstracts: Session 10—Epidemiology 2

47 - Optimizing vector capture methods on beef cow-calf farms in Mississippi.

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Bovine anaplasmosis (BA), caused by infection with *Anaplasma marginale* (AM), is a widespread hemolytic disease of cattle with an economic impact estimated at $660 per clinically affected head. Control of BA through medicated feeds requires diagnosis of active infection. Available diagnostic methods are expensive, insensitive, or time-consuming when applied to low prevalence herds. We aim to evaluate the utility of a proxy-testing strategy using vectors (*Dermacentor variabilis* [DV] ticks and tabanids) to categorize BA herd seroprevalence. The relationship between AM in free-ranging vectors and anti-msp5 antibody in cattle has not been described. Our objectives were to evaluate the feasibility of on-farm vector capture methods and to optimize capture methodology. From April–October 2024, ticks and tabanids were collected biweekly from 5 cow-calf farms in Mississippi using drag cloths and H-traps. The proportion of successful captures (defined as ≥1 target per capture attempt) per time period, species, and trapping environment were evaluated. Thirty-three DV and 110 tabanids were collected. Tabanids were captured at all sites with a trap success rate of 31.7% (Clopper-Pearson 95% CI 25.5–38.5%) while DV were captured at 3/5 sites with a drag success rate of 9.4% (Clopper-Pearson 95% CI 5.4–14.7%). Tabanid captures were highest from May–September while DV captures were highest from May–August. Pearson's chi-squared test demonstrated no significant difference in tabanid capture success among trap environments ($P = 0.259$). These results suggest tabanids are favorable candidates for proxy test development. Future work will involve correlating detection of AM in tabanids with seroprevalence of nearby cattle herds.

Financial Support: This work is supported by the U.S. Department of Agriculture, USDA-ARS grant number: 58-6064-3-017 and the Mississippi State University College of Veterinary Medicine House Office Clinical Research Grant Program.

Key Words: vector, Tabanidae, *Anaplasma marginale*, tick, horsefly, diagnostics

48 - The relationship between fecal microbiome and liver abscesses in beef-dairy crossbred cattle.

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Liver abscesses continue to be a problem for the beef industry in the United States, primarily originating from beef-dairy crossbred cattle. It is estimated that approximately 60% of livers in slaughterhouse production lines show some evidence of having been damaged by liver abscesses, with 17% having active liver abscesses. Recent estimates suggest that liver abscesses result in approximately $260 million in production and product losses to the beef industry each year. We conducted a study to investigate fecal microbial communities as predictors of liver abscesses, as well as the impact of days on feed, comparing those that reach their target weight earlier and those who spend 30 more days on feed. Fecal samples were collected from 120 cattle housed in 4 pens at a commercial feedlot at arrival and just before slaughter. Fecal microbial communities were characterized using 16S rRNA gene sequencing. Microbial communities changed over time as indicated by significant differences in richness ($P < 0.001$) and Shannon's diversity ($P < 0.01$). A total of 86% of cattle had liver abscesses at slaughter, 48% with severe (A+) abscesses. No significant differences were found in α diversity of microbial communities in association with different liver abscess scores.

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Key Words: bovine, cattle, liver abscess, microbiome, bovine gut microbiota

49 - Microbial profiling of *Mycoplasma bovis* associated with polymicrobial infections in feedlot cattle mortalities.

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Complex polymicrobial diseases such as bovine respiratory disease (BRD), arthritis, and liver abscesses are leading causes of cattle mortalities in North American feedlots. To have a better understanding of these diseases, it is important to view the animal as a whole. This project is designed to determine how *Mycoplasma bovis* might be involved with multiple disease states within individual animals. Genome Sequencing (WGS) is utilized to gain insights into the phylogenetics, as well as the distribution of virulence and antimicrobial resistance genes in *M. bovis* isolated from different body locations. Samples were taken from the following 6 locations: heart, liver, lung, nasopharynx, knee, and gut-associated lymphoid tissue (GALT) from 39 feedlot cattle mortalities in the Texas panhandle region. *Mycoplasma* was isolated (n = 70) on PPLO Amp plates, and a single colony was subcultured in PPLO Amp broth. PacBio HMW NanoBind kits were used to extract DNA from isolates. The native barcoding kit 24 v14 was used for library prep, and whole-genome sequencing was performed using GridION flow cells (version R10), with 24 isolates per flow cell. For bioinformatics, Flye was utilized to produce assemblies and Medaka was utilized for polishing. The presence of single nucleotide polymorphisms (SNPs) in the core genome was inferred using Snippy, and data were used to create a phylogenetic tree. The Virulence Factor Database was used with ABRicate to search assemblies for virulence genes, MEGARes and Resfinder were used to search for antimicrobial resistance genes. Scoary2 was used to evaluate the association of virulence genes with metadata points. This study found that *Mycoplasma bovis* in multi-site samples were similar at a strain level. Genes coding for variable surface proteins were prevalent in our samples. We did not find genes linked to antimicrobial resistance, which may be due to the computational tools used in this study. This study has shown the importance of viewing *Mycoplasma bovis* within a broader scope to determine how the pathogen interacts and is involved with different disease states. Phylogenetics, virulence genes, and antimicrobial resistance data have provided us insight to *Mycoplasma bovis* throughout multiple sample sites.

Financial Support: This research was funded by Texas A&M AgriLife Research, Texas Cattle Feeders Association, and USDA Hatch Funding NC 1192

Key Words: polymicrobial, beef cattle, *Mycoplasma bovis*, whole-genome sequencing, bovine respiratory disease (BRD)

50 - Identifying protective and disease risk-associated fecal microbiomes in neonatal calves.

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Preweaned calf diarrhea presents a significant challenge for both beef and dairy operations and leads to substantial economic losses in agriculture. This study explores the fecal microbial communities that determine susceptibility and resistance to gastrointestinal disease in neonatal calves via 16S rRNA amplicon sequencing. Ninety-eight preweaned crossbred beef-on-dairy calves housed individually in hutches were enrolled in a cross-sectional study at a large commercial calf-raising facility in the High Plains region of the United States. Fecal samples were collected into sterile centrifuge tubes using standardized, non-invasive methods (i.e., free catch, floor swab, ground collection) and stored at −80°C until processing. DNA was extracted via DNeasy PowerSoil Pro Kits and subjected to 16S rRNA gene amplicon sequencing targeting the V3–V4 hypervariable region via a Illumina MiSeq analyzer. Sequence data were processed through DADA2 for quality filtering and ASV inference, followed by taxonomic classification using the SILVA database. Statistical analyses included α (Shannon, ACE, Chao1, Fisher, InvSimpson; Wilcoxon-Rank and Kruskal-Wallis tests) and β (Jaccard, Bray–Curtis, Aitchison; PERMANOVA) diversity comparisons, and differential abundance testing method (ALDEx2) to identify taxa or community patterns that predict disease status (FDR < 0.05). Results from the α diversity analysis showed no significant differences based on sex, health status, collection method, or origin. Beta diversity analyses using Jaccard, Bray–Curtis, and Aitchison metrics showed considerable overlap across sex, health status, collection method, and origin, with no clear clustering. However, samples from Texas origin and those with higher fecal diarrhea scores displayed slightly greater dispersion. Overall, none of the variables independently influenced α or β diversity, although there may still be differences within groups. Limitations of the study include the cross-sectional design of calf raising and a limited sample size, which may have affected the ability to detect statistically significant effects. Future studies including more calves to better assess multivariable outcomes are warranted.

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51 - Factors affecting the decision of veterinary students to pursue a career in food animal practice: A survey among veterinary students.

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The purpose of this study was to identify drivers for veterinary students to pursue a career in food animal veterinary medicine (FAVM). A pre-survey among veterinary students at the University of California Davis (UCD) helped identify latent constructs associated with students' attitudes toward FAVM. A questionnaire with multiple items per construct (scales) was developed and distributed to all veterinary students at Kansas State University (KSU) for validation and then UCD and Western University of Health Sciences College of Veterinary Medicine (WU) in spring 2024. Graded-response item response theory (IRT) models were used to evaluate and validate the scales (Likert-type, from strongly disagree to strongly agree). Logistic regression models were fitted to each school to explore the relationship between each construct as the predictor and interest versus no interest in a career in FAVM as the binary outcome, controlling for confounders (year in veterinary school, size of place of origin, and experience with food animals [FA] after high school). The initial 5 constructs were (1) income, job security, and job opportunities; (2) lifestyle, work conditions, clients, and industry; (3) ethics, e.g., improving animal care/welfare, client education, purpose, and public opinion; (4) interest in population medicine/epidemiology and species; and (5) experience, exposures, and mentoring. Constructs 1 through 4 were then split into (a) importance of construct to oneself (motivation) and (b) how FAVM is viewed with respect to the construct (perception). Higher odds of interest in FAVM were associated at all 3 schools with a favorable perception of lifestyle and work conditions of FA veterinarians (KSU, $P = 0.001$; UCD, $P < 0.001$; WU, $P = 0.005$), feeling well educated in FAVM (WU, $P = 0.003$), prior experience with livestock after high school (KSU, $P = 0.0002$), and year in veterinary school (year 4 vs. year 1 had lower odds of interest in FAVM [WU, $P = 0.03$; KSU, $P = 0.04$]). The motivation and perceptions of students toward FAVM are shaped by prior experiences, their view of the work of FA veterinarians, and their desire for mentorship. Results are important for recruitment efforts of veterinary colleges to ultimately offset the current shortage of FA veterinarians in the US.

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Key Words: food animal medicine, shortage, predictor, veterinary student, item response theory

52 - Transforming livestock information systems through standardized and interoperable data for disease control and One Health.

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Ethiopia, home to Africa's largest livestock population, is implementing a transformative digital reform to overcome long-standing fragmentation in livestock data systems that has hindered disease control, traceability, and One Health coordination. Historically, platforms for animal identification, health surveillance, genetics, and market data operated in silos without shared standards or interoperability, limiting evidence-based responses to transboundary diseases, zoonoses, and climate risks. To address this fragmentation, the Ministry of Agriculture, with technical support from Development Gateway, Livestock Improvement Corporation, International Center for Tropical Agriculture launched a national livestock information system reform under the "A Livestock Information Vision for Ethiopia (aLIVE)" program. The reform aims to establish a standardized, governed, and interoperable LIS as a core component of Ethiopia's national digital infrastructure, strengthening evidence-based livestock management, traceability, and One Health coordination. It is anchored on 3 foundational pillars: the Livestock Data Standard (LDS), which harmonizes core data elements and formats based on international frameworks (ICAR) adapted to Ethiopia's context; the Livestock Data Governance (LDG) Framework, which defines institutional mandates, stewardship roles, access protocols, and quality controls aligned with national digital strategies; and the Interoperability Framework, which enables real-time and secure data exchange through shared APIs, metadata registries, and semantic alignment. Together, these components form a unified, nationally owned digital ecosystem that strengthens data integrity, accountability, and collaboration across livestock systems. The findings demonstrate improved data quality, consistency, and comparability across systems. The LDS is standardizing animal ID, health, and genetics data, while the LDG is institutionalizing responsible sharing and accountability. Together, these efforts enable real-time exchange, stronger disease surveillance, and evidence-based policymaking. In conclusion, the launch of integration pilots and sustainability planning marks a foundational shift in Ethiopia's livestock data ecosystem. The LIS is agile, scalable, and resilient—strengthening disease surveillance, traceability, genetic improvement, and policy responsiveness—and offers a replicable model for transforming livestock systems across low- and middle-income countries.

Financial Support: This work was made possible through the significant technical support, leadership, and coordination of Development Gateway (DG), with immense contributions from the Livestock Improvement Corporation (LIC) in designing the roadmap, and financial support from the Bill & Melinda Gates Foundation. The valuable contributions of government (particularly the staff of the Ministry of Agriculture), research, private, and development partners in the livestock sector are highly acknowledged.

Key Words: livestock information system, data standard, data governance, interoperability, One Health, digital transformation, Ethiopia

General Abstracts: Session 11—Swine Influenza 1

53 - The effect of swine influenza on fattening pig productivity on Japanese commercial farms.

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Swine influenza is a respiratory disease caused by influenza A viruses of swine (IAV-S), which is one of the major respiratory pathogens affecting pigs in large parts of the world. The virus typically spreads rapidly through a susceptible population due to its high transmissibility and the short incubation period that precedes virus shedding. Infection results in clinical signs of fever and coughing, which leads to decreasing fattening pig productivity. However, no study in Japan has yet quantified the impacts of IAV-S on fattening pig productivity. Therefore, the objective of this study was to compare the fattening pig productivity among different IAV-S statuses on 11 Japanese commercial farms. This study was conducted on 11 Japanese farrow-to-finish commercial farms. Active surveillance was performed in July 2024, October 2024, and January 2025 to collect nasal swab samples that were randomly collected from 30 weaned pigs by veterinarians on each farm. The samples were examined for the presence of IAV-S by real-time RT-PCR. Farms were classified into 3 groups based on the frequency of IAV-S detection. Productivity measurements used were post-weaning mortality, age at slaughter, vaccine and medication costs per market pig (VMC), and feed conversion ratio (FCR). Of the 11 farms, 5 farms had at least one IAV-S positive sample in all 3 surveillance, defined as high IAV-S farms, 4 farms had at least one IAV-S positive sample in 1 or 2 surveillance, defined as low IAV-S farms, and 2 farms had no IAV-S positive sample in all 3 surveillance, defined as IAV-S-free farms. High IAV-S farms had higher post-weaning mortality than low IAV-S farms and IAV-S-free farms (mean: 13.5% vs. 4.8% and 6.5%). Additionally, high IAV-S farms had higher age at slaughter than low IAV-S farms and IAV-S-free farms (mean: 190.4 d vs. 176.5 d and 184.9 d). No numeral difference was found in VMC and FCR among IAV-S status. In conclusion, this study quantified the effect of IAV-S on fattening pig productivity on Japanese commercial farms. Farms with high IAV-S had lower productivity than other farms. This indicates that controlling and preventing IAV-S is important for improving fattening pig productivity.

Key Words: swine influenza, productivity, commercial farm, fattening pig

54 - Longitudinal surveillance of influenza A virus at the human-swine interface in Midwest swine farms in the United States.

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Active surveillance is needed to detect emerging strains of influenza A virus (IAV) in swine and assess threats to public health. This study characterized detection of IAV in farm employees, as well as in pre-wean and nursery pigs at the human-swine interface. Active surveillance was conducted in 30 farms, targeting employees, pre-wean piglets at 15 breeding herds, and piglets in 15 downstream nurseries in the Midwest US, for a minimum of 12 mo from April 2022 to August 2025. Farm employees were surveyed to assess their age, years of experience working with swine, gender, and IAV vaccination status within the prior 12 mo. For at least 1 year, farm employees self-collected a nasal swab each month. Swine samples included 10 udder wipes and 15 nasal wipes from the sow farms and 15 nasal wipes and 4 oral fluids from the nurseries each month. Detection of IAV was determined by a commercial PCR from ThermoFisher Scientific and genetic sequencing was conducted at Iowa State University. Descriptive statistical analyses were performed to evaluate farm employee demographic data and determine the frequency of IAV RNA detection using Excel and R Studio software version 4.1.1. The project enrolled 189 farm employees, and survey results indicated 66.32% male and 33.68% female, 39.38% were included in the 26–35 age category, 79.27% worked with swine <10 years, and 43.01% received the human seasonal IAV vaccine the previous year. During the 3-year period, 7 human nasal swabs (0.47%; n = 1,484) tested IAV-positive with high Ct values from 4 farms. Five hemagglutinin sequences were generated from human samples and revealed 2 H3N2 human seasonal IAV from 22 to 23, 1 H1N1 pandemic from 2025, and 2 H1 1A.1.1.3 swine-lineage IAV from 24 to 25. Detection of IAV in swine by RT-rtPCR was 29.8% (n = 7,665) positive across all farms. Approximately 25.09% (n = 4,400) and 36.20% (n = 3,265) were IAV positive in pre-wean and nursery pig samples, respectively. Four subtypes (H1N1, H1N2, H3N1, and H3N2) were detected in swine samples from different farms with some mixed subtypes. Hemagglutinin sequencing also detected contemporary swine-lineage IAV that included H1 1A.1.1.3, 1A.3.3.3, 1B.2.2.2, 1B.2.1, and H3 3.1990.4, 3.1990.4.a, and 3.2010.1. Human seasonal IAV were not detected in swine during the surveillance period. Active surveillance involving both humans and swine is critical to understanding IAV circulation at the human-swine interface and its impact on bidirectional transmission.

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Key Words: influenza, swine, human interface

55 - Hemagglutination inhibition antibody profiles against multi-lineages of co-circulating influenza A virus in cohorts of pigs from weaning to near market.

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Influenza A virus (IAV) is a major pathogen in swine, where co-circulation of hemagglutinin (HA) lineages contributes to genetic diversity and reassortment. Population-level immunity may influence the occurrence of these lineages, yet little is known about how antibody levels vary across production stages and how immunity impacts lineage persistence. This study evaluated hemagglutination inhibition (HI) titers against 4 HA lineages circulating in 3 cohorts of pigs from weaning to near market. Serum and nasal swab samples were collected in 3 sow farms from piglets before weaning at ~3 weeks of age (woa), and from 3 cohorts of pigs after weaning in the nursery (5 and 8 woa) and the finishing stage (~16 woa). Piglets from the 3 sow farms were commingled at weaning to form a cohort. All samples were tested by RT-qPCR targeting the M gene, and HI assays were performed against 4 HA lineages (H1A.1.1.3, H1A.3.3.3-c3, H3.2010.1, H3.1990.4.a). Linear mixed-effects models with cohort as a random effect evaluated associations between HI titers and viral RNA detection. HI titers were highest in piglets before weaning, with geometric mean titers (GMT) >6 $\log_2$ (= 80) for all 4 lineages and >80% seropositivity, consistent with maternal antibody transfer. Titers decreased after weaning, averaging ~6 $\log_2$ (80) at the first nursery sampling and ~5 $\log_2$ (40) at the second, with reduced seropositivity for H1A.1.1.3 and H3.1990.4.a. In finishing, titers were lowest overall (~4.5 $\log_2$; 20), and seropositivity fell below 40% for some lineages. Differences between H1 and H3 responses indicated lineage-specific immune dynamics. Mixed-effects modeling showed that higher HI titers were generally associated with higher Ct values (lower viral load), although stage- and lineage-specific interactions were significant. In summary, HI titers were highest in younger pigs and more variable in older pigs. We identified lineage-specific differences between H1 and H3 that underscore the complex role of immunity in shaping viral circulation. While higher titers were linked to reduced viral detection, antibodies alone did not prevent infection or co-circulation. Integrating serological data with genomic surveillance is essential to understand IAV transmission and evolution in swine herds.

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Key Words: hemagglutination, inhibition, influenza, swine, immunity

56 - Dynamics of influenza virus A infection across wean-to-finish herds in the United States Midwest.

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Influenza A virus (IAV) is endemic in swine, causing economic losses and posing public health risks. Data on prevalence, incidence, and infection dynamics in growing pigs, especially wean-to-finish herds, are limited. A better understanding of IAV circulation in wean-to-finish (WTF) herds is needed to guide surveillance, biosecurity, and control strategies. This study evaluated IAV status and infection patterns in WTF herds across the Midwestern US. Sixty-three WTF herds from 10 US production companies located in the Midwest were enrolled in a prospective cohort study (Sep 2017–Dec 2018). Herds were chosen by convenience, logistics, and the producer's willingness to participate. At each site, 8 oral fluid samples were collected every 4 weeks at approximately 3, 8, 12, 16, 20, and 25 weeks post-placement using cotton ropes. The IAV RNA was detected by RT-qPCR, with Ct ≤37 considered positive, and selected samples were submitted for whole-genome sequencing. Positive sample proportions across weeks were compared using chi-squared test, with Bonferroni-adjusted pairwise comparisons ($P < 0.05$). A total of 2,585 oral fluid samples were tested for IAV, yielding 623 positives (24.1%). Positive samples were identified in 60 out of 63 WTF herds (95.2%) across all companies. Influenza A virus was detected at all 6 scheduled sampling events throughout the WTF period. Influenza A virus was detected in most herds at least once, with positivity and viral load varying across time points. The highest sample level positivity was at 8 weeks post-placement (31.0%, mean Ct 29.7), followed by 3 weeks (22.6%, Ct 30.4). Thereafter, positivity declined over time: 15.4% at 12 weeks (mean Ct 29.3), 12.0% at 16 weeks (Ct 30.0), 15.2% at 20 weeks (Ct 30.6), and 3.7% at 25 weeks (Ct 30.7). Weeks 3 and 8 showed higher positivity than weeks 12–25, with week 8 significantly higher than week 3. No significant differences were observed among weeks 12, 16, and 20. Week 25 had the lowest rate. Influenza A virus circulates widely in WTF herds in the US Midwest, peaking in the first 8 weeks post-weaning. Infection dynamics varied among herds, reflecting ongoing and new introductions. Early detection suggests piglets may be weaned IAV-positive, infected during transport, or shortly after weaning. Variable timing of infection and duration highlight complex IAV dynamics and control challenges. Findings stress the need for tailored surveillance, extended monitoring, and control strategies to reduce IAV burden in growing pigs.

Financial Support: Merck Animal Health and the University of Minnesota Swine Disease Eradication Center (SDEC)

Key Words: influenza A virus (IAV), epidemiology, oral fluid, RT-qPCR

57 - Live attenuated influenza A virus vaccines with improved safety profile induce strong mucosal immune responses in swine.

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Influenza A viruses (FLUAV) pose a threat to the global swine industry, causing significant losses. Inactivated vaccines are the main method of controlling FLUAV in swine but often fail to protect against antigenically distinct strains. Although effective at inducing systemic IgG antibodies, these vaccines have limited capacity to stimulate cellular and IgA responses, essential for mucosal immunity and cross-protection. Live attenuated influenza vaccines (LAIV) induce broader responses, but safety concerns highlight the need for improvements to minimize reassortment risk. We previously developed safe LAIV capable of inducing robust mucosal immunity, but their potential for transmission and reassortment was unknown. Thus, our objective was to modify these LAIV to improve immune stimulation while reducing transmission and reassortment in swine. We designed 2 vaccines with a rearranged genome (RAM) backbone, a genetic attenuation strategy relocating the M2 gene from segment 7 to PB1. In the first, we inserted the IgA-inducing protein (IGIP) gene into HA, and in the second, the swine interleukin-18 (IL-18) gene into NA, aiming to enhance IgA production and T cell activation. A third vaccine used a temperature-sensitive PB2 mutation (Flu-att) and included both IGIP-HA and IL-18-NA. Piglets were assigned to groups receiving RAM-IGIP, RAM-IL-18, or Flu-att-IGIP+IL-18, and compared with a non-modified Flu-att, an inactivated vaccine, or PBS. Viral replication and transmission were evaluated in vaccinated animals and sentinels, and immune responses assessed via hemagglutination inhibition, microneutralization, IgA ELISA, and flow cytometry. Our results demonstrate that RAM-backbone vaccines reduced replication compared with Flu-att, and no transmission to sentinels was detected for the RAM groups. Microneutralization and hemagglutination inhibition showed that sera from RAM-IGIP and RAM-IL-18 immunized animals neutralized the virus, indicating systemic antibody responses. The ELISA results revealed that all attenuated vaccines induced high mucosal IgA in bronchoalveolar lavage and nasal washes, whereas inactivated vaccines showed little detectable IgA. Our findings support IGIP and IL-18 as immunomodulators to enhance responses induced by LAIV in swine. The RAM-based strategy was genetically stable and safe, reducing replication and transmission. These results highlight a promising approach to improve influenza control in pigs while minimizing safety concerns.

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Key Words: live attenuated influenza vaccine (LAIV), influenza, vaccine, immunoglobulin A (IgA), molecular marker, pig

58 - Systemic and mucosal immune responses induced by adenoviral-vectored consensus H5 influenza A vaccines in mice and swine.

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The continued evolution and cross-species transmission of clade 2.3.4.4b H5Nx highly pathogenic avian influenza (HPAI) viruses underscore the need for broadly protective vaccines in swine, a key intermediate host. This study aimed to evaluate the systemic and mucosal immune responses elicited by adenoviral-vectored (Ad) vaccines encoding a centralized consensus hemagglutinin antigen (H5CC) in mice and swine. We constructed H5CC-based vaccines delivered using replication-defective (Ad5 and Ad6) and replication-competent (Ad28 and Ad48) human adenoviral vectors. Using a serotype-switched prime-boost strategy, vaccines were delivered intramuscularly (IM) or intranasally (IN) in mice and swine. We determined humoral, mucosal, and cell-mediated immune responses by hemagglutination inhibition (HI), microneutralization assay (MNA), ELISA, and IFN-γ ELISpot. Protective efficacy was evaluated by lethal H5N1 challenge in mice. All vaccine strategies and routes induced significant levels of anti-H5 immunity. The Ad5/Ad6 IM immunization elicited strong systemic IgG and MNA titers ($P < 0.0001$) and robust T cell responses ($P < 0.05$). The IN delivery with Ad5/Ad6 induced superior mucosal IgA levels in lungs and nasal secretions ($P < 0.05$). In swine, Ad5/Ad6 IM conferred the highest MNA titer and T cell responses ($P < 0.0001$), whereas the IN route enhanced mucosal IgA ($P < 0.05$). The Ad28/Ad48 vaccines induced immunity in a similar pattern as compared with the Ad5/Ad6 strategy, but to slightly lesser degree, in general. The commercial H1/H3 swine influenza vaccine failed to elicit cross-protective immunity. All H5CC vaccinated mice survived lethal H5N1 challenge without weight loss. Adenoviral-vectored H5CC vaccines elicit broad, cross-clade immunity with route-dependent immune profiles. The IM vaccination is optimal for systemic and cellular responses, whereas IN delivery enhances mucosal immunity. These findings support the advancement of adenoviral platforms for influenza control in swine and pandemic preparedness.

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Key Words: adenovirus, centralized consensus, H5Nx, highly pathogenic avian influenza (HPAI), influenza A virus, vaccine

General Abstracts: Session 12—Bovine Respiratory Disease 1

60 - Nasal microbiome markers are related to respiratory health outcomes.

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Bovine respiratory disease (BRD) is the largest cause of morbidity and mortality in stocker cattle. Increasing the understanding of how bacterial pathogens impact the nasal microbiome is crucial in improving diagnostic tools and prevention and treatment measures. The purpose of this study was to evaluate associations between changes in the nasal microbiome and health outcomes in stocker heifers and the potential impact of metaphylaxis. Stocker heifers from Mississippi were split into 2 groups: those who received metaphylaxis on arrival (META) and those who did not (NO META). Nasal samples were collected from 4 cohorts: Fall 2019 (F19), Fall 2020 (F20), Spring 2021 (S21), and Fall 2021 (F21); over 3 sampling times: day zero (D0), at the time of treatment (TRT), where applicable, and wk 3 (WK3). Cattle were categorized based on 3 health outcomes: those who remained healthy (healthy), those who became ill and recovered (sick), and those who became ill and died post-treatment (dead). In total, 216 samples were collected. Samples were analyzed using 16S rRNA gene sequencing to characterize microbial communities. Richness and phylogenetic diversity of microbial communities decreased between D0 and WK3 in healthy ($P < 0.05$) and sick ($P < 0.05$) cattle regardless of placement cohorts. However, there were differences in magnitude between the 4 cohorts. The largest changes in community structure were driven by the increased relative abundance (RA) of *Mycoplasmataceae* and subsequent decreased RA of *Pasteurellaceae* and *Moraxellaceae*. There were no significant associations between META and NO META. The decrease in the stability of the nasal microbial communities between D0 and WK3 may indicate increased disease susceptibility. Commingling is a common factor that influences cattle susceptibility to BRD and likely is also associated with these microbial changes. This study provides the groundwork for future studies to further characterize the nasal microbiome and its association with health outcomes.

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Key Words: bovine respiratory disease, nasal microbiome

61 - Role of control and detection strategies for bovine respiratory disease on feedlot cattle performance and emissions.

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Health challenges, such as bovine respiratory disease (BRD), reduce cattle growth and feed efficiency, increasing greenhouse gas emissions (GHG) per unit of beef produced (emissions intensity). Our research objective was to quantify how feedlot BRD control and detection strategies impact production outcomes and GHG emissions intensity. A feedlot-phase emissions model was applied to data from a previously published commercial feedlot trial, which compared 3 BRD control programs: whole-pen antimicrobial metaphylaxis; negative control, no metaphylaxis; and a targeted metaphylaxis control program based on individual risk (TCP). Two BRD detection strategies—a novel automated animal disease detection (ADD) system and traditional pen-riding—were also evaluated. Pen-level outcomes were analyzed to estimate emissions per kilogram of final body weight (FBW) and hot carcass weight (HCW). Linear mixed models were used for analysis with treatment main effects (control and detection strategies) and their interaction as fixed effects, and random intercepts to account for the block design. Health outcomes differed among strategies: whole-pen metaphylaxis reduced BRD morbidity, removals, and mortality compared with the negative control, whereas ADD enabled earlier treatment, fewer pen mortalities, and fewer removals. Performance outcomes aligned, as metaphylaxis increased daily weight gain (ADG) by 9% versus the negative control, and ADD increased ADG by 6% versus pen-riding. These shifts translated into differences in emissions intensity, specifically for improved BRD detection. The ADD method reduced emissions intensity by 3.1% per kg FBW ($P = 0.004$) and 3.0% per kg HCW ($P = 0.006$) compared with conventional pen-riding. No significant differences in emissions intensity were observed among control strategies ($P \geq 0.12$). These findings demonstrate that disease management strategies can improve health and performance while reducing emissions intensity, highlighting animal health as a key driver of productivity and sustainability in feedlot systems.

Financial Support: This study was funded by the College of Veterinary Medicine's Center for Outcomes Research and Epidemiology and Merck Animal Health Technical Servies.

Key Words: feedlot cattle, bovine respiratory disease, metaphylaxis, automated disease detection, greenhouse gas emissions, emissions intensity

62 - Impact of preweaning vaccination on gene expression profiles in healthy and BRD-affected cattle during backgrounding.

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Bovine respiratory disease (BRD) remains a priority in livestock research. Vaccination of preweaning calves against BRD-related pathogens is a leading management strategy, but long-term effects on health outcomes and resulting immunomodulation are poorly understood. Comparing gene expression in vaccinated and unvaccinated calves that later develop or resist BRD may identify host mechanisms associated with health and performance. Seventy-three bull calves were enrolled and sampled in a split-plot randomized controlled trial. Calves were assigned to receive vaccination (VAX) or saline (NOVAX), and then transported either directly after weaning (DIRECT) or through an auction market (AUCTION). Sampling of jugular blood (Tempus Blood RNA Blood Tubes) occurred at 4 time points: T1, vaccination (median age = 107 d); T2, 7 d after vaccination (median age = 114 d); T3, revaccination and surgical castration (median age = 183 d); and T4, weaning (median age = 230 d). VAX calves received Pyramid 5 at T1 and T3, and all calves received Covexin 8 at T1 and T3. Following T4, cattle were transported to TX for a 45-d backgrounding phase and were monitored daily by trained personnel who were blinded to treatment; cattle were either treated for BRD (BRD) or remained clinically healthy throughout backgrounding (NOBRD). Isolated mRNA from each time point was sequenced (NovaSeq 6000; ~35M reads/sample), aligned to ARS-UCD1.2 reference-guided assembly (HISAT2/StringTie2), and analyzed with edgeR and glmmSeq (FDR <0.05). Differentially expressed genes (DEGs) were assessed for Gene Ontology, Reactome, and KEGG enrichments (KOBAS-i). When evaluating time, 10,397 DEGs were identified and enriched for innate/adaptive immunity, interleukin signaling, and protein translation. Vaccination yielded a total of 112 DEGs across all 4 time points, notably at T3, where VAX calves showed increased expression of pathways related to cellular stress response, neutrophil degranulation, and antigen presentation. Bovine respiratory disease status was associated with a total of 243 DEGs, where, at T4, BRD calves possessed enriched pathways related to oxygenation, cellular metabolism, and cytokine signaling compared with NOBRD. These results identify immune and metabolic genomic mechanisms linked to vaccination and BRD susceptibility, providing insight into predictive markers and targeted management strategies for beef production.

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Key Words: bovine respiratory disease, transcriptomics, vaccination, immunity, interleukin, cellular stress

63 - Precision metagenomics: Adaptive sampling for improved detection of *Mannheimia haemolytica*.

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M*annheimia haemolytica* is considered a major bacterial agent in acute bovine respiratory disease (BRD) cases. Typically, a pathobiont within the upper respiratory tract of cattle, environmental changes, stress, and viral co-infections can trigger *M. haemolytica* colonization of the lower respiratory tract and increase disease susceptibility. Understanding intra- and inter-host dynamics of *M. haemolytica* would reveal drivers of disease variation within herds. We used adaptive sampling to selectively enrich *M. haemolytica* DNA from metagenomic samples, enabling strain-level resolution that traditional metagenomics lacks. A total of 72 nasopharyngeal swabs from a trial evaluating metaphylaxis effects on the respiratory microbiome were stratified into 3 groups based on *M. haemolytica* DNA concentration (negative, low, high; n = 24/group), quantified using a QuantStudio Absolute Digital PCR system. Samples were randomly assigned into pools (n = 8 samples/pool), prepared with the SQK-NBD114-24 kit, and sequenced on a PromethION 2 Integrated device over 72 h. Adaptive sampling was enabled via MinKNOW (v24.11.8) using reference genomes for serotypes A1, A2, and A6. Passed reads were aligned using Minimap2 (v2.28) to assess alignment accuracy. Linear and logistic generalized estimating equations were used to evaluate the effect of time and *M. haemolytica* concentration on total and mapped reads. Results showed heterogeneous mapped reads across pools within groups, with no significant correlation to initial *M. haemolytica* DNA concentration. A median of 97.2% of reads mapped to reference genomes. Cumulative reads significantly increased over sequencing time ($P < 0.05$), whereas unique reads significantly decreased over time in high-concentration pools ($P < 0.05$ across most time points). Low and negative groups showed fewer significant differences between time points. Total recovered reads followed the trend of high > low > negative, with negative samples yielding the lowest number of *M. haemolytica* reads. Our findings demonstrate that adaptive sampling effectively enriches *M. haemolytica* DNA from metagenomic samples, increasing sequencing efficiency and reducing reliance on costly, labor-intensive methods. This approach offers a powerful tool to investigate *M. haemolytica* ecology and strain diversity, deepening our understanding of its role in BRD pathogenesis.

Financial Support: Texas AgriLife Research Animal Health and Disease Capacity Funding.

Key Words: adaptive sampling, *Mannheimia haemolytica*, sequencing method, enriched sequencing

64 - Broadly protective bovine parainfluenza 3 virus and bovine viral diarrhea virus vaccine.

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The objective of this project is to develop broadly protective vaccines against bovine parainfluenza virus 3 (BPI3V) and bovine viral diarrhea virus (BVDV). Consensus envelope (E2) and nonstructural (NS2-5) antigens, designated E2NS2-5, were designed from >200 BVDV1-2 genomes. Similarly, consensus fusion (F2) and hemagglutinin-neuraminidase (HN2) antigens, designated F2HN2, were derived from 97 BPI3V genotypes A, B, and C. Using a BPI3V genotype C (BPI3Vc) vector, recombinant viruses expressing the novel F2HN2 (BPI3Vc-F2HN2) and E2NS2-5 (BPI3Vc-E2NS2-5) antigens were generated, along with control vectors (BPI3Vc-GFP and BPI3Vc-TMSP67). In vitro characterization of the BPI3Vc-GFP virus confirmed vector attenuation as judged by temperature sensitivity, while animal studies assessed safety, immunogenicity, and efficacy. In the first study, calves (n = 5) were immunized intranasally with BPI3Vc-F2HN2. A commercial BPI3Va vaccine served as a positive control (n = 5), and sham-immunized calves served as negatives (n = 5). Antibody responses were evaluated by indirect ELISA, and serum was evaluated by VNA for cross-neutralization of diverse BPI3Va–c strains, and protection was determined by challenge with wildtype BPI3Vc. In the second study, calves (n = 5) received BPI3Vc-E2NS2–5, with a commercial BVDV vaccine as the positive control (n = 5) and BPI3Vc-TMSP67 as the negative control (n = 5). Protection was tested by challenge with wild-type BVDV1b, and sera were screened by VNA against multiple BVDV1-2 isolates. Data were analyzed by two-way ANOVA. The BPI3Vc-F2HN2 and BPI3Vc-E2NS2-5 prototype vaccines were safe in calves and elicited robust antibody responses. Importantly, BPI3Vc-F2HN2 induced significantly ($P < 0.0001$) higher neutralizing titers against representative BPI3Va–c strains, whereas BPI3Vc-E2NS2-5 produced broader neutralization against representative BVDV1-2 strains ($P = 0.0031$ and $P = 0.0025$, respectively) compared with commercial vaccines. Both vaccines conferred superior protection upon viral challenge. In conclusion, BPI3Vc-F2HN2 and BPI3Vc-E2NS2-5 represent promising prototype vaccines. They not only protected calves from challenge but also induced sera capable of neutralizing diverse BPI3V and BVDV strains, supporting their potential as broadly protective vaccine platforms for improved 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.

Key Words: bovine parainfluenza-3 virus, bovine viral diarrhea virus, temperature-sensitive vector, vaccine, surface display, broadly neutralizing antibody

Featured Speaker: Session 13—CRWAD Council Keynote and Special Symposium

65 - The dark alliance: Co-evolution of antimicrobial resistance and bacterial virulence.

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Antimicrobial resistance presents a global threat to both animal and human health. An alarming trend is the convergence of antimicrobial resistance with bacterial virulence, creating a “dark alliance” that leads to rapid expansion of antibiotic-resistant and hypervirulent bacterial clones. These clones can disseminate rapidly, cause severe diseases, and are difficult to treat. The co-evolution of antimicrobial resistance and virulence is a dynamic process and is driven by selective forces from both host defenses and antimicrobial use. As bacteria acquire resistance traits, they often gain genetic changes that make them more virulent to animal hosts. These resistance and virulence determinants may be co-located on mobile genetic elements, facilitating their simultaneous transfer and persistence within bacterial populations, or evolved separately via genetic modifications of bacterial chromosomes. In this presentation, examples will be given to illustrate how bacteria evolve to become multidrug resistant and hypervirulent, what selection pressures contribute to their emergence and expansion, and how acquisition of both traits influence their transmission across various animal hosts. Future perspectives on prevention and control of antimicrobial resistant pathogens will also be discussed at the end of the talk.

Featured Speaker: Session 14—CRWAD Special Symposium

67 - Artificial intelligence in population medicine and disease dynamics.

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University of California, Karen C. Drayer Wildlife Health Center, School of Veterinary Medicine, Davis, CA

No abstract text provided.

68 - Integrating AI in animal health research and graduate education: Challenges and opportunities.

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Artificial intelligence (AI) tools are rapidly transforming the landscape of animal health research and education of veterinary and graduate students. The term AI is broad and incorporates predictive models, large language models (LLMs), machine-learning classifiers, computer-vision systems, and a variety of specialized tools. Adoption of AI is accelerating across disciplines, but the opportunities, limitations, and best-use strategies are poorly defined. This presentation synthesizes current examples of AI applications in veterinary medicine and explores how AI can both enhance scientific research and support effective student training. Using AI in research can take a variety of forms, including ideation, literature synthesis, data management, predictive modeling, and improved communication. Effective AI use begins with well-structured prompts, clarity of task goals, and recognition that the objective is for AI to be complimentary rather than a substitute for domain expertise. Multiple AI platforms exist, and careful consideration is required to ensure the selected technology aligns with the specific task. Predictive models have been frequently applied in research settings, including behavioral monitoring, disease risk prediction, disease prognosis, and computer vision analysis. Classifier performance varies widely across data sets, and researchers should start with a specific question and clearly defined target variable. Within graduate and veterinary education, AI tools present meaningful opportunities to enhance learning efficiency, promote higher-order reasoning, and simulate real-world decision-making. However, AI-generated summaries or study guides may be superficial or inaccurate when used by novice learners, underscoring the need to teach verification skills and critical thinking. Clear guidelines regarding authorship, attribution, accountability, and ethical use are essential as students increasingly incorporate AI into grant preparation, manuscript writing, and clinical decision support. Overall, AI has the potential to improve efficiency, strengthen research quality, and support more interactive education. Establishing best-practice frameworks for prompt design, model evaluation, data stewardship, and intellectual attribution will be critical to maximizing value while maintaining scientific rigor and educational integrity.

Featured Speaker: Session 15— ACVM Symposium

69 - Molecular characterization of a virulence factor of *Leptospira*, Lig proteins.

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Clones expressing novel immunoreactive leptospiral immunoglobulin-like proteins (LigA, LigB, and LigC) from *Leptospira interrogans* serovar Pomona were identified by screening a genomic DNA library with serum from a mare that had recently aborted following leptospiral infection. Lig proteins function as surface-exposed adhesins that mediate bacterial attachment to host cells through specific interactions with extracellular matrix (ECM) components. Among these, LigB exhibits broad binding activity, interacting with calcium ions (Ca^{2+}) and several ECM proteins, including fibronectin (Fn), fibrinogen (Fg), laminin, collagen, and C4b-binding protein (C4BP). A high-affinity Fn-binding site was mapped to the central region of LigB (LigBCen2), comprising the partial eleventh and complete twelfth immunoglobulin-like repeats (LigBCen2R) together with 47 amino acids of the adjacent non-repeat region (LigBCen2NR). LigBCen2NR specifically bound the N-terminal domain (NTD) of Fn with a dissociation constant (K_D) of 379 n*M*, as determined by ELISA and isothermal titration calorimetry (ITC). Circular dichroism spectroscopy revealed that LigBCen2NR adopts a β-strand-rich conformation upon NTD binding, consistent with the β-zipper mechanism characteristic of NTD–ligand interactions. In addition to Fn, Lig proteins were found to interact with human tropoelastin (HTE) and elastin through overlapping domains, including LigBCon4, LigBCen7′–8, LigBCen9, and LigBCen12. LigB bound specifically to exons 17–27 of HTE (17–27HTE), and this interaction was enthalpy-driven and sensitive to pH, suggesting that electrostatic forces contribute to host tissue adhesion during infection. Moreover, the fibrinogen-binding region of LigB was localized to LigBCen2 (amino acids 1014–1165), with the minimal binding domain confined to LigBCen2R (amino acids 1014–1119). LigBCen2R interacted with the C-terminal αC domain of fibrinogen (FgαCC; amino acids 392–644 of the α chain) with K_D values of 0.375 m*M* (ITC) and 0.364 m*M* (fluorescence spectroscopy). LigBCen2R binding to the FgαCC RGD motif induced conformational changes that prevented integrin αIIbβ3 recognition on platelets, thereby inhibiting platelet aggregation and reducing clot formation. These findings suggest that LigB interferes with fibrinogen's αC domain, potentially disrupting host hemostasis and providing a mechanistic explanation for the pulmonary hemorrhage and thrombocytopenia observed in severe leptospirosis.

70 - *Brachyspira* spp. infections in animals.

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Currently there are 9 officially named species in the genus *Brachyspira.* These anaerobic spirochetes colonize the large intestines of multiple animal species and humans, resulting in a range of outcomes from inapparent infections to severe clinical disease, depending upon the bacterial species and a variety of host factors. This presentation will discuss the common host range of recognized species, typical disease manifestations, and commonly available methods for laboratory detection and differentiation. *Brachyspira pilosicoli* has the broadest host range and has zoonotic potential. Intestinal spirochetosis has been described in pigs, poultry, and humans in association with *B. pilosicoli* infection, and isolates of human origin have been experimentally inoculated into pigs, chickens, and mice. Pigs are a major host for *Brachyspira* spp. and can be colonized by at least 7 of the officially named species. The most significant disease in pigs, swine dysentery, is a severe mucohemorrhagic colitis caused by strongly β-hemolytic isolates of *Brachyspira hyodysenteriae*, *Brachyspira hampsonii*, or *Brachyspira suanatina.* In chickens, infections with *Brachyspira intermedia*, *Brachyspira alvinipulli*, and *B. pilosicoli* have been associated with diarrhea and reduced egg production. Modern sequencing technologies have afforded insights into the genetic diversity of members of this genus as well as the epidemiology of infection and geographical spread. Regional differences in detection and antimicrobial resistance in *Brachyspira* spp. further complicate mitigation and control efforts globally.

General Abstracts: Session 16—One Health and Public Health 1

71 - Strengthening foot-and-mouth disease preparedness in Puerto Rico.

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Puerto Rico, a United States territory, is vulnerable to high-consequence foreign animal diseases such as foot-and-mouth disease (FMD), given its location and sizable cattle and small-ruminant sectors. An FMD outbreak on the island could disrupt its production and threaten food security. Strengthening awareness and preparedness among producers and veterinarians is therefore critical for early detection, prevention, and response. The objective of this study was to develop, implement, and evaluate FMD educational workshops for producers, stakeholders, and veterinarians and determine its effectiveness in improving awareness, knowledge, and attitudes. Between June 20 and 27, 2025, 3 interactive producers' workshops and 1 trainers' workshop were conducted across Puerto Rico. The workshops covered content on FMD recognition, biosecurity practices, and outbreak response preparedness. Guided by the Kirkpatrick's evaluation model, a mixed-methods evaluation design was employed, utilizing self-administered instruments at pre-, mid-, and post-workshop stages to assess participants' reaction, learning, and immediate intention to change (proxy for behavior). Additionally, facilitator observation forms were completed by 4 independent observers for each session to capture participants' level of engagement. Descriptive analyses and a mixed-effects logistic regression of recognition of FMD-related terms (0/1) on time (pre vs. post), with random intercepts for participant and term (10), was conducted in R software. A total of 81 participants attended the workshops, with the majority being extension workers and producers. For producers' workshops, pre/post-evaluation using Wilcoxon signed-rank indicated significant gains in term recognition and understanding ($P < 0.001$). Median recognized terms doubled from 5 to 10, with a median improvement of 4.5 (95% CI: 4.0–5.0), indicating substantial learning. Mixed-effects logistic regression showed a large post-training gain in recognition of FMD related terms (OR = 39.9, 95% CI 23.2–68.5, $P < 0.001$). In the reflection forms, participants most frequently identified FMD clinical signs as a new concept learned (n = 29) and the farm biosecurity model as the most liked component of the workshop (n = 28). Facilitator observation data highlighted high levels of engagement and participation. These workshops reinforce the need for sustained investment in training and systematic follow-up evaluations to build and maintain long-term preparedness capacity.

Financial Support: USDA's Animal and Plant Health Inspection Service through the National Animal Disease Preparedness and Response Program, AP grant AP24VSSP0000C110

Key Words: foot-and-mouth disease, foreign animal disease, response preparedness, Puerto Rico

72 - Neuraminidase-mediated antibody immunity against highly pathogenic avian influenza H5N1 in humans with occupational exposure to swine.

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Since the emergence of highly pathogenic avian influenza (HPAI) H5N1 clade 2.3.4.4b in North America in 2022, 70 cases of human infection have been reported. Although there was no reported human-to-human transmission, the outbreak in dairy cattle and infections in other wild mammals increase the risk for mammalian adaptation and an H5N1 pandemic in humans. The risk of HPAI H5N1 to domestic swine is unknown but is a concern for swine health and the potential for reassortment with swine adapted strains and further mammalian adaptation. Swine farm workers and veterinarians would be the front line of human exposure if transmission of H5N1 HPAI to swine occurred. Cross-reacting neutralizing antibody responses in humans from H1 or H3 seasonal vaccination or infection is limited against the HPAI H5 hemagglutinin (HA). In contrast, antibodies to the N1 subtype neuraminidase (NA) protein may have increased cross-reactivity as circulating avian N1, human N1, and swine N1 share a common ancestor. To investigate HA and NA reactivity in occupationally exposed swine veterinarians and swine farm employees, sera from these cohorts were tested by hemagglutination inhibition (HI) and neuraminidase inhibition (NI) assays. Test antigens included H5 HA clade 2.3.4.4b and human seasonal H1 in HI assays and representative avian (HP.N1-c1, HP.N1-c5), human (N1pdm), and swine (N1.C.3.2) N1 lineages in NI assays. No standardized correlate of protection exists for NI titers, so responses were interpreted relative to the human N1pdm reference antigen. HI results indicated that veterinarians lacked antibodies (<40) to H5 2.3.4.4b. However, veterinarian sera showed similar NI geometric mean titers (GMT) between HP.N1-c1 (GMT = 67) and N1pdm (GMT = 62). Detection of N1 specific antibodies suggests that prior exposure or vaccination to seasonal N1 may provide cross-protection against H5N1 HPAI via NA-mediated immunity, even in the absence of HA-specific cross-protection. Future studies will include swine farm employee sera and additional swine and avian N1 antigens to more fully characterize cross-reactive antibody patterns against N1.

Key Words: highly pathogenic avian influenza, swine, occupational exposure, serosurveillance, pandemic preparedness

73 - Influenza D virus at the wildlife–livestock interface: Emerging cross-species dynamics.

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Influenza D virus (IDV) is a member of the *Orthomyxoviridae* discovered in swine in 2011. Although cattle are considered a reservoir for IDV, evidence of IDV infection has been confirmed in several animal species and humans. There are 5 lineages of IDV: D/OK, D/660, D/CA2019, D/Yama2016, and D/Yama2019. The envelope glycoprotein, hemagglutinin esterase fusion (HEF) of D/CA2019 exhibits broader antigenicity. IDV undergoes rapid evolution and extensive genetic diversification, likely driven by its broad host range. White-tailed deer (WTD), one of the most widely distributed mammals in the United States, often shares pastureland with cattle. Spillover of cattle pathogens such as *Mycobacterium bovis* and bovine viral diarrhea virus between WTD and cattle has been documented. A recent study on a limited number of WTD sera suggested IDV seroprevalence, but the role of WTD on IDV ecology and evolution remains unclear. This study aimed to investigate the evidence of IDV infections in wild WTD and the susceptibility of WTD airway epithelial cells to IDV. We used sensitive pseudovirus neutralization assay (pVNT) to detect IDV antibodies (Abs). Pseudoviruses (pVs) were generated using third-generation lentivirus to carry IDV HEF. D/CA2019 HEF pV was used to determine IDV Abs in serum. We tested 90 sera from WTD residing in New Jersey, collected during February 2025. In pVNT, 20 sera showed = 80% neutralization, indicating 22% seroprevalence. Titration of a subset of positive sera (n = 10) against IDV lineages showed higher IC_{50} to D/Yama2016, D/OK, and D/660, indicating cocirculation of lineages in WTD. To investigate the susceptibility of respiratory cells to IDV, we used an in-house developed tracheal epithelial cell culture (DTEC) model. We infected DTEC cells with 0.1 MOI of D/bovine/Oklahoma/660/2013. Infected cell culture supernatants were harvested at 24-h intervals for 5 dpi and analyzed by PB1 qRT-PCR and $TCID_{50}$ to assess replication. In DTEC, IDV replicated to 10^4 $TCID_{50}$/mL and 3.5×10^4 copies/reaction, ~50-fold increase compared with 0 hpi. We performed anti-NP Ab-based IFA on IDV-infected DTEC cells at 48 hpi, which yielded fluorescence signal, indicating virus replication. Our findings show that WTD are susceptible to IDV, and there are ongoing transmissions between cattle and WTD, which could potentially serve as a wildlife reservoir for IDV. Our study warrants One Health surveillance for monitoring IDV spillover and evolution in WTD and cattle.

Financial Support: This work was supported by the Agriculture and Food Research Initiative Competitive Grant no. 2024-09438 from the USDA National Institute of Food and Agriculture

Key Words: influenza D virus, cattle, white-tailed deer, surveillance, co-circulation

74 - Tick-borne diseases in Illinois: A One Health analysis.

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Illinois is known to have established populations of 4 vector ticks of human health concern: *Ixodes scapularis*, *Dermacentor variabilis*, *Amblyomma americanum*, and *Amblyomma maculatum*. These ticks can transmit pathogens causing 8 reportable human tick-borne diseases (TBDs); of these, the Companion Animal Parasite Council (CAPC) reports data on anaplasmosis, ehrlichiosis, and Lyme disease in dogs based on results from the IDEXX 4DX Snap test. The incidence of these diseases is spatially varied and has been changing over time. The purpose of this research is to describe factors associated with incidence of the various tick-borne diseases in Illinois, and comparing the factors among humans and dogs. All cases of tick-borne diseases in humans reported to Illinois Department of Public Health (IDPH) between 2004 (when reporting began) and 2022 were collated with all cases of tick-borne diseases in dogs reported by CAPC between 2012 and 2022 and the county and year level. Comparison with known risk factors was conducted using zero-inflated Poisson modeling for anaplasmosis, ehrlichiosis, and Lyme disease in humans and combined TBDs in dogs, using county-level human population as the offset. There were 6,423 cases of TBDs reported to IDPH between 2004 and 2022, and 167,847 canine cases reported by CAPC between 2012 and 2022. Every county in Illinois reported at least 1 human case of a TBD within this period, and 79/102 reported at least 1 canine case. The majority of cases were Lyme disease, followed by SFGR in humans and ehrlichiosis in dogs. Canine TBD incidence and human Lyme disease and anaplasmosis were significantly higher in the Northern region than in the Central region. Human anaplasmosis was also higher in the Southern region than in the Central region. All human TBDs were higher in counties with higher rurality, and Lyme disease and ehrlichiosis were higher in counties with higher deer harvest. Case distributions were spatially similar to known vector tick distributions. TBDs have been present and increasing in incidence across the state of Illinois in humans and animals. This may be explained by the relative abundance of vector ticks, which is expected to change due to the interacting effects of range expansion, host and reservoir populations, human behaviors, and climate change.

Financial Support: Cooperative Agreement Number U01CK000651 from the Centers for Disease Control and Prevention

Key Words: tick, tick-borne disease

75 - Understanding public knowledge, attitudes, and practices toward PFAS exposure in Michigan communities with and without known environmental contamination.

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Per- and polyfluoroalkyl substances (PFAS) are highly persistent environmental contaminants associated with adverse health effects in both humans and animals. This study assessed awareness, attitudes, and preventive practices related to PFAS exposure among Michigan residents from communities with and without documented contamination. A Knowledge, Attitudes, and Practices (KAP) survey was administered over 2 years to 431 participants, including veterinary faculty, staff, and students at the Michigan State University College of Veterinary Medicine, as well as dog park visitors in 2 Michigan communities with confirmed PFAS contamination (Belmont/Rockford and Parchment). The survey evaluated PFAS awareness, perceived exposure sources, preventive behaviors, and preferred information channels. Veterinary professionals were not significantly more aware of PFAS (χ^2, $P = 0.30$) nor more likely to use preventive measures such as water filters (χ^2, $P = 0.22$) compared with residents of contaminated communities. However, on a scale of 1 to 5, veterinary participants reported significantly greater familiarity with PFAS (ANOVA, $P < 0.001$). When the veterinary cohort (n = 160) was excluded, no significant differences in awareness or familiarity were found between individuals living inside versus outside contaminated areas (χ^2, $P = 0.54$; ANOVA, $P = 0.48$). Having an immunocompromised household member did not increase PFAS awareness (ANOVA, $P = 0.59$) but was associated with greater use of water filtration or other preventive measures (χ^2, $P = 0.003$). Participants most frequently identified industrial waste (90%) as a contamination source, while only 4% recognized rain or snow. Preferred information channels included the internet (53%), medical or veterinary professionals (49%), and news media (38%), followed by social media and word of mouth. Despite widespread discussion of PFAS, many respondents—particularly in affected communities—remained uncertain about exposure routes and reported limited adoption of risk-reducing behaviors. Targeted outreach through trusted professionals and accessible digital platforms is needed to strengthen environmental health literacy and promote practical risk-reduction behaviors that protect both human and animal health.

Financial Support: Michigan Animal Health Foundation

Key Words: PFAS; Knowledge, Attitudes, and Practices (KAP); Michigan; environmental health

76 - Proof-of-concept: Engineered *Escherichia coli* Nissle 1917 to stably express bacteriophage-derived CD27L endolysin enables targeted and sustained treatment of *Clostridioides difficile* infection.

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Bacterial infections are becoming harder to treat due to rising antimicrobial resistance (AMR), which reduces the effectiveness of conventional antibiotics. Bacteriophages offer a potential alternative, but delivering the complete phage as a therapeutic is challenging. Bioengineered probiotics expressing phage endolysin genes present a promising solution. As proof of concept, we engineered *Escherichia coli* Nissle 1917 to stably express CD27L endolysin with activity against a broad range of *Clostridioides difficile* serotypes, enabling targeted *C. difficile* infection (CDI) control without antibiotics. We selected *E. coli* Nissle 1917 (EcN) as a probiotic chassis and delivery system due to its unique combination of safety, colonization potential, and therapeutic versatility. Native plasmids were cured using CRISPR-Cas9 to enable stable integration of a synthetic pMUT2 plasmid carrying the optimized cd27l gene. After overexpression in BL21(DE3) with pET28a, the gene was cloned into pMUT2 under FNRS and J23101 promoters via Gibson assembly. Engineered EcN strains were validated with GFP and tested against *C. difficile* using 1:1 and 4:1 coculture assays and spent media. Antimicrobial efficacy was evaluated by quantifying *C. difficile* colony-forming units (cfu). CD27L, a phage-derived endolysin, showed strong lytic activity against 30 *C. difficile* strains, including ribotype 027, without affecting commensal bacteria. After CRISPR-Cas9-mediated curing of native plasmids, a synthetic pMUT2 plasmid encoding CD27L was introduced under either constitutive (J23101) or anaerobic (FNRS) promoters. GFP confirmed expression and plasmid stability without antibiotics. In 1:1 coculture assays, J23_CD27L_EcN significantly reduced *C. difficile* R20291 viability ($P < 0.01$), whereas FNRS_CD27L_EcN showed no significant effect. No reduction was observed at a 4:1 ratio. Spent media from J23_CD27L_EcN also significantly inhibited *C. difficile* growth ($P < 0.05$), suggesting extracellular CD27L activity. This establishes engineered *E. coli* Nissle 1917 (EcN) as a versatile platform for delivering targeted antimicrobial proteins. Stable expression of CD27L endolysin enabled effective inhibition of *C. difficile* without antibiotic selection, showing therapeutic potential. These findings support the broader use of engineered EcN as a modular chassis for expressing diverse genes to develop precision bacteriotherapies against multidrug-resistant pathogens.

Financial Support: Oklahoma State University

Key Words: engineered probiotic, bacteriophage endolysin, antimicrobial resistance (AMR), *Clostridioides difficile* infection

General Abstracts: Session 17—Artificial Intelligence and Data Analytics

77 - Random forest imputation is less biased than other imputation methods when capturing antimicrobial resistance trends over time.

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Idiosyncrasies in antimicrobial resistance (AMR) data—including missing data and changes in antibiotics and concentrations tested over time—make characterizing AMR trends challenging. This study developed random forest models (RF) to impute missing minimum inhibitory concentrations (MIC) and compared their performance to other common imputation methods for missing data. MICs corresponding to 14,605 cattle-associated *Escherichia coli* isolates collected by the National Antimicrobial Resistance Monitoring System (NARMS) between 2002 and 2023 were used for model building. RF models were designed to predict the MIC of a given isolate for the drugs amoxicillin-clavulanic acid (AMC) and ciprofloxacin (CIP), using year of isolate collection (year) and MICs of other antimicrobials as predictors. Models were trained on 70% of the isolates and evaluated on the remaining 30% by comparing predicted MICs against the laboratory-derived values. To assess whether RF imputations helped capture AMR trends, a simulation was designed to randomly delete a percentage of the test data (truncated data set) and impute MICs with the RF, median, and mode MIC value. The simulated missingness ranged from 5% to 40%. Proportional odds (PO) models were then fit to predict the MIC of the respective drug based on year and whether the isolate was collected before or after the Veterinary Feed Directive was enacted in 2017. PO models were fit on original test data (baseline), imputed data sets, and truncated. Each simulation was repeated 1,000 times. The relative bias of the year coefficient was calculated by comparing imputed/truncated model coefficients to the baseline. The exact accuracy (EA) and within-1-MIC-category accuracy (±1) of each RF model are as follows: AMC (EA: 52%, ±1: 96%) and CIP (EA: 85%, ±1: 99%). For both the AMC and the CIP PO models, the mean relative bias across iterations for the year coefficient in the RF-imputed data sets was generally closer to 0 across missingness percentages than the truncated, median-imputed, and mode-imputed data sets. The average relative bias across missingness percentages are: AMC (RF: 5, truncated: 156, median: 222, mode: 222) and CIP (RF: 248, truncated: 549, median: 2,090, mode: 2,090). This indicates that RF-imputed data sets captured AMR trends by year more accurately than the other methods. These results suggest that RF MIC imputations allow for better evaluation of AMR trends over time than leaving the data missing or using simpler imputation approaches.

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Key Words: antimicrobial resistance, machine learning, random forest, imputation, missing data

78 - Spatiotemporal analysis of highly pathogenic avian influenza A (H5N1) occurrence in wild birds in the United States, 2021–2025.

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Highly pathogenic avian influenza (HPAI) A (H5N1) virus poses a threat to poultry, livestock, wild birds, and humans worldwide. HPAI H5N1 has been a concern in wild birds across the United States since 2021. Understanding HPAI H5N1 patterns in wild birds across space and time can help policymakers develop targeted surveillance and mitigation measures to reduce the risk of cross-species transmission of H5N1. This study investigated spatial and temporal patterns of HPAI H5N1 in wild birds at the US state level. Data on HPAI H5N1 in wild birds from December 2021 to May 2025 were obtained from the US Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS) surveillance. The data included 13,134 records, aggregated by state, month, and species. Global Moran's index (I) statistics were computed for wild bird species to detect global spatial autocorrelation. Local Moran's indicators of spatial association (LISA) were computed to identify local clusters of HPAI H5N1 in wild bird species. We conducted a Poisson regression model within the generalized linear modeling framework to investigate the relationship between season and the total case count. Choropleth maps of HPAI H5N1 incidence by wild bird species were generated using ArcGIS Pro version 3.0.3. Global Moran's test showed significant positive spatial autocorrelation in mallards (I = 0.47, *P* = 0.001), green-winged teals (I = 0.16, *P* = 0.043), black vultures (I = 0.22, *P* = 0.002), blue-winged teals (I = 0.16, *P* = 0.044), and bald eagles (I = 0.24, *P* = 0.009). LISA results showed HPAI H5N1 hotspots among mallards in the northeastern US, green-winged teals, black vultures, and blue-winged teals in the southeastern US, and bald eagles in the midwestern US. H5N1 incidence was higher in fall (incidence rate ratio [IRR] = 3.89; 95% CI: 3.6, 4.17), winter (IRR = 3.36; 95% CI: 3.13, 3.60), and spring (IRR = 1.70; 95% CI: 1.58, 1.84) compared with summer. Future work will investigate the relationship between wild bird H5N1 counts and migratory flyways, poultry density, and temperature using geographically weighted Poisson regression to capture spatially varying relationships at the state level of wild bird H5N1 frequency. These findings can help guide targeted strategies in high-risk areas and seasons, for example, by providing educational outreach to poultry and dairy farmers about how HPAI H5N1 spreads and what steps they can take to prevent it.

Financial Support: Lincoln Memorial University College of Veterinary Medicine

Key Words: avian influenza H5N1, spatial epidemiology, temporal pattern, surveillance, wild bird

79 - AstraMEV (AI-guided structural assembly of multi-epitope vaccines) against infectious bronchitis virus.

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Infectious bronchitis virus (IBV) continues to threaten the poultry industry due to its rapid mutation and genetic diversity. We introduce AstraMEV, a predictive structural immunoinformatics platform that leverages genomic screening of 56 IBV genomes to identify conserved epitopes from the nucleocapsid (N) and spike (S) proteins. Two multi-epitope vaccine constructs, SmallTope and BigTope, were designed and refined using AI-driven methods such as RFdiffusion and ProteinMPNN, to optimize structural stability and immunogenicity. Molecular dynamics simulations confirmed the constructs' stability and effective binding to chicken toll-like receptor 4 (chTLR4). Crucially, Poisson-Boltzmann calculations revealed that certain computationally derived variant epitopes significantly improved binding enthalpy, highlighting key interaction domains. Our comprehensive approach demonstrates the feasibility of leveraging computational immunoinformatics for targeted vaccine design. These findings underscore AstraMEV's robust potential for next-generation IBV vaccines and broader applications in combating rapidly evolving pathogens with significant global impact.

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Key Words: artificial intelligence, chicken infectious bronchitis virus (IBV), multi-epitope vaccine, vaccine design

80 - Assessing connectivity and biosecurity compliance of personnel and vehicles among a Spanish poultry production system.

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Compliance with external biosecurity practices is crucial for preventing pathogen introduction and spread within animal production systems. Frequent human and vehicle movements between poultry facilities present significant biosecurity challenges, emphasizing the need for robust monitoring systems. This study aimed to investigate human and vehicle movement networks within a Spanish poultry production system to characterize network interconnectivity and quantify biosecurity breaches. Movement data from 24 poultry sites were collected using Biorisk®External, using QR codes and GPS geolocation. A total of 9,534 visits were recorded from June 2024 to March 2025. Human and vehicle movement networks were constructed, and metrics including average degree, density, clustering coefficient, diameter, and average path length were calculated. Biosecurity compliance was assessed by comparing authorized and nonauthorized visits with biosecurity protocols, focusing on downtime between visits. Analyses were performed in R. The vehicle network showed higher average degree and density (7.13 and 0.078) compared with the human network (5.68 and 0.028), indicating that vehicles are more interconnected among poultry sites than people. The average path length and diameter were slightly shorter for vehicles (5.36 and 35) compared with the human network (5.79 and 45), suggesting faster potential pathogen spread through the vehicle network, though outbreak sizes might be more limited. About 16.6% of movements were authorized despite not meeting biosecurity requirements, mostly involving egg (44.1%) and carcass collectors (16.2%). Additionally, 1,026 unauthorized visits were enforced, highlighting the system's usefulness. The findings underscore the importance of integrating technological solutions into biosecurity management to optimize protocol compliance. Because vehicles were more interconnected, biosecurity plans should focus on vehicle decontamination and limiting proximity to animal areas. Enhanced monitoring and stricter downtime enforcement, particularly for egg collectors and carcass collection workers, are essential to minimize pathogen transmission. This study demonstrates the critical role of data-driven approaches in managing biosecurity risks, advocating for broader adoption across food animal industries.

Key Words: information technology, truck movement, SNA, chicken, commercial poultry

81 - Planners, balancers, and drifters: A forward induction approach to understanding learning archetypes in biosecurity decision-making.

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This study employs forward induction to estimate a learning model for the decision-making process of livestock producers when investing in biosecurity. Computational models of disease spread often assume that producer behavior is static, yet real people learn over time and adjust their choices in response to internal and external factors. In future models, this behavioral dynamism should be carefully considered. Using data from a biosecurity investment game, we classify different types of player learning and compare behavior to predictions from backward induction. Participants (n = 533 after cleaning) made biosecurity investment decisions in a simulated disease spread scenario under varying biosecurity-investment thresholds for conditional indemnity. We assumed that players tried to maximize their profits and took exploratory or greedy actions according to a Softmax distribution. Using maximum likelihood estimation, we fit the a (learning rate) parameter of a temporal difference learning model and the β (inverse temperature) parameter of the Softmax distribution for exploratory/greedy behavior. Parameters were fit by minimizing the negative log-likelihood of observed actions using the L-BFGS-B algorithm. We clustered player behavior with k-means (k = 3 based on elbow and silhouette analyses). Results from forward induction (predicting behavior step by step as players learn) revealed similar patterns to backward induction (theory-based reasoning from the end of the game backward): in most cases, the optimal strategy is to invest up to the indemnity threshold. We identify 3 distinct clusters of player learning behavior. Planners (low a, high β) update predictions of future rewards slowly and act consistently; balancers (moderate a, β) balance learning with exploration; and drifters (high a, low β) update estimates rapidly but behave erratically. This forward induction analysis lends additional support to previous backward induction findings and offers novel insights into the different ways decision-makers learn over time. These findings could inform policy strategies to enhance biosecurity practices and better manage disease risks in the agriculture and animal health fields. Future work will explore ways to incorporate these findings into a national-level agent-based model of producer behavior and disease spread and investigate other aspects of learning, including foresight and memory.

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Key Words: biosecurity, learning, agent-based modeling, survey, forward induction, indemnity

82 - Beyond the flavor: Shotgun metagenomics for functional insights and MAG recovery in bovine liver abscesses.

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Liver abscesses (LAs) are a prevalent and costly challenge in the North American beef industry, leading to condemnation, and reducing carcass yield and feed efficiency. Although 16S rRNA amplicon sequencing has shown that LAs contain distinct microbial community types—or flavors—its limited resolution and lack of functional insight make shotgun metagenomics a more powerful alternative. Prior work from our group showed that LA communities can be broadly categorized into 3 types: high *Fusobacterium*, high *Bacteroides*, or high diversity. Here, we applied shotgun metagenomics to characterize prevalent LA microbial species, compare functional profiles for the first time, recover whole genomes, and guide the design of a target-enrichment bait set for dominant taxa. Based on a previous study, we selected 8 samples from each LA flavor (n = 24). Shotgun metagenomic libraries were prepared and sequenced on an Illumina NovaSeq 6000 instrument using paired-end chemistry at the North Texas Genome Center. Raw sequence reads were processed using the AMR++ pipeline, and functional profiling was performed using HUMAnN3. Metagenome-assembled genomes (MAGs) were recovered using MEGAHIT and MetaBAT2 and assessed for quality using CheckM. Statistical analyses were conducted in R Studio. Each of the 3 previously defined LA types displayed a distinct species-level taxonomic profile. Analysis of functional profiling revealed significant differences in gene family composition among LA types, with LA type explaining 55% of variance (n = 8; PERMONOVA, $P < 0.001$). Twenty-nine genes were identified to be differentially abundant between LA types by ANCOM-BC, with nearly all differences observed between the high-*Fusobacterium* LA type and the high-diversity and high-*Bacteroides* LA types. We recovered 134 high-quality MAGS, spanning microbial lineages traditionally associated with LAs (e.g., *Fusobacterium necrophorum*, *Trueperella pyogenes*) and including putative new species of *Porphyromonas*, *Bacteroides*, and *Desulfovibrio*. By refining our understanding of the microbes linked to LA formation and their functional capacity, this research highlights potential mechanisms of disease pathogenesis, and the challenges related to LA treatment and prevention. Ongoing work is applying shotgun metagenomics to our entire library of LA samples (n >800) and using target-enriched metagenomics to trace prevalent LA taxa back to their origins throughout the bovine gut.

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Key Words: liver abscess, beef cattle health, shotgun metagenomics, *Fusobacterium necrophorum*, *Bacteroides*

General Abstracts: Session 18—Immunology 1

83 - Induction of secondary and tertiary lymphoid microstructures in fish.

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In mammals, antibody (ab) responses are induced in organized lymphoid tissue areas (e.g., lymph nodes, germinal centers) as well as in non-lymphoid organs (e.g., liver, fat, kidney) where organized lymphoid tissue termed as tertiary lymphoid organs (TLOs) are induced upon infection and other stimuli. Whether fish contain TLOs remains unknown; thus, an objective of this work was to evaluate the presence of TLOs in rainbow trout. Although the presence of secondary or tertiary lymphoid structures analogous to GCs or TLOs has been thought to be absent in teleost fish, we have recently shown that, upon immunization or infection, fish develop organized lymphoid microstructures (LMs) in the spleen that are functionally analogous to germinal centers. Our more recent data points to the presence of previously unrecognized TLOs in fish. These TLOs were discovered through the use of splenectomized (SPLT) fish in combination with immunofluorescence microscopy studies. Interestingly, SPLT fish were still able to generate strong primary antibody responses, and memory responses were dramatically reduced. This led to the hypothesis that additional sites other than the spleen were contributing to the induction of fish primary immune responses. The study of these responses during the last year has led to the discovery of an additional potential inductive site of the fish immune response, namely the mesenteric fat tissue (MFAT), which was identified through immunofluorescence microscopy studies. We observed a significantly higher number of proliferating IgM+ B cell zones in MFAT from immunized fish compared with those of control animals (*t*-test, $P < 0.001$). As MFAT is not a lymphoid organ, the LMs found therein can be considered as TLOs. In conclusion, our studies point to the discovery of the first TLOs in a non-mammalian species. These findings have important implications for the development of fish vaccines that stimulate not only splenic LMs but also non-splenic TLO structures.

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Key Words: fish, germinal center, tertiary lymphoid organ

84 - Exploring and exploiting immune priming and its specificity in Eastern oysters, *Crassostrea virginica*.

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For decades, the aquaculture industry has suffered economic losses via unexplained mortality. Although many stressors have been implicated, perhaps the most historically significant are infectious diseases. Yet, current disease management practices suffer from many issues, including in Eastern oysters, where distinct pathogens hamper production during critical periods of development. To assuage these concerns, there is great potential to leverage "immune priming," which may improve host resistance/tolerance in the face of varying pathogen pressures before disease onset. Here we test this hypothesis and predict that homologous challenges will lower infection intensities and increase survival. Life stage–specific activation of oyster defenses was performed using heat-inactivated stimuli. Homologous and heterologous challenges were created from cultured stocks of *Vibrio coralliilyticus*, *Vibrio alginolyticus*, and *Tenacibaculum ascidiaceicola* grown on selective agar or yeast-peptone media (YP-30). Single colonies were then inoculated into YP-30 broth until desired working concentrations were obtained (i.e., 5×10^5 cfu/mL). Treatments were then heat-killed for 1 h at 75°C. Larvae were then placed in 96-well plates (~75 individuals/mL) and primed with 1 of 4 heat-killed treatments. Six hours post-priming, larvae were given either a live pathogen or control, representing all possible priming-by-pathogen combinations. Preliminary work has revealed immune priming specificity at the larval stage of development, where not only is survival improved by homologous challenges (χ^2 = 29.33, d.f. = 1, $P < 0.001$), but protection is pathogen species–specific (χ^2 = 144.73, d.f. = 4, $P < 0.001$). Work is still ongoing to reveal how responses differ across hatchery sources, and distinct life stages. The ubiquity of pathogens in marine wildlife necessitates preventative strategies that effectively combat disease. Because there are distinct differences in the infectious agents that oysters face, leveraging immune priming in a life stage–specific manner is critical for Eastern oyster health at all lines of commercial production. Preliminary work thus far has revealed specificity in protection at the larval stage, and, although work is ongoing, exploring and exploiting the efficacy of this evolved phenomenon in Eastern oysters has great potential to benefit aquaculture disease management practices at both regional and national scales.

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Key Words: immune priming, Eastern oyster, infectious disease

85 - Nebulized mRNA encoding an anti-VapA monoclonal antibody provides partial protection from *Rhodococcus equi*.

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Rhodococcus equi is a facultative intracellular pathogen that causes severe pneumonia in foals worldwide. The virulence-associated protein A (VapA) is necessary for *R. equi* to cause disease in foals. Currently, the standard method for preventing *R. equi* pneumonia is passive immunization by transfusion of hyperimmune plasma (HIP), which is costly, labor- and time-intensive, and carries risks for foals. As an alternative to HIP transfusion, our laboratory has developed mRNA encoding the heavy (H) and light (L) chains of an immunoglobulin G_1 (IgG_1) monoclonal antibody (mAb) targeting the VapA of *R. equi*. When formulated with poly-β-amino-thio-ester (PBATE) and nebulized into the lungs of foals, the VapA mAb was translated from the nebulized mRNA and detectable in bronchoalveolar lavage fluid ≥19 d and in serum for up to 40 d after nebulization. In a second arm of the experiment, our objective was to determine whether nebulization of mRNA encoding the VapA mAb IgG_1 could protect foals against intrabronchial *R. equi* infection by comparing the incidence of pneumonia between nebulized foals and controls. At age 5 d, 8 foals were nebulized with mRNA-polymer complex encoding the anti-VapA mAb, and 3 non-nebulized foals served as controls. Two days later (at age 7 d), all foals were challenged intrabronchially with 5 × cfu of virulent *R. equi*. Foals were monitored for evidence of pneumonia by twice-daily physical examinations and weekly thoracic ultrasonograms and hemograms. The incidence of pneumonia was compared between the 2 groups using Fisher's exact test. Clinical pneumonia developed in 5 of 8 (62.5%) nebulized foals and in all 3 (100%) control foals (P = 0.49). These results indicate that nebulization of 10 mg of anti-VapA mRNA as formulated with PBATE provided only partial protection against intrabronchial infection with *R. equi*. Future studies will focus on methods to improve potency of the expressed antibody.

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Key Words: *Rhodococcus equi*, mRNA, monoclonal antibody, VapA, pneumonia, foal

86 - A novel polymeric carrier improves transfection of foals nebulized with mRNA encoding a monoclonal antibody targeting the pulmonary pathogen *Rhodococcus equi*.

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Rhodococcus equi is a bacterial pathogen that causes severe pneumonia in foals worldwide. The virulence-associated protein A (VapA) is necessary for *R. equi* to cause disease in foals and thus represents a rational therapeutic and preventative target. The current standard for preventing rhodococcal pneumonia in foals is passive immunization by transfusion of hyperimmune plasma, which is costly, labor- and time-intensive, and carries risks for foals. As an alternative to transfusion, we aim to deliver an mRNA encoding a VapA-targeting monoclonal antibody (mAb) directly to the lungs of foals by nebulization. Our initial design used 2 mRNAs encoding an IgG_1: 1 encoding the heavy (H) and another encoding the light (L) chain, with a nanoluciferase tag fused to the L chain to enable sensitive detection and to distinguish from endogenous IgG_1 by luminescence. Because lipid nanoparticles are unstable as mRNA carriers during nebulization, the mRNA was formulated with a poly-β-amino-thio-ester (PBATE) polymer. In this formulation, the VapA mAb was undetectable in bronchoalveolar lavage fluid (BALF) 5 d post-nebulization and absent from serum at all time points. Therefore, we reformulated the mRNAs (including the L chain with the nanoluciferase sequence) in a novel, optimized PBATE polymeric compound and compared its performance with the original formulation. Foals were nebulized at age 2 d with the new (n = 3) or original (n = 2) PBATE-mRNA formulations; 3 non-nebulized foals served as controls. BALs were performed at 2, 5, 12, and 26 d after nebulization, and serum was collected at the same time points; for the new formulation and control groups, serum was also collected 40 d after nebulization. Serum and BALF were tested by luminescence ELISA to detect the relative concentration of mAb. The new PBATE carrier resulted in significantly ($P < 0.05$) higher mAb concentration (≈100-fold) for longer duration in BALF (≥19 d after nebulization in all 3 foals) than the original formulation. The mAb was not detected in serum at any time with the original formulation but was detected up to 40 d after nebulization with the new formulation. Moreover, the time required for nebulization was reduced by ≈50% using the new PBATE. The novel PBATE greatly improves the efficiency and effectiveness of nebulizing mRNA to the lungs of foals.

Financial Support: This work was supported by a grant from the Foundational and Applied Science Program, project awardnumber 2022-67015-36335, from the U.S. Department of Agriculture's National Institute of Food and Agriculture. Additional support was provided by the Link Equine Research Endowment, Texas A&M University and the Glenn Blodgett Chair in Equine Studies.

Key Words: *Rhodococcus equi*, mRNA, poly-β-amino-thio-ester, monoclonal antibody, VapA, IgG_1

87 - Intestinal immune responses in foals to enteral immunization with live virulent *Rhodococcus equi*.

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Rhodococcus equi is an intracellular bacterium that causes severe pneumonia in foals worldwide. Gavage with live virulent *R. equi* has been shown to protect foals against *R. equi* pneumonia after intrabronchial challenge, but the mechanisms involved in the gut-lung axis in foals are not well characterized. Recent evidence demonstrates that RNA-sequencing (RNA-Seq) analysis of equine fecal exfoliated intestinal cells (exfoliome) closely mirrors the gastrointestinal mucosal transcriptome and provides a noninvasive approach to investigate intestinal cellular and molecular pathways. The exfoliome, therefore, is a potentially useful tool to study the local intestinal immune responses following immunization with *R. equi* by gavage in foals. Our objective was to compare the exfoliome from foals gavaged with either live virulent *R. equi* or saline in the first week after birth, to identify mechanistic pathways that might be associated with protection against *R. equi* pneumonia. Healthy Quarter Horse foals (n = 5) were randomly assigned to receive either live virulent *R. equi* (VRE; 2×10^9 *R. equi* in 100 mL of NaCl 0.9%; n = 3) or saline (100 mL of NaCl 0.9%; n = 2) by gavage at ages 1 and 3 d. Fecal samples were collected at ages 1, 14, and 28 d, and stored in DNA/RNA Shield for subsequent analysis of RNA-Seq of exfoliated cells. Data will be analyzed and integrated using multiple computational approaches and underlying regulatory mechanisms will be inferred from features identified through the exfoliome analysis. Gavage of foals and sample collection have been completed. RNA-Seq analyses of exfoliated intestinal cells from foals and associated pathways are pending but will be presented at the conference. The exfoliome is a noninvasive tool for evaluating intra-intestinal immune responses to enteral immunization in foals and might help in determining the protective mechanisms behind the gut-lung axis in foals.

Financial Support: USDA-NIFA, Glenn Blodgett Chair In Equine Studies, Link Equine Research Endowment, Grayson Jockey Club Research Foundation

Key Words: *Rhodococcus equi*, exfoliome, gut-lung axis, gastrointestinal immune response, gavage, foal, pneumonia

88 - Analysis of porcine γδ thymocyte development using a new single-cell γ and δ chain TCR profiling assay.

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γδ T cells are a population of innate-like T lymphocyte that serve as a crucial link between the innate and adaptive immune systems. The γδ T cell receptor (TCR) is composed of γ and δ protein chains. When assembled, it recognizes a broad spectrum of antigens, usually without the aid of major histocompatibility complex molecules. For unclear reasons, pigs, like other artiodactyls, possess high concentrations of γδ T cells. To better understand porcine γδ T cell biology, including their development, phenotypes, and function, we developed porcine-specific γδ TCR enrichment primers that are compatible with the 10× Genomics V(D)J sequencing platform to perform paired single-cell RNA and γδ TCR sequencing. Using this assay, we profiled the γδ TCR repertoire in the pig thymus from d 60 of gestation to 7 mo of age. We successfully mapped γ chain V, J, and C gene segments to the IMGT reference database (δ chains are not yet fully annotated). We observed (1) high expression of γ and δ chain transcripts in double-negative (DN) thymocytes that far exceeded pre-TCR and b chain expression; (2) limited γ chain V–J segment usage and recombination diversity throughout development; (3) sustained DN γ chains expression across life, in contrast to humans, in which DN γ chain expression declines markedly after birth; and (4) a substantial shift in V–J segment usage and recombination patterns between fetal and postnatal thymi samples. Together, these findings provide novel insights into the development of porcine γδ T cells and demonstrate the utility of our new single-cell–based high-throughput γδ-TCR assay.

Financial Support: NIH HD092286 and AI158477

Key Words: pig, single cell, γδ TCR, immune system development

General Abstracts: Session 19—Virology 1

89 - Divergent porcine astrovirus 4: Distinct respiratory and gastrointestinal genetic clades.

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Porcine astrovirus 4 (PoAstV4) is a non-enveloped, positive-sense, single-stranded RNA virus that has been primarily associated with gastrointestinal (GI) disease. In 2016, PoAstV4 was first reported in pigs presenting with acute respiratory disease. Around the year 2019, additional cases emerged, involving pigs with respiratory disease, and its association with lesions characteristic of epitheliotropic viral infection was confirmed. Retrospective analyses from the Iowa State University Veterinary Diagnostic Laboratory suggest that respiratory-associated PoAstV4 strains are genetically distinct from those detected in the GI tract. The genetic dissimilarity between these 2 clades has been quantified at ~22.5–26%. Both ORF1ab and ORF2 regions contribute to the observed divergence, with homology at 74–76% in ORF1ab and 62–69% in ORF2 between the 2 clades. Phylogenetic methods were used to infer a time to the most recent common ancestor for the respiratory-associated clade, which was found to be 1996 (1989–2002, 95% HPD). The substitution rate for the respiratory lineage was estimated to be ~4.2 nucleotide sites per year across the ORF2, which encodes for both the spike and the capsid proteins. Comparison of genetic sequences revealed 46 distinct nucleotide mutations that differ between the 2 clades, 17 in ORF1ab and 29 located in ORF2. Additionally, we identified a 13–14 amino acid difference located in the C-terminal end of the capsid-coding region in ORF2 that may contribute to functional variation between strains of the 2 clades. This region includes an insertion of 5–6 amino acids and a nearby deletion of similar length, which may serve as a potential target for PCR-based differentiation. These regions in the structural protein were mapped onto predicted protein structures using AlphaFold3 and visualized in PyMOL. Although the functional role of these mutations in tissue tropism remains unconfirmed, this study provides a foundation for exploring tissue-specific adaptation in PoAstV4. Our findings offer new insight into the molecular differentiation of PoAstV4 strains, which may improve diagnostic resolution and inform future detection strategies for emerging respiratory variants.

Key Words: porcine astrovirus 4, tissue tropism shift, capsid polyprotein, genetic divergence

90 - Mutations in the macrodomain 1 of NSP3 attenuate PEDV virulence in vivo without impairing in vitro replication.

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The conserved macrodomain 1 (Mac1) within non-structural protein (NSP) 3 of coronaviruses (CoVs) can reverse ADP-ribosylation of proteins and contributes to viral virulence. Although Mac1 has been characterized in several CoVs, its role in porcine epidemic diarrhea virus (PEDV) remains unknown. Because D445 and N462 are functionally important residues for several β-CoVs, we mutated the 2 residues for PEDV Mac1 protein and the infectious virus (icPC22A-NSP3-D445A and icPC22A-NSP3-N462A) and analyzed them in vitro and in vivo. Sequence alignment revealed that N462 is highly conserved, whereas D445 is less conserved among porcine CoVs. The N462A significantly impaired ADP-ribose binding and hydrolysis activity of Mac1, whereas D445A had minimal effect. Compared with wild-type PEDV, both PEDV mutants replicated efficiently in Vero and LLC-PK1 cells. Unexpectedly, both mutated viruses induced significantly lower levels of type I and type III interferons (IFNs) in LLC-PK1 cells, in contrast to previously characterized Mac1 mutants of SARS-CoV-2 and mouse hepatitis virus (MHV)-JHMV strain. Moreover, the icPC22A-NSP3-N462A virus showed significantly reduced sensitivity to IFNβ treatment in Vero cells. In gnotobiotic neonatal piglets, icPC22A-NSP3-N462A showed reduced peak viral shedding and attenuated virulence compared with wild-type PEDV. These results demonstrate that N462, but not N462, is important for Mac1's ADP-ribose binding and hydrolysis function. Although the N462 residue is insignificant for PEDV replication in vitro, it modulates viral replication and pathogenesis in vivo. These results may suggest CoV host- or tissue-specific adaptations. Our findings identify Mac1 N462 as a rational target for live attenuated vaccine design and highlight Mac1 as a potential target for antiviral therapeutics against PEDV. This study provides the first in vitro and in vivo evidence for the function of key residues in the Mac1 domain of a porcine enteric CoV and represents the first in vivo characterization of Mac1 function of α-CoVs.

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Key Words: coronavirus, porcine epidemic diarrhea virus, non-structural protein 3, Mac1, ADP-ribosylation, interferon

91 - Prevalence estimation and phylogenetic analysis of porcine rotavirus C in Hungarian pig herds.

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Rotaviruses (RVs) belong to the *Sedoreoviridae* family and are significant pathogens associated with acute gastroenteritis in both animals and humans. The genus *Rotavirus* comprises 9 species (A to I), and their genome consists of 11 segments of double-stranded RNA, encoding 6 structural (VP) and 5 nonstructural (NSP) proteins. All strains are classified into VP4 (P, protease-sensitive) and VP7 (G, glycoprotein) genotypes. RVs are considered endemic in swine populations worldwide. RVC was first identified in swine in 1980 and has since been detected in several species, including cattle, ferrets, dogs, and humans. In pigs, it is mainly associated with diarrhea outbreaks in suckling and weaned piglets, but it also circulates among postweaning animals with potential pathogenic relevance. This study aims to determine the prevalence and genetic diversity of porcine RVC in Hungary. To assess RVC prevalence, 228 oral fluid samples were collected from 27 asymptomatic pig farms and tested by RT-qPCR. For genetic characterization, 97 fecal swabs were collected from 12 herds experiencing diarrhea outbreaks. Samples with high viral load were subjected to nanopore next-generation sequencing for genotyping. Phylogenetic trees were generated in MEGA X using the maximum likelihood method with 1,000 bootstrap replicates. RVC RNA was detected in 27% of oral fluid samples, across 75% of farms, indicating widespread subclinical circulation. Analysis of fecal swabs from diarrheic pigs showed that 79% contained at least 1 RV, with RVC detected in 55% of cases. VP7 genotyping identified G1 and G5–7 types, and the phylogenetic analysis of these strains showed close relatedness to RVC strains from several European countries, suggesting a regional lineage. VP4 genotyping identified 3 genotypes (P[1], P[4], and P[6]), but 1 sequence showed less than 85% identity to known VP4 genes, suggesting the presence of a novel P genotype. For the G1P[6] and G6P[X] strains, full RVC genomes were successfully obtained, displaying a common Cowden-like porcine backbone constellation. These findings enhance our understanding of RVC epidemiology and evolution in Hungarian pig populations. As RVC was frequently detected in co-infections with other viral and bacterial enteric pathogens in diarrheic piglets, our future work will focus on clarifying its role within the multifactorial etiology of diarrhea outbreaks in pigs.

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Key Words: rotavirus C, swine, nanopore sequencing, prevalence, genetic diversity, epidemiology

92 - Prevalence and genetic characterization of porcine rotavirus B in Hungarian pig farms.

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Rotaviruses (RVs) are small RNA viruses belonging to the *Sedoreoviridae* family and are among the most significant viral pathogens causing acute gastroenteritis in both animals and humans. The genus *Rotavirus* comprises 9 species (A–I), distinguished by the antigenic properties of VP6. The RV genome consists of 11 segments of double-stranded RNA enclosed in a triple-layered particle (TLP), encoding 6 structural (VP) and 5 or 6 non-structural (NSP) proteins. RVs are considered endemic in swine populations worldwide. Rotavirus B (RVB, *Rotavirus betagastroenteritidis*) was first detected in pigs with diarrhea and later in cattle, lambs, rats, and horses. In pigs, RVB has been reported in Brazil, the United States, and Japan, as well as in several European countries, typically in older animals. To assess RVB prevalence in Hungary, 228 oral fluid samples were collected from 27 asymptomatic pig farms and tested by RT-qPCR. For genetic characterization, 33 fecal swabs were collected from 6 herds experiencing diarrhea outbreaks. Samples with high viral load were genotyped using nanopore next-generation sequencing. Viral RNA was detected in oral fluid samples from approximately 35% of the farms, indicating widespread subclinical circulation. RVB was also detected in 10/33 (30.3%) fecal swab samples, of which we obtained 4 VP7 sequences that were classified as G20 or G12 genotypes, and 1 VP4 sequence that belonged to the P[4] genotype. In one case, a full RVB genome was successfully obtained, displaying the following constellation: G20-P[4]-I11-R4-C4-M4-A7-N5-T4-E4-H4. This configuration closely resembles those described in North American RVB strains, which often share conserved internal gene segments such as VP1, VP2, VP3, NSP3, NSP4, and NSP5. Based on nBLAST analysis, the NSP1, NSP2, and NSP3 genes showed high identity with porcine-associated genogroups. Co-infection with RVA and RVC strains was detected in 8 samples, and RVB was also frequently found alongside other viral and bacterial enteric pathogens, suggesting a multifactorial etiology in diarrheic piglets. These findings enhance our understanding of RVB epidemiology and evolution in Hungarian pig populations. We detected RVB on more than one-third of the farms surveyed, which emphasizes the need for regular screening. In the future, we plan to examine a much larger sample pool across 5 age groups to assess the susceptibility of newborn, young, and older animals to different RVs.

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Key Words: gastroenteritis, diarrhea, rotavirus B, phylogeny, nanopore sequencing, epidemiology

93 - The association of rotavirus species with gastrointestinal and respiratory illness in suckling and weaned pigs under field conditions.

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Rotavirus A (RVA) and C (RVC) cause acute gastroenteritis (AGE) in young children and animals, including piglets. Rotavirus B (RVB) causes AGE in adults and older animals. Multiple studies have demonstrated RVA/RVC presence in blood and different visceral organs (including lungs) of children and piglets. Additionally, some studies have reported on respiratory signs concurrent with RVA/RVC shedding in piglets. However, knowledge on RVA/RVC respiratory tropism and pathogenesis remains limited, and even less is known about RVB intestinal and extraintestinal involvement. We screened nasal and rectal swabs from suckling and weaned piglets (n = 427, from 6 Ohio swine farms: 4 commercial and 2 research, F1–F6) that were either healthy or had diarrhea or respiratory signs for RVA, RVB, and RVC. Swabs collected from different tissues of 16 dead piglets (aged 1–3 weeks, all-cause mortality) from F2 were also tested for RVA/RVB/RVC. Samples were analyzed using RT-qPCR to detect each virus. For all comparisons, statistical analysis was conducted using one-way ANOVA with Bonferroni's multiple comparison test ($P \leq 0.05$). We found 88%, 9%, and 29% of piglets positive for RVA, RVB, and RVC, respectively, and the prevalence varied greatly between different farms (RVA: 67–100%, RVB: 0–46%, RVC: 7–56%). Consistent with prior research, the highest RVA titers were shed by diarrheic weaned piglets on most farms. Of special interest, F6 suckling piglets with respiratory signs had increased RVA titers, suggesting that increased respiratory RVA tropism can be observed in this age group. We confirmed RVA presence in various (including respiratory tract) samples of 12 out of 16 dead piglets. In sharp contrast to RVA, RVC shedding was either at baseline level (suggestive of no ongoing outbreak) or higher in suckling piglets with diarrhea on all farms but one. In addition, RVB was found on 3 farms, F1, F4, and F6, with most cases clustering on F1 and almost exclusively affecting weaned piglets. Additionally, 67% of F1 piglets with respiratory signs were RVB+, whereas only 44% of healthy piglets and 37% of diarrheic piglets were RVB+. These data suggest that RVA/RVC remain highly prevalent diarrheagenic pathogens in piglets; however, the mechanisms of RVA and RVC transmission and pathogenesis are distinct. Also, although RVB prevalence was substantially lower, our data suggests its possible association with respiratory pathology in weaned piglets requiring further investigation.

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Key Words: rotavirus, respiratory illness, diarrhea, suckling pig, weaned pig, field conditions

94 - Prevalence of rotavirus A and C in Polish pig herds.

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Rotaviruses (RV) are known to cause diarrhea in various species, including pigs. Although they have zoonotic potential, there is limited data on their prevalence in Poland. This study aimed to determine the prevalence of rotavirus A and C (RVA and RVC) on 33 Polish farms (F1–F33). Among them, 17 were large-scale sow farms (1,400 to 5,200 head) operated by major pork producers in Poland, whereas the remaining 16 were small-scale farms (70 to 420 animals) owned by independent private farmers. Between 2016 and 2024, feces, rectal swabs, and ileum contents were collected from animals up to 22 weeks of age. All the samples were tested for RVA and RVC using real-time RT-PCR assays. Fisher's exact test was used to compare prevalence between farm sizes. This study was based on diagnostic submissions, and, as a result, the number of samples collected per farm varied greatly (1–61). Of the 434 submitted specimens, RVA was found in 12.0% (52/434), and RVC in 41.2% (179/434) of samples. In the large-scale farms, the percentages of positive samples for RVA and RVC were 11.5% (30/260) and 58.5% (152/260), respectively. On small-scale farms RVA was present in 12.6% (22/174) samples, whereas RVC was detected in 15.5% (27/174). The difference in RVC prevalence between both studied groups was statistically significant ($P < 0.05$). The prevalence of both RVA and RVC varied among individual farms. Prevalence of RVA on the farm level ranged from 0% to 57.1% on farm F11 (4/7), whereas the occurrence of RVC ranged from 0% to 100% on farm F1 (5/5). To estimate RV distribution by age, samples were grouped into neonates (≤1 week), piglets (2–4 weeks), and weaned pigs, including both growers (5–8 weeks) and finishers (9–22 weeks). RVC was more common in neonates (61.5%, 139/226) and weaned pigs (22.3%, 33/148), whereas RVA dominated in piglets (20%, 12/60). Understanding the variations in the occurrence of rotavirus species across various farms and age groups may help improve the control of diarrhea in suckling piglets. Further studies are needed to assess the dynamics of rotavirus infections in Poland and comprehend their significance as a factor, or a co-factor, of piglet diarrhea.

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Key Words: rotavirus A, rotavirus C, pig, prevalence, Poland

General Abstracts: Session 20—Disease Pathogenesis—Avian

95 - *Clostridium paraputrificum*-metabolized chicken bile reduces *Clostridium perfringens* virulence.

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Clostridium perfringens is the main pathogen of chicken necrotic enteritis (NE). Dietary bile acid deoxycholic acid (DCA) protected chickens against NE and improved growth performance, whereas chicken bile (cheno-DCA and cholic acid [CA]) did not. It remains largely unclear what molecular mechanism of bile acids differently influencing *C. perfringens*-induced NE. In this study, we hypothesized that chicken bile metabolized by microbiota altered *C. perfringens* pathogenesis. We aimed to investigate the relationship of *Clostridium paraputrificum*-metabolized chicken bile on *C. perfringens* virulence. *Clostridium perfringens* was incubated with 0.1, 0.25, and 0.5 m*M* chicken bile in BHI or with *C. paraputrificum* and 0.25 m*M* chicken bile or ox bile (tauro- and glyco-CA). After 24 h, *C. perfringens* was enumerated by serial dilution and plating on *C. perfringens* agar plates tryptic soy agar with sodium thioglycolate, D-cycloserine, and sheep blood. *Clostridium perfringens* virulence gene expression was measured by qPCR and H_2S disc assay. Differences between treatments were analyzed using one-way ANOVA followed by Tukey correction using Prism 7.0 software. Differences were statistically significant if *P*-values were <0.05. *Clostridium perfringens* growth in BHI (control) reached 7.35 $\log_{10}$ cfu/mL after 24-h culture. Compared with the control, bacterial growth was 7.38, 7.29, and 7.09 ($P < 0.05$) $\log_{10}$ cfu/mL in 0.1, 0.25, and 0.5 m*M* chicken bile, respectively. In a separate experiment, *C. perfringens* growth in BHI (control) reached 7.13 $\log_{10}$ cfu/mL after 24-h culture, whereas its growth was significantly reduced by 2.95, 9.96, and 100.00% in *C. paraputrificum* (Cpara), 0.25 m*M* chicken bile (ch_bile), and chicken bile plus *C. paraputrificum* (Cpara+ch_bile), respectively. Importantly, the Cpara+ch_bile group significantly inhibited *C. perfringens* growth compared with either Cpara or ch_bile. In addition, Cpara+ch_bile or Cpara reduced *C. perfringens* H_2S production. Gene expression of *asrA1*, responsible for H_2S production, was significantly reduced by 33% and 42% in Cpara+ch_bile and ch_bile groups, respectively. Gene expression of *virT* and *cpe* was significantly reduced in Cpara, ch_bile, and Cpara+ch_bile groups. Interestingly, ox bile metabolized by *C. paraputrificum* only reduced 0.95 $\log_{10}$ cfu/mL *C. perfringens* growth. We found that *C. perfringens* virulence was inhibited by ch_bile, but not ox bile, metabolized by *C. paraputrificum*. This finding could be used as an effective alternative to antibiotics against chicken NE.

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Key Words: bile acid, *Clostridium paraputrificum*, *Clostridium perfringens*, metabolite, virulence

96 - Developing a bile acid-metabolizing enzyme expression system.

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Clostridium perfringens is the main pathogen of necrotic enteritis (NE) and causes $6 billion loss to the global poultry industry yearly. The primary bile acid microbial metabolite deoxycholic acid (DCA) mitigates *C. perfringens*-induced NE. Approaches to bio-transform DCA or other bile acids are limited. This study aimed to clone and express bile acid-metabolizing enzymes to bio-transform bile acids. *Clostridium scindens* genomic DNA was extracted, and the bile acid-metabolizing genes *baiE* and *baiA2* were amplified by PCR. After gel electrophoresis, *baiE* and *baiA2* bands were excised and purified. The *baiE* and *baiA2* PCR inserts and pET28a plasmids were digested with *Bam*-HI-HF and BmtI-HF restriction enzymes and ligated, followed by transformation into *Escherichia coli* DH5a. After PCR genotyping, the resulting plasmids were named *baiE*-pET28 and *baiA2*-pET28, respectively. The plasmids were transformed into *E. coli* BL21 cells. After overexpression using 0.4 m*M* isopropyl β-D-thiogalactopyranoside (IPTG), the proteins in the BL21 cells were lysed and then subjected to SDS-PAGE, followed by Coomassie Blue staining and Western blotting with Histag. *Clostridium scindens* genomic DNA was separated on 1% gel, and bands with sizes more than 1.0 kb were collected and purified. The *baiE* and *baiA2* PCR products were around 500 bp and 800 bp, respectively, which was consistent with the literature. After digestion, ligation, and DH5a transformation, colonies were genotyped by PCR, and the positive colonies were cultured for *baiE*-pET28 and *baiA2*-pET28 plasmid amplification. The plasmids were then transformed into BL21 cells, after which *baiE* and *baiA2* proteins were overexpressed with IPTG induction. After lysing the BL21 cells, the crude cell extracts were subjected to SDS-PAGE. In the Coomassie Blue stained gel, clear bigger bands around 26 kDa and 34kDa were present in cells with *baiE*-pET28 and *baiA2*-pET28 plasmids, respectively. The proteins in another SDS-PAGE gel were transferred onto cellulose membranes. Western blotting with the Histag antibody showed proteins around 26 kDa and 34 kDa for *baiE* and *baiA2*, respectively. We are expressing other bile acid-metabolizing enzyme genes and will evaluate their enzyme activities on bile acid metabolism. We have successfully cloned and expressed bile acid-metabolizing enzymes *baiE* and *baiA2* genes. After these bile acids enzyme activities are evaluated, specific bile acid derivatives could be bio-transformed in vitro and in animals.

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Key Words: bile acid-metabolizing enzyme, PCR, *Clostridium scindens*, protein overexpression

97 - The effect of exosomes from tumor-bearing chickens (TEX) on innate immune signaling.

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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 lifelong 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 their contribution to lymphomagenesis, tumor progression, and 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. In the examination of hypothesis (3), we have purified exosomes from the serum of tumor-bearing chickens via size-exclusion chromatography. These were all characterized by TEM, nanotracking analysis (NTA), and proteomic as well as whole-transcriptome sequencing. This year, we examined the effect of TEX pre-treatment on innate immune signaling of HD11 cells and those programmed to be mature macrophages and dendritic cells in response to innate immune agonists LPS and Poly I:C. Using chicken kinome arrays, we examined phosphorylation patterns at 1 and 3 h post-treatment. In pathway analysis of this signaling, we identified that TEX predisposed cells to more proliferative (growth factor, MAP kinase) signaling and interferon receptor recycling. Our data suggest a novel innate immune evasion pathway mediating decreased response to type I interferons through targeted downregulation of a receptor subunit.

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Key Words: Marek's disease, Marek's disease virus, exosome, immune suppression, pathogenesis, innate immunity

98 - The role of chuA on *Campylobacter jejuni* responding to bile acid 7β cholic acid.

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Campylobacter jejuni is a major foodborne pathogen worldwide, with limited understanding of the mechanism of its pathogenesis. Bile acids are weak acids and mediate *C. jejuni* infections in *Il10*$^{-/-}$ mice. In this study, we aimed to elucidate the effect of acidity and bile acids on *C. jejuni* pathogenesis. Growth of *C. jejuni* 81–176 in the presence of 7β cholic acid (CA) was measured in brain heart infusion (BHI) broth at pH 7 of neutral environment and 5.75 of small intestine lumen. *Campylobacter jejuni* growth was enumerated by serial dilution and plating under microaerobic conditions for 48 h (h). *Campylobacter jejuni* virulence gene expression was evaluated by quantitative PCR (qPCR). PCR was used to assemble a linear construct containing the kanamycin resistance cassette flanked by *chuA* (heme receptor) homologous regions. After the methylated linear construct was electroporated into *C. jejuni* 81–176, Δ*chuA* mutant was generated. The Δ*chuA* mutant was inoculated in BHI at pH of 5.75 and 5, and its growth was evaluated. Differences between groups were analyzed using one-way ANOVA followed by Tukey correction using Prism 7.0 software. Differences were statistically significant if *P*-values were <0.05. *Campylobacter jejuni* grew to 6.85 and 4.53 $\log_{10}$ cfu/mL in BHI at pH 7.5 and pH 5.75, respectively, whereas addition of 1.5 m*M* 7βCA altered the bacterial growth to 6.81 and 0 ($P < 0.05$) $\log_{10}$ cfu/mL in BHI and pH 5.75, respectively, compared with the controls. *chuA* expression of *C. jejuni* in pH 5.0 was 4.2-fold higher ($P < 0.05$) compared with that of BHI, whereas the gene expression significantly increased to 9.7- and 11.0-fold in the presence of 1.5 m*M* 7βCA of BHI and pH 5.0, respectively. A Δ*chuA* mutant was then generated using a linear kanamycin cassette and PCR sequencing confirmation. Notably, Δ*chuA* mutant growth at pH 5.75 significantly reduced to 2.2 $\log_{10}$ cfu/mL compared with 6.12 $\log_{10}$ cfu/mL of *C. jejuni* wild type (WT), whereas at pH 5, Δ*chuA* mutant growth was 100% inhibited compared with 5.98 $\log_{10}$ cfu/mL of WT. We found that bile acid 7βCA and acidity reduced *C. jejuni* growth. The *chuA* gene played an important role in *C. jejuni* responding to acidity and potentially bile acids.

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Key Words: bile acid, acidity, methylated linear transformation, gene expression

99 - Identifying *Clostridium perfringens* genes responding to bile acids using single mariner-based mutants.

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Chicken necrotic enteritis (NE) caused by *Clostridium perfringens* is reduced by dietary bile acid deoxycholic acid (DCA). The underlying mechanism of DCA inhibition remains elusive. This study aimed to identify *C. perfringens* genes responding to inhibitory bile acids using single mariner-based mutants. *Clostridium perfringens* HN13 was made competent and transformed with plasmid pHLL24 by electroporation. The transformants were plated on BHI plus erythromycin and chloramphenicol. The bacterium was incubated with lactose to induce transposase expression, followed by dilution and galactose broth incubation. The bacterium was then plated on the selective agar plates. The mutant library (ΔmLib) with more than 500 colonies was collected, and we had 4 libraries. Seventy single colony mutants in the libraries were also individually collected. Thirty single mutants were selected for initial DCA screening based on growth. Three mutants (Δm1, Δm2, and Δm3) resistant to DCA were reported here. Wild-type HN13 (WT), ΔmLib, and the selected single mutants with triplicates were cultured in BHI with 0, 0.25, or 0.5 m*M* DCA for 24 h, and *C. perfringens* was enumerated by serial dilution and plated on BHI agar plates. Differences between groups were analyzed using one-way ANOVA followed by Tukey correction using Prism 7.0 software, and were considered statistically significant if *P*-values were <0.05. As expected, WT HN13 grew to 6.50 cfu $\log_{10}$ CFU/mL after 24 h. In separate experiments, WT and ΔmLib growth reached 7.73 vs. 6.59, 0 vs. 3.23, and 0 vs. 3.28 ($P < 0.05$) $\log_{10}$ (CFU)/mL in 0, 0.25, and 0.5 m*M* DCA, respectively, showing significant DCA resistance in the ΔmLib group compared with WT. Similarly, WT and Δm1 growth reached 6.44 vs. 6.27 and 0 vs. 4.11 ($P < 0.05$) CFU/mL in 0 and 0.25 m*M* DCA, respectively. Growth of WT and Δm2 reached 7.50 vs. 6.36 and 0 vs. 5.87 $\log_{10}$ CFU/mL in 0 and 0.25 m*M* DCA, respectively. Growth of WT and Δm3 reached 7.58 vs. 6.61 and 0 vs. 3.23 CFU/mL in 0 and 0.25 m*M* DCA, respectively. The mutants will be evaluated with other bile acids, and our group is working on genotyping the single mutants using PCR and Sanger sequencing. In conclusion, we have generated a mariner-based *C. perfringens* mutant library, and some of the single mutants were resistant to inhibitory DCA. After genotyping the single mutants, we may be able to reveal the molecular mechanism of *C. perfringens* responding to bile acids.

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Key Words: bile acid inhibition, growth, mariner library, transformation

100 - Evaluating components of *Lactobacillus johnsonii*-metabolized chicken bile against *Clostridium perfringens* growth.

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Clostridium perfringens causes chicken necrotic enteritis (NE), and bile acids such as deoxycholic acid (DCA) reduce NE. We found that *Lactobacillus johnsonii*-metabolized chicken bile reduced *Clostridium perfringens* growth, but the underlying mechanism remains unknown. This study aimed to evaluate components of *L. johnsonii*-metabolized chicken bile against *C. perfringens* growth. *Clostridium perfringens* was cultured with 0.37 m*M* chicken bile, ch_bile_W, and ch_bile_L; 0.25, 0.37, 0.5 m*M* chenodeoxycholic acid (CDCA); 0.37 m*M* Tauro-CDCA; or 0.37 m*M* lecithin or bilirubin in the presence of *L. johnsonii*. After 24 h, *C. perfringens* growth was enumerated by serial dilution and plating on *C. perfringens* agar plates with sodium thioglycolate, D-cycloserine, and sheep blood. Differences between groups were analyzed using one-way ANOVA followed by Tukey correction using Prism 7.0 software. Differences were statistically significant if *P*-values were <0.05. After 24-h culture in BHI broth, *C. perfringens* growth reached 7.5, 7.2, 7.4, and 0 $\log_{10}$ cfu/mL in control, 0.37 m*M* chicken bile (ch_bile), *L. johnsonii* (Lj), and ch_bile+ Lj, respectively. Notably, the significant inhibition of *C. perfringens* growth in ch_bile+ Lj group compared with either the Lj or the ch_bile group. In separate experiments, *C. perfringens* grew to 7.52, 7.46, 7.41, and 7.35 $\log_{10}$ cfu/mL with CDCA of 0, 0.25, 0.37, and 0.5 m*M*, whereas the bacterium grew to 7.45, 7.39, 7.69, and 7.47 $\log_{10}$ cfu/mL in groups with addition of Lj. Interestingly, *C. perfringens* growth was 7.55, 7.54, 7.43, and 7.60 $\log_{10}$ cfu/mL in control, Lj, 0.37 m*M* TCDCA, and TCDCA+Lj groups. *Clostridium perfringens* growth was 7.54, 7.32, and 7.25 $\log_{10}$ cfu/mL in control, Lj, 0.37 m*M* lecithin, and lecithin +Lj groups. *Clostridium perfringens* growth was 7.80, 7.42, 7.54, and 7.65 $\log_{10}$ cfu/mL in control, Lj, 0.37 m*M* bilirubin, and bilirubin +Lj groups. We found that ch_bile coupled with Lj inhibited *C. perfringens* growth, whereas the pathogen growth was not inhibited by Lj plus chicken bile components of TCDCA, CDCA, lecithin, or bilirubin.

Financial Support: This project was supported by USDA- NIFA Hatch project 1012366, Hatch/ Multistate project 1018699, NIFA project 2020-67016-31346, NIFA SAS 2019-69012-29905, NIFA 2024-67015-4204, and Arkansas Biosciences Institute, USDA.

Key Words: bile component, TCDCA, lecithin, bilirubin

General Abstracts: Session 21—Tick-Borne Diseases

101 - Infection with 4 geographically distant isolates of *Anaplasma marginale* in 2-year-old heifers caused a severe form of bovine anaplasmosis.

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Bovine anaplasmosis resulting from *Anaplasma marginale* infections in erythrocytes is a major economic burden to cattle industry in the United States and globally. High mortalities are common in adult cattle. Despite the economic losses in billions of US dollars, safe and efficacious vaccines for controlling the disease are not available. Recently, we developed a genetically modified *A. marginale* strain that is attenuated, and prior infection with it protects cattle against wild-type challenge independent of intravenous (IV) infection and tick transmission. Vaccine protection against diverse isolates remains to be tested. To support this goal, we tested the pathogenicity of 4 *A. marginale* isolates originating from Virginia, Missouri, Idaho, and Washington. Four healthy 2-year-old heifers were IV infected with 1 isolate each and monitored for clinical signs, hematological changes, and systemic infection progression. By ~30 d post-infection, all 4 heifers developed severe clinical signs, which included lethargy, fever, pale mucous membranes, and enlarged lymph nodes. Blood smear analysis confirmed *A. marginale* inclusions in red blood cells (RBC) as early as d 7 post-infection, with bacteremia peaking between 20% and 38% by the terminal day. Disease severity was associated with a significant drop in the packed cell volume (total drop ranged between 49% and 70%), RBC, and hemoglobin, along with developing anisocytosis and reticulocytosis. Two heifers developed rickettsemia 1 week earlier, and these animals had evidence of an apicomplexan parasite. Metagenomic sequencing identified it as *Theileria orientalis* Ikeda, a genotype associated with bovine theileriosis and transmitted by the longhorned tick, *Haemaphysalis longicornis*; it was undetectable in the heifers before *A. marginale* infection. Due to the disease severity, all animals needed euthanasia. Gross pathology revealed splenomegaly and lymphadenomegaly, with no major changes seen by histology. This study demonstrates the severe pathogenicity of the *A. marginale* isolates tested and highlights the importance of bovine anaplasmosis. This work also suggests that *T. orientalis* Ikeda persists, as undetectable levels and coinfection with *A. marginale* may exacerbate bovine anaplasmosis severity. Future studies will focus on evaluating the protective efficacy of the genetically modified live vaccine against heterologous strain infection challenges with or without the presence of *T. orientalis* Ikeda.

Financial Support: This study was supported by the Russell L. Rustici Rangeland and Cattle Research Endowment, UC Davis, and the University of Missouri

Key Words: bovine anaplasmosis, *Anaplasma marginale*, tick-borne pathogen, *Theileria orientalis* Ikeda, bovine theileriosis, live genetically modified vaccine

102 - Farmers' perceptions of and attitudes toward tick control strategies in communal areas of Eastern Cape Province, South Africa.

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Ticks and tick-borne disease are important impediments to communal livestock production and health, causing significant economic losses due to treatment and control. The aim of the study was to assess farmers' knowledge, perception, and awareness related to control of ticks and tick-borne disease in communal livestock farms in Eastern Cape, South Africa. A structured close-ended questionnaire was used to conduct face-to-face interviews with communal livestock farmers (n = 60) across different habitats and 3 local municipalities in Amathole district municipality. High prevalence of tick-borne diseases (TBDs) was recorded from all participants, and 85% was associated with skin damage. Approximately 70% of the farmers reported Redwater as the most problematic TBD in the area, followed by Heartwater and gall sickness, with 60% indicating widespread TBD awareness. Although 50% of the respondents employed monthly winter dipping, 45% encountered challenges such as weak acaricides and poor mixing of acaricide solution. Climate change was perceived by 70% of respondents as the main factor influencing the increase of TBDs, with 50% expressing serious concerns about its effects and 60% observing a rise in TBDs during long rainfall seasons. Knowledge of tick species was high among respondents, with 70% recognizing blue ticks, 65% bont ticks, and 60% acknowledging public health risks posed by ticks. There is a need for farmer education on the use of acaricidal drugs to improve tick control and TBD prevention. The findings show that despite awareness, inconsistent farming practices hinder effective tick management.

Financial Support: The research is supported Unisa research grant

Key Words: tick, tick-borne disease, cattle, farmer, perception, attitude

103 - Dual RNA-seq reveals the transcriptomic landscape of *Ehrlichia canis* during macrophage infection.

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Ehrlichia canis is a tick-borne obligate intracellular bacterium that causes canine monocytic ehrlichiosis, a debilitating disease in dogs. The disease can have broad manifestations, from mild asymptomatic illness to acute or chronic life-threatening conditions. Currently, there are no vaccines available to prevent infection. The obligate intracellular nature and inability to grow the bacteria in axenic cultures limit advancements in this field. Hence, understanding the transcriptome during infection is critical for advancing the pathogenesis of this bacterium. Therefore, we performed dual RNA sequencing of infected and uninfected DH-82 (canine macrophage cells). Custom ribodepletion probes targeting both canine and *E. canis* genomes were used to enrich host and pathogen transcripts. Reads were aligned to the *E. canis* reference genome using HISAT2, quantified with FeatureCounts, and analyzed for differential expression with DESeq2. We observed a clear distinction between infected and uninfected groups and tight clustering of replicates with principal component analysis, indicating consistent biological differences across replicates. Differential gene expression analysis revealed widespread changes in *E. canis* during infection. We observed abundant transcripts of many antigenic-surface proteins, uncharacterized hypothetical proteins, and genes involved in metabolic adaptation and stress response. Importantly, several of the transcripts overlapped with proteins previously identified as immunogenic by peptide microarray screening, supporting their role in *E. canis* pathogenesis and may have potential as diagnostic markers or vaccine candidates. This study presents the first comprehensive transcriptomic landscape of *E. canis* in a macrophage infection model, offering novel insights into its pathogenesis and adaptive strategies. These findings lay the groundwork for future investigations into host-pathogen interactions and translational applications in diagnostics and vaccine development.

Financial Support: American Kennel Club

Key Words: *Ehrlichia canis*, dual RNA sequencing, antigens, vaccines, host-pathogen interactions

104 - Towards novel acaricide development against cattle fever tick: Transcriptomics and GPCR localization with labeled neuropeptides.

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The overall goal is to validate cattle tick neuropeptide G protein-coupled receptors (GPCRs) as potential targets for tick control. Aims were (1) to silence the kinin receptor to uncover the gene network regulated by kinins in the gut vs. carcasses using transcriptomic analyses; and (2) to validate a fluorescently labeled pyrokinin neuropeptide to specifically localize the cognate GPCR in tick tissues. Female ticks were injected with dsRNA targeting the (leuco) kinin receptor (LKR), β-actin (positive control), or β-lactamase (negative control), and placed on calves alongside non-injected control ticks. Photographs of ticks on calves were obtained for phenotypic comparisons. Tick midgut and carcass tissues were dissected 5 d post-attachment, and extracted mRNA was subjected to RNA-seq. Transcriptomes were sequenced using Illumina NextSeq2000, and differential expression was analyzed by DESeq2, after quality control and mapping to the *Rhipicephalus microplus* reference genome. To confirm the activity of a custom-synthesized tetramethylrhodamine-labeled (TMR) pyrokinin peptide, its activity on the recombinant receptor was assessed by measuring intracellular calcium bioluminescence in comparison to the unlabeled peptide. The localization of the recombinant receptor expressed in CHO-K1 cells was analyzed by fluorescence microscopy imaging. Results were as follows. (1) Photographs showed the tick sizes of actin- and LKR-silenced ticks were smaller than those of controls. A total of 30,947 unique transcripts were identified in tick libraries. Principal component analysis of the 70 libraries showed libraries of midgut and carcass separated along the PC1 axis. Preliminary analysis of gut transcripts identified 4 neuropeptide GPCRs related to osmoregulation, reproduction, and/or excretion, and 3 related to feeding regulation. (2) The TMR-labeled pyrokinin was active and elicited intracellular calcium release, as expected of activation of the pyrokinin receptor, with an EC_{50} of ~211 n*M*, whereas the unlabeled peptide EC_{50} was 35 n*M*. Fluorescence microscopy confirmed membrane localization of recombinant pyrokinin receptors in the transformed CHO-K1 cell line. We conclude that the GPCRs and neuropeptide transcripts identified in the gut are candidates for future cellular localization. Moreover, the observed activity and receptor localization by the TMR-labeled pyrokinin peptide validated this tool to localize the receptor in tick tissues to clarify their role in tick physiology.

Financial Support: This work was supported by the Agriculture and Food Research Initiative Project Award no. 2022-67015-36336, from the USDA National Institute of Food and Agriculture (2022-2025).

Key Words: cattle fever tick, midgut transcriptome, receptor localization, fluorescent neuropeptide, acaricide target

105 - Habitat characteristics and tick diversity: A focus on invasive tick, *Rhipicephalus microplus* (Acari: Ixodidae), impacting communal grazing cattle in the Eastern Cape Province, South Africa.

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Ticks thrive in habitat conditions, primarily needing moisture and cover to survive and reproduce. This study established the seasonal abundance and species composition of ixodid ticks under different habitats in the Eastern Cape Province of South Africa. Adult ticks were collected from 2 communal diptanks: Wesley community under Albany Coastal Belts (ACB) and Gcinisa community under the Great Fish Thickets (GFT). A total of 9,193 ticks were collected from cattle. The tick species were *Rhipicephalus microplus* (49.67%), *Amblyomma hebraeum* (17.76%), *Rhipicephalus evertsi evertsi* (10. 76%), *Rhipicephalus appendiculatus* (9.70%), *Rhipicephalus decoloratus* (7.52%), and *Hyalomma rufipes* (4.59%). The *A. hebraeum* male ticks were significantly more abundant ($P < 0.05$) during summer (2.72 ± 0.194) and spring (2.47 ± 0.194) at ACB. Regarding *H. rufipes*, both sexes had higher counts (2.22 ± 0.140 and 1.94 ± 0.124, respectively) in GFT during the summer season and were absent in ACB in all seasons. *Rhipicephalus appendiculatus* and *R. evertsi evertsi* had no significant difference ($P > 0.05$) at either locality across all seasons. Comparison on *Rhipicephalus* (*Boophilus*) spp. during the 12-month study period showed that *R. microplus* (86.14%) partially outcompeted *R. decoloratus* (13.14%). *Rhipicephalus microplus* recorded higher prevalences (>80%) in ACB and GFT habitat types, whereas *R. decoloratus* (<20%) was less prevalent at these habitats. Moreover, *R. microplus* female ticks had high counts (4.57 ± 0.215) during the summer and male ticks during the summer and autumn (4.14 ± 0.210 and 3.14 ± 0.210, respectively) in ACB compared with GFT. Generally, lower tick counts were observed for the *R. decoloratus*, but ACB had a high count of *R. decoloratus* female ticks during the summer season (2.18 ± 0.167) and male during the spring (1.99 ± 0.147) and summer (1.90 ± 0.147) seasons. The results demonstrate that habitat type such as ACB can influence tick infestation on hosts. Habitat-specific surveillance and integrated tick management strategies are recommended to mitigate the potential health and economic impacts of tick-borne diseases.

Financial Support: The Eastern Cape Department of Agriculture and University of South Africa are acknowledged for financial assistance throughout the study period

Key Words: Albany Coastal Belts, cattle, Eastern Cape Province, habitat, *Rhipicephalus microplus*, tick diversity

106 - Shifts in the distributional ranges of *Rhipicephalus* (*Boophilus*) ticks in South Africa: Displacement of the native tick, *Rhipicephalus decoloratus*, by the invasive cattle tick *Rhipicephalus microplus* in communal areas of the Eastern Cape.

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In addition to the threat of infestation by indigenous ticks and the diseases they transmit, cattle in several countries on the African continent are put at risk by the introduction and spread of the pantropical blue tick, *Rhipicephalus microplus.* The objective of this study was to establish to what extent the native tick species *Rhipicephalus decoloratus* has been displaced by the invasive introduced tick *R. microplus* at 2 communally grazed areas in the Eastern Cape Province, South Africa. To this end, ticks were collected quarterly from 20 cattle over a period of 12 mo at each locality. Statistical Analysis System version 9.1 (SAS, 2003) was used to analyze the data. The mean separation of the least squares means was performed using the PDIFF procedure and statistical significance was tested at a 95% level, with all results with $P < 0.05$ considered to be statistically significant. A total of 17,691 ticks were collected; among these, about 17,393 were *R. microplus* (98.32%), followed by 298 *R. decoloratus* (1.68%). Significantly ($P < 0.0001$) higher numbers of *R microplus* were found throughout the 4 seasons at Wesley, summer (144.70 ± 7.27), autumn (101.35 ± 10.31), winter (46.75 ± 4.23), and spring (143.45 ± 9.65), and similar results were obtained from Gcinisa for summer (108.35 ± 4.27), autumn (117.75 ± 2.83), winter (67.20 ± 5.01), and spring (140.10 ± 4.77). Between the 2 localities compared, *R. decoloratus* was only significant ($P < 0.05$) in Wesley. These results support the phenomenon of displacement of indigenous ticks by the pantropical blue tick *R. microplus*. It is evident that *R. microplus* continues to displace the native tick, and this has implications for diseases transmission caused by its pathogen, *Babesia bovis*, which is more virulent than the African counterpart.

Financial Support: This work was supported by the University of South Africa through their Post-Doctoral fellowship grants.

Key Words: cattle, *Rhipicephalus decoloratus*, *Rhipicephalus microplus*, displacement, South Africa

Featured Speaker: Session 22—ACVM Symposium

107 - *Leptospira* infection: Conventional wisdom vs. current perception.

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Leptospirosis is a globally underrecognized zoonotic and economically significant disease that poses a serious threat to both animal and human health. Vulnerable populations, including agricultural workers, urban slum residents, and individuals exposed to contaminated water, are disproportionately affected. A major challenge in controlling leptospirosis is the chronic, asymptomatic colonization of animal kidneys and reproductive tracts, which leads to persistent environmental contamination and ongoing transmission. Current vaccines are limited by short-lived immunity, poor cross-protection across diverse serovars, and an inability to prevent renal colonization or urinary shedding. These limitations are compounded by significant gaps in our understanding of *Leptospira* biology, epidemiology, and pathogenesis. To address these challenges, we have adopted a systems biology framework that integrates genomics, transcriptomics, proteomics, and metabolomics. Our efforts include hybrid genome assemblies for multiple *Leptospira* strains to improve serovar and strain identification, immunoinformatic profiling to identify novel antigenic proteins as candidates for vaccines and diagnostics, transcriptome sequencing using Direct cDNA and Direct RNA protocols, revealing unexpected evidence of prokaryotic mRNA polyadenylation, and metagenomic analysis of environmental samples, demonstrating the effectiveness of culture enrichment followed by sequencing to assess *Leptospira* abundance and diversity. Additionally, our animal studies challenge long-standing assumptions about reservoir status and resistance in mouse models, offering new insights into host-pathogen interactions. Together, these integrated approaches provide a deeper understanding of *Leptospira* biology and pathogenesis. This systems-level strategy is essential for developing more effective diagnostics, vaccines, and control measures for this neglected disease affecting both humans and animals.

108 - *Edwardsiella ictaluri* attachment mechanisms: A novel approach to prevent enteric septicemia of catfish.

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Aquaculture represents the fastest growing component of global agriculture, and pond-raised catfish is the largest aquaculture industry in the United States. Enteric septicemia of catfish (ESC) is caused by *Edwardsiella ictaluri*, a gram-negative species that is highly infectious for channel catfish (*Ictalurus punctatus*). Losses in fingerlings to market-size fish account for about 50% of the total disease losses to catfish producers annually, and mortalities resulting from ESC have been reported in over 78% of all operations. Like mammalian skin, catfish skin acts as a primary physical barrier against pathogens, but, in fish, skin is also part of the mucosal immune system and contains mucus, antimicrobial peptides, and immune cells. Our group showed that *E. ictaluri* can attach to abrasions on catfish skin as a portal of entry. The objective of this research was to identify mechanisms of *E. ictaluri* skin attachment for development of a preventive strategy against ESC. We constructed a mutant library by random insertion of the Mar2xT7 transposon into the chromosome of *E. ictaluri* harboring the bioluminescence plasmid pAKgfplux1. This library was then screened through a series of 3 consecutive challenges for mutants showing a decreased ability to colonize catfish skin. Eighteen mutants were identified that have decreased adhesion and virulence. DNA sequences flanking each transposon insertion site were identified by single-primer PCR and sequencing, and deletion mutations were constructed in the genes by allelic exchange. Mutants were characterized for skin attachment and virulence in experimental infections. The 18 transposon mutations were in 15 unique genes. Based on predicted protein function, 3 gene categories were identified: genes encoding regulatory proteins, chemotaxis proteins, and potential adherence proteins. Some predicted proteins have unknown functions. Of the 15 isogenic deletion mutants, 11 had significantly impaired attachment to catfish skin, and 10 of the 15 mutants were significantly attenuated compared with wild-type *E. ictaluri*, using experimental infection by bath immersion. Due to the nature of the aquatic environment, catfish skin serves not only as a physical barrier against bacterial pathogens, but it is also a critical organ in the catfish mucosal immune system. Thus, identification of *E. ictaluri* mechanisms of skin attachment offers a unique opportunity to prevent ESC through mucosal immunity. We have discovered *E. ictaluri* genes with potential involvement in regulating skin attachment, chemotaxis, and adherence to skin abrasions, and some of the deletion mutants show potential as live attenuated vaccines to stimulate skin mucosal immunity.

General Abstracts: Session 23—One Health and Public Health 2

110 - Prevalence, resistance, and genomic characterization of *Staphylococcus aureus* from animal, food, and environmental sources: A One Health perspective.

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Staphylococcus aureus is an opportunistic pathogen that can cause severe infections in both humans and animals. Its ability to acquire antibiotic resistance (AMR), including methicillin resistance, makes it a major One Health concern. This study investigated the prevalence, antibiotic resistance, and virulence traits of *S. aureus* isolated from companion animals, food products, and environmental sources in Kentucky. A total of 551 samples were collected, comprising 210 from dogs, 128 from horses, 83 from retail meats (beef and chicken), and 130 from wastewater plants. Samples were enriched and cultured on Mannitol salt agar. *Staphylococcus aureus* was confirmed by PCR targeting the *nuc* gene, and methicillin-resistant strains were identified by PCR targeting the *mecA* gene and phenotypic testing on CHROMagar. Isolates were tested for susceptibility to 11 antibiotics across 8 classes and evaluated for biofilm formation, hemolysis, catalase, and coagulase activity. *Staphylococcus aureus* prevalence was 28.2% in animals, 29.7% in food, and 10.5% in water, with no statistically significant difference among sources ($P > 0.05$). Among these isolates, 94% were methicillin-resistant and exhibited multidrug resistance, including complete resistance to azithromycin and greater than 90% resistance to clindamycin, tetracycline, and chloramphenicol. All isolates produced biofilms and exhibited catalase and coagulase activity, and most displayed β-hemolysis, indicating their potential virulence. To complement these phenotypic findings, 48 representative isolates have been submitted for whole-genome sequencing (WGS), which will provide further insight into resistance determinants, virulent genes, and mobile genetic elements. Together, these results reveal the widespread circulation of *S. aureus* across animal, food, and environmental reservoirs, highlighting the importance of integrating surveillance, stewardship, and genomic approaches to address its role in the spread of AMR within a One Health framework.

Financial Support: Center for Biomedical Research Excellence (COBRE), Center for Pharmaceutical Research and Innovation (CPRI, NIH P20 GhdsbM130456), and U.S. Department of Agriculture (USDA) National Institute for Food and Agriculture (NIFA) (grant number 2022-09086)

Key Words: *Staphylococcus aureus*, antimicrobial resistance, One Health, animal, food safety, zoonosis

111 - Characterization of outreach and communication from mosquito abatement districts and local public health.

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Outreach and communication regarding mosquito activity and its associated disease risk are crucial to inform the public of risks to their health. Outreach is often done by mosquito abatement districts (MADs) and other public health agencies, but it remains unclear what information is provided and what formats they use for communication. The objective of this study was to characterize how these organizations conduct their outreach and what information they provide. Recruitment was done via email, and the interviews were conducted on Zoom with the primary facilitators of communication for 13 organizations across 5 states. An interview guide was specifically developed with questions that gauged mosquito activity, organizational responses to mosquito activity, disease risk, communication formats used, public awareness of their organization, and barriers to perform outreach. Interviews did not strictly follow only these questions. Interviews were recorded and transcribed on Zoom, then analyzed in MAXQDA. Custom key themes were applied to transcripts, and their frequency and interactions were evaluated to assess organizational outreach. We found that the most common formats for outreach were social media, in-person events, and physical materials. Organizations reported that their communications centered on mosquito habitats in residents' immediate housing environment, personal protection measures against mosquitoes, and the disease risk from mosquitoes. Mosquito-borne disease risk tended to be narrowly communicated via websites, whereas other information was broadly communicated via physical materials and in-person interactions. The public demand from MADs instead focused on lowering mosquito bite frequency, wanting treatment services, and generally learning more about mosquitoes, and the rationale behind the organizations' work. There were barriers that restricted the capacity of these organizations to do outreach and effectively communicate with the public. These include a lack of diversity in communication formats, the public's lack of knowledge about mosquitoes, and competing work duties. Understanding how these organizations communicate with the public is important to find ways to improve public health messaging regarding vectors. Additionally, the findings of this research will inform the development of a KAP survey to understand the difference in awareness and behaviors among residents in the Midwestern region between areas with and without MAD coverage.

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Key Words: qualitative analysis, interview study, mosquito control, human behavior

112 - Identifying dominant trade distributors and potential co-mingling events in native and exotic species trade between deer farms across Florida (2014–2020).

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Florida's deer farm industry includes more than 400 deer farms statewide. In 2014, the Cervidae Health Research Initiative (CHeRI) was formed to work in collaboration with Florida deer farmers to promote the health of captive deer. White-tailed deer (WTD) are a dominant, native game species in Florida and deemed one of the most economically important species in the state, though many exotic (non-native) bovid and cervid species are farmed. This includes translocation events between farms through sales and trades, which can be modeled as trade networks. These translocations could expose naïve populations to different circulating environmental pathogens or introduce carried pathogens into new locations. The objectives of this study were to (1) identify the differences in native and exotic captive ruminant trade networks, (2) determine regional and temporal patterns in trade, and (3) investigate co-mingling of species within trade movements. To address these aims, we obtained movement permit data (2014–2020) from the Florida Fish and Wildlife Conservation Commission and the Florida Department of Agriculture and Consumer Services. Directed, weighted trade networks were constructed from these data and evaluated using spatial and aspatial network metrics (degree centrality, modularity) and temporal analyses. A species co-occurrence network was developed to assess potential co-mingling among native and exotic species. Network analyses identified major distributors (hubs), changes in exotic trade networks, and potential species co-mingling events. We found dominant distributors were conserved in the general species trade and the exclusively exotic-involved trade networks. Alternatively, one of the large distributors was reduced in the exotic network, suggesting minimal or no exotic trade. Regional and statewide patterns were detected across different subnetworks - discovering central distributors that are vital in safe translocation practices and disease mitigation efforts. The species co-occurrence network identified co-mingling events between native and exotic species occurs frequently and in high quantities with specific species, suggesting future targeted studies to determine the impact of their interactions with native WTD and if that affects disease incidence.

Financial Support: This research was funded by the University of Florida, Institute for Food and Agricultural Sciences, Cervidae Health Research Initiative, funded through the Florida State Legislature, grant number LBR2199.

Key Words: deer, trade network, livestock disease

113 - First insight into human–animal *Campylobacter* epidemiology in Southeast Nigeria.

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Food-producing animals are key reservoirs of zoonotic *Campylobacter* species, mainly *Campylobacter jejuni* and *Campylobacter coli*, causing human gastroenteritis and reproductive problems in animals. Epidemiological data on these pathogens in Nigeria are scarce, limiting control efforts. This study reports the first molecular epidemiology and clonal analysis of *Campylobacter* from humans and animals in Enugu State, Nigeria. Isolates were obtained using standard microbiological methods. Molecular identification employed conventional PCR, and genetic relatedness was assessed via enterobacterial repetitive intergenic consensus (ERIC)-PCR. *Campylobacter coli* (61.1%) was the predominant species isolated in this study. The overall prevalence of *Campylobacter* species infections (CSI) was 18.5% (224/1,212). The host-specific prevalence was 16.6% (67/406), 20.4% (85/416) and 18.5% (72/390) for slaughtered cattle, poultry, and humans, respectively. In slaughtered cattle, CSI was significantly [χ^2 (1) = 5, *P* = 0.032] associated with seasons but not with breed, age, sex and location. The infection was significantly [χ^2 (1) = 8.9, *P* = 0.003] higher in broilers (26.2%, 56/214) when compared with other poultry types. Human CSI were more in those aged ≥18 yr (19.9%, 48/241), males (20.5%, 34/166), and slaughterhouse workers (25.5%, 26/). In children, *Campylobacter* infection was prevalent in males (23.8%) than in females (10.5%). In pregnant women, CSI increased with both age and parity levels. *Campylobacter* colonization was significantly higher (*P* = 0.032) during the rainy/wet season than in the dry/hot season in all 3 species studied. The ERIC-PCR fingerprinting provided evidence of interspecies and geospatial genetic relatedness in the 20 *C. coli* isolates tested. The findings warrant a One Health control approach against CSI in Nigeria, to limit the possible public health and socioeconomic consequences.

Financial Support: I'm very grateful to The Department of Agriculture and Animal Health, University of South Africa for sponsoring me to attend this great conference.

Key Words: *Campylobacter* jejuni, *Campylobacter* coli, ERIC-PCR fingerprinting, human-animal interface, zoonosis

114 - Spatial-distribution of dog rabies in Gauteng Province using the average nearest neighbor from 2009–2025.

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Contact between rabid animals, including dogs, with the human population in Gauteng continues to be recorded, which leaves the community at risk of contracting the virus. However, the spatial-distribution of dog rabies is in Gauteng has not been described; hence, this study seeks to describe the spatial-distribution of dog rabies, as this would help in identifying areas within the province where control efforts can be directed. This study is an observational study which involves 144 rabies positive animals that were confirmed positive with the direct fluorescence antibody test at the accredited rabies reference Ondersterpoort laboratory Institute (OVI), from 2009 to 2025. Data of the 144 cases were captured using the global positioning system (GPS) to record the location of the rabies. Secondary data obtained as GPS data were inputted into MS Excel and converted into shapefile form using the ArcGIS version 10.3 (ESRI) which was used to construct maps and conduct the spatial analysis using the average nearest neighbor (ANN) method. Analysis using the ANN, showed that rabies distribution pattern in Gauteng Province over 15 years was clustered (Z-score = −7.620172 [<−1.65], *P* = 0.000000 [<0.1]; nearest neighbor ratio = 0.646547 [<1]). Rabies cases recorded over the study period showed clustering in Gauteng Province specifically in West Rand municipality where the observed mean center and the expected distance was estimated around Krugersdorp and Roodepoort. The average nearest ratio for the city of Johannesburg is significantly random tending toward clustering, while the nearest neighbor ratio for city of Tshwane and Sedibeng >1, and only 1 rabies case reported in the city of Ekurhuleni. The domestic dogs were responsible for 53% of the rabies cases, wild animals for 30%, livestock animals and horses 13%, and the cats for 5% for rabies cases over the study period. Gauteng Province being the economic hub of South Africa a lot of people migrate to the province in search of better job opportunities in the Minning for example, and as they migrate, they bring along their dogs, with a poor history of vaccination against rabies. This is why clustering of rabies cases was observed around the Roodepoort and Krugersdorp areas (including Soweto). Intensification of rabies control through vaccinations and other means in the dogs and wildlife (Jackal) population within these areas is crucial in Gauteng Province.

Financial Support: University of South Africa.

Key Words: rabies, dog rabies, Gauteng Province, clustering, observational study

General Abstracts: Session 24—Highly Pathogenic Avian Influenza 1

115 - Understanding knowledge and perception of dairy producers regarding HPAI in dairy cattle.

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The emergence of highly pathogenic avian influenza (HPAI H5N1) in dairy cattle has raised concerns about public health, disease transmission and response capacity within the dairy industry. Understanding producers' perception and response to this threat is essential for designing effective communication strategies and policy development. This study aims to assess the knowledge and perception of dairy producers regarding HPAI. A structured questionnaire on perceived risk of HPAI, self-reported exposure, and preparedness was mailed to all Michigan dairy producers and obtained 15.3% (120/781) response rate. Descriptive statistics were used to summarize the responses, one-way ANOVA, and Mann–Whitney U tests were employed to evaluate variations across perception and biosecurity groups. Herd sizes averaged 600 cows, ranging from fewer than 50 to more than 3,000 mostly operating conventional (93%) dairies. The average bulk-tank SCC was about 159,000 cells/mL. Most farms (83%) milked in parlors, whereas 17% used robots. A 76% of farms were closed herds, and the estimated average employee turnover was about 33%. Most respondents (76%) were moderate to very familiar with HPAI and its potential effect on dairy farms and considered receiving enough guidance (83%). The most trusted sources of information were veterinary professionals (71%), followed by government health agencies (51%), and industry associations (47%). Producers largely attributed the risk of HPAI introduction to environmental and animal-related factors, identifying wildlife (73%) as the most likely source, followed by air transmission (33%), cattle movement (25%), and shared employees (12%). Producers with greater familiarity with the disease ($P < 0.001$) had higher implementation of biosecurity measures than those with less familiarity. Meanwhile, producers who perceived themselves as being at a lower risk than others of acquiring HPAI showed significantly greater confidence in their existing biosecurity practices and were less likely to implement biosecurity changes compared with those who perceived their risk similar to others ($P = 0.003$). Other variables did not differ significantly among groups. Overall, most producers considered wildlife as a top driver of HPAI spread and demonstrated good general awareness and perception of HPAI risks in dairy cattle. Results presented here represent an initial step for understanding these groups' perceptions and preparedness to guide more effective outreach strategies.

Key Words: HPAI H5N1, risk perception, outbreak preparedness, biosecurity practice

116 - Survey of impacts of avian influenza type A on a convenience sample of California dairies.

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The emergence of type A avian influenza (AI) H5N1 in US dairy cattle in 2024 marked its first detection in bovines. Spreading across 18 states, it challenged the dairy industry and increased zoonotic risk. An in-person survey on a convenience sample of 8 California dairies assessed clinical, production and management including demographics, reproduction, milking hygiene, and biosecurity across 4 periods: 30 d before quarantine, quarantine, 30 d after quarantine, and, if applicable, after second quarantine. Data were analyzed using multiple factor analysis (MFA) and hierarchical clustering on principal components (HCPC). Milk yield declined by 12.0% (±1.8) in the first 2 wk of quarantine. Somatic cell counts increased by 14.6% (±2.4), whereas DMI decreased 7.6% (±1.5). Abnormal milk was reported in 75% (±15.3) of herds for 3 to 6 d (±2.5), with reduced yield persisting 24.1 d (±2.9). Abortions were reported in all 8 herds, and reproductive disorders lasted 41.2 d (±3.6). Mortality among lactating cows averaged 1.48% (±0.3). Outbreak responses included isolation pens, increased disinfection, and restricted access (75% ± 15.3). Sick cows received anti-inflammatory drugs and electrolytes (100%), rumen therapy (75% ± 15.3), IV fluids (37.5% ± 17.1), and antibiotics in advanced cases (12.5% ± 11.6). The MFA identified 2 main dimensions: (1) biosecurity and external risk exposure (27.4% variance), including between farm contact, wildlife, feeding feed refusals, and proximity to livestock such as poultry, sheep and goats; and (2) disease monitoring (26.3% variance), defined by prolonged clinical signs, SCC changes, and reproductive disorders. Personal protective equipment use and clinical signs durations contributed to both axes. The HCPC classified herds into 3 clusters. Cluster 1 herds (n = 2) showed longer clinical signs durations and daily calf pickup compared with the other clusters. Cluster 2 herds (n = 2) had reduction in milk yield, frequent observation of respiratory signs, and higher SCC compared with the other clusters. Cluster 3 herds (n = 4) had more visitors, employee contact with other farms, and greater proximity to dairies compared with the other clusters. In conclusion, the AI outbreak in the surveyed dairies had a heterogeneous effect that varied by herd management and biosecurity practices such as use of an isolation pen and restricting personnel access to premises.

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Key Words: dairy cattle, avian influenza type A, H5N1, outbreak, epidemiology, biosecurity

117 - Epidemiology of avian influenza type A in lactating California dairy cattle.

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Avian influenza type A (H5N1 B3.13) was first identified in dairy cattle in California in August 2024. Ninety-nine Holstein cows across 4 California dairies were enrolled in a 10-wk study monitoring their serological and virological profiles. Of the 99 cows, 54 were clinically normal at enrollment. Of the clinically normal cows, 55% (n = 27; SE 6.80) were previously H5N1-exposed as evidenced by H5N1 shedding by PCR (nasal, oral, or milk) or presence of antibodies by ELISA (serum or milk). Of the clinical cows, 84.44% (n = 38; SE 5.40) shed H5N1 virus or had antibodies against it. Clinical illness at enrollment was associated with 5.45 (SE 2.51; $P < 0.01$) times increased odds of shedding H5N1 compared with nonclinical cows. By 10 wk, 11 of the 99 cows enrolled (11.11%; SE 3.16) remained PCR negative, 3 (3%; SE 1.72) were positive only on nasal swab, 6 (6%; SE 2.40) were positive only on oral swabs, 14 (14.14%; SE 3.50) were positive only on milk, and 65 of the 99 (65.65%; SE 4.77) had mixed specimen positivity. Ten of the 99 cows were sold or died throughout the study. Interval-censored survival models were used to estimate time to H5N1 virus shedding. Early lactating cows unexposed to H5N1, defined by their initial negative H5N1 status on any test and being nonclinical, had a median of 4 d (SE 5.03) to PCR test positivity compared with 42 d for unexposed late lactating cows. Only milk specimens had a positive relationship between parity and virus shedding. Hazard of shedding H5N1 in milk was 2.54 times ($P = 0.016$; SE 0.99) higher in multiparous (3+ parity) compared with primiparous cows. Cows unexposed to H5N1 had a median of 4.6 d to test positive on ELISA (SE 0.90). Stage of lactation increased this estimate, peaking at 5.5 d ($P = 0.024$; SE 1.26) during mid-lactation (60–200 DIM). Parity was included as it confounded the association between clinical status and ELISA positivity. Pregnancy status did not affect either ELISA or PCR outcomes. Our study identified differential test positive rates by cow parity, stage of lactation, and specimen tested. Reliance on clinical signs alone underestimates H5N1 shedding or exposure despite being a significant risk factor for shedding and seropositivity. Targeted risk mitigation in high-risk cows based on the study findings may strengthen outbreak response by providing actionable guidance for long-term surveillance and control of H5N1 on dairies.

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Key Words: highly pathogenic avian influenza H5N1 B3.13, dairy cow, viral spillover, One Health, interval-censored regression

118 - Risk factors and shedding patterns on milk, nasal, and vaginal samples for HPAI H5N1 in dairy cattle.

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Highly pathogenic avian influenza (HPAI) H5N1 in dairy cattle has raised concerns for animal health, food safety, and potential zoonotic transmission. Although the virus is well described in wild birds and poultry, little is known about its dynamics in dairy herds. Information on detection patterns and risk factors for shedding is needed to guide surveillance and control. This study evaluated (1) detection patterns across sample types and (2) associations between cow-level characteristics and shedding of HPAI in milk. A repeated cross-sectional study was conducted on 4 commercial dairy farms in Michigan with confirmed HPAI outbreaks. Cows were randomly selected within strata based on clinical status reported by farm personnel. Milk, nasal, and vaginal samples were tested by quantitative PCR at the Michigan State University Veterinary Diagnostic Laboratory. Cow-level data, including parity, DIM, pregnancy status, and clinical information, were obtained from farm management software. Directed acyclic graphs were used for model adjustment, and mixed-effects logistic regression was applied to evaluate associations between cow-level characteristics and HPAI qPCR positivity in milk samples. Separate models were developed for each outcome of interest. Shedding was most frequently detected in milk, with an overall qPCR positivity of 11.1% (117/1321) and an average cycle threshold value of 35.7. In contrast, nasal and vaginal samples showed very low positivity rates (0.4% each), with only 4 and 2 positive detections out of 936 and 451 samples, respectively. Cow-level analyses revealed that animals in their third or greater lactation had higher odds of viral shedding compared with ≤2 lactation cows (odds ratio [OR]: 3.66; 95% CI:1.39–9.66). Stage of lactation was also significant: mid-lactation (OR: 3.96; 95% CI: 2.14–7.33) and late-lactation cows (OR: 2.68; 95% CI: 1.41–5.08) showed greater odds of shedding compared with early-lactation cows (<100 DIM). Conversely, pregnancy status (OR: 1.20; 95% CI: 0.70–2.05) and clinical status (OR: 1.14; 95% CI: 0.67–1.94) were not significantly associated with shedding in milk. These findings provide critical insights into designing practical surveillance protocols in dairy herds. The HPAI shedding was primarily detected in milk, with minimal detection in nasal and vaginal samples, representing a meaningful diagnostic challenge in nonlactating cows. Surveillance should prioritize milk sampling, particularly in mid- and late-lactation cows.

Financial Support: USDA, APHIS and CEIRR.

Key Words: HPAI H5N1, dairy cattle, viral shedding, surveillance, risk factor

119 - Association between comorbidities and HPAI-H5N1 virus shedding during outbreak investigations in dairy cattle.

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In March 2024, the United States faced the first case of high pathogenic avian influenza (HPAI) H5N1 in dairy cattle. Susceptibility, viral-shedding dynamics, and clinical presentation of HPAI-H5N1 in dairy cattle vary widely among animals. Comorbidities, such as chronic or systemic infections, or respiratory diseases, may compromise immune function, increase susceptibility, and prolonging viral shedding. Investigating these relationships is essential to improve diagnostic approaches, mitigate spread, and predict severity and potentially affect clinical outcome. Here, we aim to assess the association among comorbidities and HPAI-H5N1 shedding patterns in milk in cows from outbreak herds. We conducted a cross-sectional study on 4 HPAI-H5N1 dairy herds outbreaks in Michigan confirmed by genome sequencing performed at the National Veterinary Services Laboratory, USDA. We collected milk, nasal swabs, blood, and serum samples from individual cows (n = 1,342) and submitted them to the Michigan State University Veterinary Diagnostic Laboratory for testing against 10 infectious diseases commonly affecting US dairy cattle. A mixed-effect logistic regression model was used to assess the strength of association among comorbidities diagnostic results and HPAI-H5N1 milk shedding, adjusting for parity. A mixed-effect linear logistic regression was used to assess the association between individual comorbidities detected and HPAI-H5N1 cycle threshold (Ct) values. Results indicated that 5 out of 10 comorbidities were detected, including bovine leukemia virus (BLV; 49%), Johne's Disease (JD; 12%), *Mycoplasma hemobos* (59%), *Mycoplasma weyonii* (62%), and infectious bovine rhinotracheitis (1%). Cows seropositive to BLV or JD were more likely to shed HPAI-H5N1 virus than seronegative cows, with OR of 2.00 (95% CI: 1.16–3.45) and 2.79 (95% CI: 1.22–6.37) respectively. No association was identified between HPAI and the other detected diseases or between comorbidities and HPAI-H5N1 Ct values. Findings indicated that co-infections with systemic and chronic diseases may contribute to the spread of HPAI-H5N1 by increasing viral shedding in milk. Evidence presented here can guide control strategies targeting most likely shedders during HPAI-H5N1 outbreaks in dairy cattle.

Financial Support: This research was made possible through the collaboration of participating farm owners and industry workers and was supported by funding from the United States Department of Agriculture's APHIS VS, the Michigan Alliance for Animal Agriculture, and the Centers of Excellence for Influenza Research and Response

Key Words: influenza A, risk factor, coinfection, diagnostics, disease control

120 - Epidemiologic investigation of disease burden and transmissibility of highly pathogenic avian influenza in Michigan dairy cattle.

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Highly pathogenic avian influenza (HPAI) H5N1 is a zoonotic disease recently identified in US dairy cattle. Nationwide bulk-tank milk surveillance has improved detection of infection in lactating dairy herds and enhanced understanding of geographic spread. However, estimates of infection frequency and disease affect at the individual cow level remain limited and variable across regions. Understanding infection extent and transmissibility in affected herds is critical to guide surveillance, biosecurity, and risk assessments for animal and public health. We aimed to estimate within-herd prevalence and incidence, assess agreement between molecular and serological diagnostics, and estimate transmission using the average number of secondary infections (reproductive number, R_0). A repeated cross-sectional study was conducted on 4 Michigan dairy farms with confirmed HPAI outbreaks. Milk and serum samples were collected twice weekly for approximately 2 mo after outbreak onset and tested at the Michigan State University Veterinary Diagnostic Laboratory. Viral detection used reverse-transcription quantitative polymerase chain reaction (RT-qPCR) in milk, and antibody detection ELISA in milk (ELISAm) and serum (ELISAs). Across the 4 farms, the number of lactating cows tested varied by diagnostic for logistic constraints. Farm 1: 323 (19%) PCR in milk, 322 (19%) ELISAm, 48 (3%) ELISAs; Farm 2: 293 (9%), 172 (5%), 233 (7%); Farm 4: 213 (19%), 209 (19%), 213 (19%); Farm 3: 88/976 PCR in milk and 68/976 ELISAs. Cumulative incidence ranged from 1.9% (95% CI: 0.0–4.6%; n =) to 29.8% (16.7%–42.9%; n = 47) by RT-qPCR in milk; from 15.3% (11.2%–19.5%; n = 112) to 46.7% (40.4%–53.0%; n = 240) by ELISAm; and from 84.0% (69.6%–98.4%; n = 25) to 93.8% (81.9%–100.0%; n = 16) by ELISAs. Diagnostic concordance was moderate, highest between ELISAm and ELISAs ($\kappa = 0.41$; 95% CI: 0.33–0.48). R_0 estimates varied across diagnostic tests used for calculations, ranging from 1.01 to 1.12 for RT-qPCR in milk, 1.08 to 1.41 for ELISAm, and 1.27 to 2.31 for ELISAs, indicating sustained transmission regardless of diagnostic used. These findings highlight rapid HPAI spread in affected herds and support combining RT-qPCR and ELISA to capture both active infection and prior exposure. Consistent diagnostic and transmission patterns across farms provide insight for targeted surveillance and biosecurity strategies.

Financial Support: This research was made possible through the collaboration of participating farm owners and industry workers and was supported by funding from the United States Department of Agriculture's APHIS VS, the Michigan Alliance for Animal Agriculture, and the Centers of Excellence for Influenza Research and Response.

Key Words: HPAI, H5N1, dairy cattle, disease frequency, diagnostic agreement, reproductive number

General Abstracts: Session 25—Immunology 2

121 - The use of a fluorescent bead-based multiplex assay to measure cytokine concentrations in bovine quarter milk samples with different culture outcomes.

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Mastitis is a highly prevalent and costly disease that affects the well-being and productivity of dairy cows. Management of intramammary infections typically relies on aerobic culture for the identification of a causative pathogen. Cytokines regulate inflammatory processes in the infected quarter. Their production and secretion patterns are influenced by the invading pathogen and by the host's immune system. We hypothesize that cytokines can be used as biomarkers for mastitis. Our goal was to use a fluorescent bead-based assay to measure concentrations of IL-10, TNF-a, and IFN-γ in quarter milk samples with different culture outcomes. We selected quarter milk samples submitted to Quality Milk Production Services at the Animal Health Diagnostic Center, Cornell University (Ithaca, NY) for routine aerobic culture. We enrolled 239 quarter milk samples identified with major mastitis pathogens including coliform bacteria (*Escherichia coli, Klebsiella* spp., n = 51), bacteria from the *Streptococcus* family (*Streptococcus uberis*, *Streptococcus dysgalactiea,* and *Lactococcus* spp., n = 53), *Staphylococcus aureus* (n = 36), samples from mastitic quarters without bacterial growth after 48 h aerobic culture (n = 44), and as controls 55 quarter samples from healthy quarters (California Mastitis Test negative, somatic cell count <200,000 cells/mL, no growth after 48 h aerobic culture). We detected measurable concentrations of all cytokines in most healthy quarters. The highest concentrations of IL-10 and TNF-a were measured in samples with coliform bacteria. Quarters identified with bacteria from the *Streptococcus* family had higher concentrations of TNF-a and IFN-γ compared with *Staphylococcus aureus*, no growth, and healthy quarters. Samples identified with *Staphylococcus aureus* did not differ from samples with no bacterial growth and samples from healthy quarters. Healthy and no growth quarters did not differ from each other for any of the cytokines. Our results demonstrate the expected cytokine patterns for various culture outcomes. Although there were differences in cytokine concentrations among different mastitis pathogen groups, the ranges overlapped. We also demonstrated that milk from healthy quarters can contain measurable amounts of IL-10, TNF-a, and IFN-γ. Further studies are needed to investigate the potential use of these cytokines as biomarkers for mastitis.

Financial Support: This work was funded by the USDA grant number NI24AHDRXXXXG038 and the Cornell University College of Veterinary Medicine Research Grants Program in Animal Health.

Key Words: dairy cow, mastitis, cytokine, multiplex assay, inflammation, biomarker

122 - Effect of zinc on lymphocyte functions relevant to vaccine responsiveness *in vitro.*

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Calves possess the machinery necessary for an effective immune response, but often fail to fully respond to immune stimuli until 6 mo of age. This limits the effective use of parental vaccines for disease prevention. Immune responses are bioenergetically demanding, and lymphocytes depend on metabolic outputs for their functionality. Calf CD4 T-cells are less effective at upregulating glycolysis than adults when activated. We previously showed that zinc (Zn) is able to increase glucose availability in calf CD4 T-cells, but the extent to which this translates into enhanced lymphocyte effector functions remains to be demonstrated. The objective of this experiment was to determine the extent to which Zn supplementation improves lymphocyte functions relevant to vaccine responsiveness in vitro. Whole blood was collected from 6- to 8-wk-old dairy calves (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 $ZnCl_2$ (0, 50, 250, 750, or 1,500 µ*M*) and activated using 20 ng/mL phorbol 12-myristate 13-acetate and 1 µg/mL ionomycin for 24h at 37°C. Gene expression of *IFNG*, *IL10*, and *IL4* were assessed using RT-qPCR. Data were analyzed using $2^{\Delta\Delta Ct}$. Protein expression of IFNg, IL10, and IL4 were assessed using a bead-based multiplex immunoassay. Because the Shapiro–Wilk test indicated non-normality, the data were transformed according to the results of a Box–Cox analysis. Results were analyzed using a mixed model with individual as a random effect and treatment as a fixed effect. Studentized residuals greater than 2 were used to identify and eliminate outliers. Pairwise comparisons were performed using Tukey's honestly significant difference test with an α of 0.05. The lowest Zn treatment (50 µ*M*) was not different than the control for IFNg, IL10, and IL4 ($P \geq 0.61$). Above 50 µ*M* Zn reduced gene ($P \leq 0.0006$) and protein ($P \leq 0.0001$) expression for IFNg and IL10. Zinc supplementation greater than 50 µ*M* reduced IL4 gene expression ($P \leq 0.05$); however, protein expression was below the limit of detection for all samples. In conclusion, Zn supplementation did not increase cytokine expression in calf CD4 T-cells. To fully understand the effect of Zn on lymphocyte functions relevant to vaccine responsiveness future, research on additional functions is needed.

Financial Support: USDA-NIFA-2023-67011-40483

Key Words: immunity, T-helper, CD4 T cell, cytokine

123 - The impacts of colostrum on systemic immune response in dairy calves.

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This study investigates the effect of colostrum processing on its capacity to protect calves against disease by evaluating passive transfer of pathogen-specific antibodies and cell-mediated immune responses. Twenty-four multiparous Holstein cows were vaccinated at −67 and −37 d relative to calving with *Salmonella* Dublin SRP vaccine. Twenty-four Holstein calves born from vaccinated cows were randomly assigned to one of 3 treatments: fresh colostrum (FSH, n = 8), frozen colostrum (FZN, n = 8), or colostrum-deprived (DPR, n = 8). Calves in FSH and FZN groups received colostrum equivalent to 8% of BW within the first 2 h of life and 6% BW at 8 to 12 h after birth, whereas DPR calves were fed milk replacer at the same equivalent amount. All calves were vaccinated with *Salmonella* Dublin SRP vaccine at d 7, 28, and 70 of life. Blood samples were collected from the jugular vein at birth and on d 2, 14, 35, and 77 for cell proliferation analysis, and on d 1, 7, 28, 35, 42, 70, and 84 for IgG measurements. Flow cytometry was used to assess the proliferation capacity of CD4+, CD8+, and γδ T cells against *Salmonella* Dublin antigen. Data were analyzed using R 4.4.1 with a linear mixed-effects model. Colostrum-fed calves showed successful passive transfer of *Salmonella* Dublin antibodies, while the DPR had no detectable IgG against *Salmonella* Dublin on d 1 ($P < 0.01$) and began producing IgG only after the second vaccine on d 28. After the third dose of the vaccine, DPR calves produced higher amounts of antibodies compared with colostrum-fed calves ($P < 0.01$). Regarding cell proliferation, colostrum-fed calves showed a lower division index in CD8+ T cells compared with the DPR ($P = 0.04$). Calves in the FSH group had a lower proliferation index of CD4+ T cells ($P = 0.03$) and γδ T cells ($P = 0.06$) compared with FZN. Calves depend on maternal antibodies for protection for approximately 3 mo; however, these antibodies interfere with endogenous antibody production, underscoring the need for effective strategies that prioritize dry cow vaccination. In addition to transferring antibodies, colostrum promotes more balanced immunity by limiting excessive cell proliferation, as reflected in vitro by a lower CD8+ T cell division index on d 2, suggesting that colostrum supports more regulated cytotoxic responses before vaccination. Furthermore, fresh colostrum enhanced this balanced immunity by reducing the proliferation index of CD4+ and γδ T cells compared with frozen colostrum.

Financial Support: Dairy Innovation Hub, University of Wisconsin - Madison

Key Words: calf, colostrum, cell proliferation, T lymphocyte

124 - Assessment of immune-related microRNAs in bovine colostrum and newborn calves' circulation.

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Bovine colostrum provides nutrients and bioactive factors including immunoglobulins, growth factors, cytokines, viable leukocytes, among other compounds that are essential for newborn calves' immune function. Recent studies have identified microRNAs (miRNAs) in bovine colostrum, highlighting their key role in gene regulation, immune modulation, and cell differentiation. However, the underlying mechanisms by which miRNAs may affect calf health are yet underexplored. Our hypothesis was that miRNAs in bovine colostrum may be absorbed by newborn calves to support immune development and metabolic regulation. This study aimed to (1) assess inflammatory and metabolic-related miRNAs in bovine colostrum and whether they are absorbed from colostrum by the neonatal calf; and (2) evaluate the role of circulatory microRNAs in neonatal calf health. To address these aims, newborn beef-on-dairy calves were randomly assigned to a colostrum-fed or colostrum-deprived group after birth (n = 10/group). Calves in the colostrum group were tube-fed pooled colostrum (10% BW/calf) approximately 4 h after birth, whereas calves in the deprived group were fed 2.8 L of a commercial milk replacer (MR) as their first feeding. Whole blood was collected from all calves before and after their first feeding. Colostrum, MR, and blood samples were subjected to total RNA extraction using commercial kit, small RNA sequencing using Illumina platform, and differential expressed miRNAs were analyzed using DESeq2 in R (v.4.4.2). Significance was declared at *P*-adj ≤0.05 and Log2 fold change ≥2. MicroRNAs miR-22–3p and miR-2285 were upregulated in bovine colostrum and in blood of calves that were colostrum fed relative to colostrum-deprived after first feeding. In contrast, circulatory miR-6775 and miR-21–5p were downregulated post colostrum-feeding compared with colostrum-deprived calves. MicroRNA miR-22–3p is involved in T cell differentiation and may contribute to early immune maturation in calves. MicroRNA miR-2285 has been linked to milk fat metabolism in mammary epithelial cells suggesting a role in metabolic homeostasis. Overall, these findings highlight functional miRNAs that may modulate immune and metabolic pathways in neonatal calves. However, the ability of a calf's intestinal microRNAs absorption from colostrum remains unclear.

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.

Key Words: small RNA-seq, calf, colostrum, immunity

125 - Effect of isoprostane class on bovine monocyte-derived macrophage function under *in vitro* inflammatory and oxidative stress challenge.

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Isoprostanes (isoP) are formed during conditions of oxidative stress (OS) through the oxidation of cell membrane polyunsaturated fatty acids (PUFAs). For example, the oxidation of n-6 PUFA results in the formation of F2-isoP whereas oxidation of n-3 PUFA produces F3-isoP. Isoprostanes have been extensively studied as a biomarker of OS, but there is a gap in knowledge regarding the biological activity of these molecules. However, our laboratory has recently shown that supplementation with F3-isoP improved neutrophil function in vitro. Building on this, we now seek to investigate their role in monocyte-derived macrophages (MDM), which are essential for initiating, maintaining, and resolving inflammation during the periparturient period. During this period however, MDM function is impaired in the presence of OS. Lipopolysaccharides serve as a model of inflammation, and 2,2'-azobis dihydrochloride (AAPH) is effective in mimicking in vivo OS conditions, in vitro. The objective of this study was to compare in vitro effects of F2- and F3-isoP on macrophage function under both in vitro inflammatory and OS challenges. Monocytes were isolated via magnetic activated cell sorting separation and differentiated into macrophages from 6 healthy mid-lactation dairy cows and cultured for 12 h with a biologically relevant concentration (250 n*M* + vehicle control) of F2- or F3-isoP, in the presence or absence of LPS (100 ng/mL) or AAPH (5 m*M*). Following incubation, MDM phagocytosis and cell viability were assessed by flow cytometry. Mixed models were built for all outcome variables. The IsoP treatment and challenge condition were included as fixed effects, and animal was treated as a random effect to account for individual variability. Tukey's honest significance test was used for post hoc pairwise comparisons. A numerical increase in phagocytosis was observed in the F3-isoP treatment group relative to control, but this did not reach statistical significance. In conclusion, under our experimental conditions, isoP class did not alter MDM phagocytosis under basal, inflammatory, or OS conditions. In this study, phagocytosis was the primary function assessed, but to determine whether favoring F3- or F2-isoP production during periods of OS could improve the impaired function of MDMs observed in periparturient cattle, other MDM functions will need to be evaluated in the future.

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Key Words: immunity, oxidative stress, innate immunity, inflammation

126 - Systemic perturbations in immunity elicited by SARA in dairy cattle.

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Subacute ruminal acidosis is a significant digestive disorder in weaning calves fed highly fermentable diets, causing significant reduction in ruminal pH, which leads to epithelial erosion and inflammation in gastrointestinal tract. The economic cost of SARA in cattle totals over $500 million annually, however early diagnostic tools for SARA are lacking. Blood-based biomarkers are promising due to relative ease of access, though currently the systemic effects of acidosis remain unexplored. To dissect the systemic effects of ruminal acidosis, we used our established model of diet-induced SARA in young calves. Ten Holstein bull calves were randomly assigned to either acidosis-inducing (AC; 43% starch and 15% NDF) or -blunting (CON; 35% starch and 25% NDF) diets at birth until 17 wk of age. Beginning 5 wk of age, AC group showed significant decrease ($P < 0.05$) in BW and ruminal pH. Blood transcriptome analysis revealed pronounced differences in expression profiles between CON and AC groups at both 8 and 16 wk of age. A total of 809 differentially expressed genes (DEGs; $P < 0.01$) were identified between treatment groups at wk 8, whereas 417 DEGs ($P < 0.01$) were identified at wk 16. Gene ontology analysis of identified DEGs indicated that defense responses to bacterium were enriched at both time points ($P < 0.01$), whereas antigen presentation ($P = 0.02$) and lymphocyte mediated immunity ($P = 0.01$) were enriched only at wk 8. Several GBP genes were also upregulated in AC calves at wk 16. Although these genes are involved in viral responses, they have also recently been implicated in direct bacterial killing. To further hone our search for biomarkers associated with ruminal acidosis we performed a correlation analysis between ruminal pH values (RPV) and gene expression levels. Of note, inflammatory genes and innate recruitment chemokines, including IL1A, IL1B, CXCL5, and CXCR1, showed significant, negative correlation ($|cor| > 0.7$, $P < 0.05$) with RPV, while antigen presenting genes and costimulatory molecules displayed the contrary, indicating immune dysfunction during acidosis. Our findings demonstrated that the consequences of ruminal acidosis extended far beyond the GI tract and can be captured by blood transcriptome analysis as early as 8 wk of age. Future studies will center on the validation of systemic biomarkers and underlying microbial factors related to adaptive immune response.

Financial Support: We would like to acknowledge the following funding support: Agricultural Research Service USDA under National Program 101 Food Animal Production Current Research Information System (CRIS) funds (project no. 5090-31000-028-00D) and National Institute of Food and Agriculture (NIFA) (award no. 2023-67015-39693).

Key Words: dairy cattle, ruminal acidosis, mucosal immunity, microbiome, inflammation

General Abstracts: Session 26—Virology 2

127 - Antiviral activity of kaempferol and isoquercetin against bovine respiratory syncytial virus: Evidence for interference in the replication cycle and high-affinity binding to viral proteins.

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Bovine respiratory syncytial virus (BRSV) is a leading cause of respiratory disease in calves and a key contributor to bovine respiratory disease (BRD), responsible for significant economic losses to the cattle industry. Despite its effect, no specific treatments are currently available. Natural products, particularly plant-derived flavonoids, are promising antiviral candidates due to their structural diversity and multitarget mechanisms. This study evaluated the antiviral activity of kaempferol and isoquercetin against BRSV using in vitro assays and molecular docking approaches. Cytotoxicity was determined by MTT assay in bovine turbinate (BT) cells, establishing nontoxic concentrations of 31.25 µ*M* for kaempferol and 7.8 µ*M* for isoquercetin. Antiviral, attachment, and penetration assays were performed in BT cells infected with BRSV (0.1 MOI). Viral median tissue culture infectious dose (TCID50/mL) were quantified using the Reed–Muench method, and viral gene expression (N and F proteins) was assessed by RT-qPCR. Data from 3 independent experiments were analyzed by one-way ANOVA followed by Tukey's test ($P < 0.05$). Additionally, molecular docking was performed using AutoDock Vina with protein models predicted by ColabFold and Phyre^2, with binding affinities ≤−7.0 kcal/mol considered significant. Both flavonoids significantly reduced BRSV titers in the antiviral assay, with kaempferol decreasing titers from $^{.88}$ to $10^{2.8}$ TCID 50/mL and isoquercetin from $^{.55}$ to $10^{2.8}$ TCID 50/mL. The RT-qPCR confirmed a 4-fold reduction in F protein expression with kaempferol and a 2-fold reduction with isoquercetin treatment. No significant effects were observed in virus attachment or penetration assays. Docking analyses revealed strong affinities of both flavonoids with replication- and structural-associated proteins, including the polymerase L, matrix M, and nucleoprotein N, whereas weaker interactions were found with G and M2–2 proteins. In conclusion, kaempferol and isoquercetin exhibit antiviral effects against BRSV in vitro, acting mainly on the replication cycle rather than viral entry. These findings highlight their potential as natural antiviral candidates for veterinary medicine.

Financial Support: Sitlington Endowment and funds from the USDA-NIFA Capacity: Animal Health and Disease Research Program, award OKL03237.

Key Words: flavonoid, veterinary, bovine respiratory syncytial virus (BRSV)

128 - Pandemic M gene reassortment in canine H3N2 influenza virus acquired amantadine resistance.

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A novel reassortant variant of the canine influenza virus H3N2 (cH3N2), designated as the M variant, was identified with a matrix (M) gene segment originating from the 2009 pandemic H1N1 virus. To characterize the phenotypic and functional consequences of this reassortment, we conducted a series of in vitro, in vivo, and in silico experiments. In cell-based assays, viral replication kinetics and drug susceptibility were evaluated in Madin-Darby canine kidney (MDCK) cells treated with amantadine, oseltamivir, and zanamivir. The half-maximal inhibitory concentrations (IC50) for each antiviral were determined by cytopathic effect and immunofluorescence assays (IFA). For animal studies, mice were experimentally infected with either the parental cH3N2 strain or the M variant following oral administration of antiviral drugs. Viral shedding and postmortem lung pathology were assessed. Viral loads in lung tissues were quantified by real-time RT-PCR. In structural modeling analyses, the M2 ion channel proteins of both strains were predicted using AlphaFold2 and subjected to comparative structural analysis. The M variant exhibited amino acid substitutions at key residues associated with the drug-binding pocket, resulting in conformational changes predicted to disrupt amantadine binding. Collectively, these data demonstrate that genetic reassortment with pandemic H1N1 conferred amantadine resistance in the cH3N2 M variant through structural alterations in the M2 ion channel. Importantly, the retained sensitivity to neuraminidase inhibitors highlights these drugs as effective therapeutic alternatives. Our findings underscore the importance of continuous molecular surveillance and integrative antiviral efficacy testing to guide control strategies for emerging influenza variants.

Financial Support: This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korean government (MSIT) (RS-2024-00432287)

Key Words: influenza A virus, reassortment, amantadine, antiviral drug, resistance

129 - The characterization of viral diversity in white-tailed deer (*Odocoileus virginianus*) respiratory tracts: Implications for cross-species transmission.

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The presence and diversity of respiratory viruses in white-tailed deer (WTD) can have significant impacts for livestock production and public health. Transmission and maintenance of viruses in WTD raise public health concerns due to frequent interactions with open-grazing livestock and humans. Hunting and deer farming results in over 5 million WTD-human interactions annually. The ongoing widespread circulation of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in WTD emphasizes the need for continued characterization and surveillance of pathogens in this species. To investigate how viral diversity correlates with SARS-CoV-2 in the WTD upper respiratory tract, 98 SARS-CoV-2 negative, and 335 SARS-CoV-2 positive nasal swabs were selected for viromic characterization. The samples were opportunistically collected from hunter-harvested WTD in New Jersey and Pennsylvania by USDA Wildlife Services from 2021 to 2022. Following nucleic acid extraction from the swabs, sequencing libraries, were prepared using the Twist EF 2.0 kit followed by Twist's comprehensive viral research panel enrichment and sequenced on an Illumina NextSeq2000. Viral genomes were assembled de novo, taxonomically classified by Kraken2, annotated by geNomad, and verified by BLAST. Excluding SARS-CoV-2, a total of 16 different animal viruses were detected, 70 samples contained at least one animal virus. 53 samples had co-infections of 2 or more viruses. Viruses that correlate to respiratory illness included bovine parainfluenza virus (0.69%) and bovine rotavirus (0.46%). Bovine coronavirus, detected in 2.54% of samples, is known to cause enteric and respiratory illness in cattle. Other recovered enteric viruses such as equine picobirnavirus (1.62%) and bovine torovirus (0.46%) are common causative agents of diarrhea in young animals. Hemorrhagic diseases including recently discovered Mobuck and CHeRI orbiviruses accounted for 4.39% of detections. This study demonstrates that white-tailed deer harbor a wide range of viruses that have known implications for livestock and human health. The presence of livestock-associated viruses alongside SARS-CoV-2 circulation in WTD underscores the need for continued surveillance at the wildlife–livestock interface. Understanding wildlife roles as reservoirs and the potential for spillover and spillback is critical for managing disease risks in livestock and protecting animal and human health.

Key Words: white-tailed deer, virus, bovine respiratory disease complex (BRDC), sequencing, SARS-CoV-2

130 - Investigating the mechanisms underlying Senecavirus A persistence.

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Senecavirus A (SVA), an emerging picornavirus causing major vesicular disease outbreaks in pigs can establish persistent infections (pi) in pigs following clinical resolution of the disease. To investigate the molecular basis of SVA persistence, we established cell culture models of SVA persistence in vitro and evaluated cell survival and host responses to infection using transcriptomic approaches. Senecavirus A persistently infected humans (H1299) and porcine kidney cells (Pk15) were generated following infection at different multiplicities of infection 0.1, 1, 5 and 10 MOI. Cells that survived lytic infection were maintained for up to 40 passages (280 d) with viral RNA and infectious virus confirmed in H1299 cells. Immunofluorescence assay was used to detect dsRNA and SVA in piPK-15 cells. Similarly, porcine tonsil (PTO) cells infected at 5 MOI were maintained for 5 passages (35 d) and infectious virus was confirmed in H1299 cells. We performed differential gene expression analysis in Pk15 cells using bulk RNA-seq. Follow up studies evaluated apoptosis through TUNEL-assays and measuring degradation of poly (ADP-ribose) polymerase-1 (PARP-1) and caspase 3 (2 hallmarks of apoptosis) using western blot. The SVA persistence was established in all cell culture models and maintained up to 40 passages (280 d in culture) in H1299 and PK15 cells. Intracytoplasmic punctate staining for viral capsid proteins and dsRNA in SVA piPK-15 cells, RT-qPCR for SVA RNA and virus titrations demonstrated continuous production of infectious virus by SVA piH1299 and piPK15 cells. Transcriptomic analysis revealed upregulation of several negative regulators of apoptosis (inhibitors, LCN, TIMP2), downregulation of pro-apoptotic, autophagy (CFTR) and inflammation (C5, RBP1, PTGFR) during SVA persistence. Unlike acute infection, persistent SVA infection did not induce DNA fragmentation and cleavage/degradation of poly (ADP-ribose) polymerase-1 (PARP-1) and caspase 3. Moreover, piPK-15 cells did not undergo apoptosis upon staurosporine (apoptosis inducer) treatment showing anti-apoptotic state of piPK-15 cells. The scRNA-Seq of piPTO cells revealed that only a subpopulation of piPTOs harbored SVA transcripts and that infected and bystander cells within the infected group had distinct transcriptional profiles. Our study shows that SVA modulates apoptosis during persistence, resulting in cell survival and ensuring long-term viral replication.

Financial Support: This work was funded by the USDA-NIFA

Key Words: Senecavirus A, persistence, swine

131 - Evaluation of PEDV non-S INDEL and S INDEL spike gene-based differential PCR assays and epidemiological analysis of the detection frequency of these 2 PEDV strains in US swine.

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Porcine epidemic diarrhea virus (PEDV) in US swine mainly includes non-S INDEL (clinically virulent) and S INDEL (clinically mild) strains, which differ mainly in the spike (S) gene. PEDV screening PCR detects both strains but cannot distinguish them. S gene sequencing is reliable but slower, costly, and with variable success. Differential real-time RT-PCR targeting the S gene offers a faster option, whereas evolving PEDV strains can escape detection. Additionally, the prevalence of both strains in US herds remains unclear. This study evaluated 3 in-house-developed and one published PEDV S gene differential real-time RT-PCR assays for analytical sensitivity, specificity and diagnostic performance. In addition, 1,747 archived screening PCR-positive samples from September 2024 to April 2025 were tested with assays 1 and 2 to determine detection frequencies. A sample was considered positive for a strain if either assay detected it. All 4 differential PCR assays showed no cross-reaction with 27 non-PEDV swine pathogens. When tested on gBlock DNAs respectively covering the partial S genes of CV777, non-S INDEL, and S INDEL PEDVs, as well as the US non-S INDEL and S INDEL PEDV isolates, all assays differentiated the strains correctly, except assay 3, whose S INDEL primers/probe also detected CV777 strain. Based on testing serial dilutions of non-S INDEL and S INDEL PEDV isolates, analytical sensitivity was comparable across the assays. All assays detected and differentiated both strains in mixtures containing different ratios of non-S and S INDEL isolates, although some variability was observed. All 4 assays missed notable positives during the diagnostic performance test using 87 randomly selected screening PCR-positive samples (Ct 14.6–35.9). Among 1,747 archived samples, 947 (54.2%) were positive for non-S INDEL, 381 (21.8%) positive for S INDEL, 14 (0.8%) positive for both strains, and 405 (23.2%) negative for both strains. Epidemiological data (sample collection time, state, pig age) will be presented at the conference. Notably, 162/1747 (9.3%) samples had Ct <30 by screening PCR but could not be identified as non-S INDEL or S INDEL strain by both assays 1 and 2. The diagnostic performance results indicate that none of the 4 assays are fully reliable for detecting and differentiating PEDV non-S INDEL and S INDEL strains, highlighting the need for further refinement to accurately monitor the prevalence of these 2 strains in US swine.

Key Words: porcine epidemic diarrhea virus, S INDEL strain, non-S INDEL strain, PCR, epidemiology

132 - Culture-independent whole-genome reconstruction of porcine astrovirus 4 from oral fluid samples.

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Astroviruses (AstVs) infect a wide range of mammals and birds and have been associated with gastrointestinal, neurological, and respiratory clinical presentations, as well as asymptomatic infections. Astroviruses are difficult to culture and very few genomes are available, which limits our ability to understand the genomic selection pressures driving the evolution of this virus, including associations with virulence, transmission and host factors. In pigs, 5 genotypes (PAstV1 through PAstV5) have been identified worldwide. Recently, the ability of PAstV4 to cause clinical respiratory signs as a primary pathogen was confirmed. However, detection has primarily relied on using targeted genomic regions through RT-PCR on individual samples, leading to a gap in knowledge about the herd-level genomics of PAstV4. Our objective was to use a novel culture-independent sequencing workflow (TELSVirus) to reconstruct and analyze PAstV4 genomes from oral fluids (OF). Oral fluid samples (n = 144) collected from 59 wean-to-finish herds across 10 systems in the Midwestern United States were subjected to the TELSVirus workflow, which generates high-depth culture-independent long-read sequences for a panel of >40 porcine viruses from metagenomic samples. TELSVirus sequences were aligned to all available PAstV4 genomes for whole-genome sequence analysis. Genome robustness was evaluated using coverage statistics, and high-quality genomes were used for phylogenetic analysis with RAxML-NG. To evaluate the population structure of PAstV in relation to the sampled swine herds, we analyzed pairwise distances within sites and across systems. PAstV4 was the most abundant virus recovered using the TELSVirus workflow. Most samples yielded a high genome fraction (median: 82.45%, range: 10.3%–99.6%), and high average per-base coverage depth (median: 746.9, range: 0.11 to 1,848.5) for the PAstV4 genome. ORF1ab was the most well-sequenced region of the genome achieving >80% coverage for 81 samples. Using ORF1ab for phylogenetic analysis, we observed statistically significant clustering of genomes by site and system. Using a culture-independent workflow, we reconstructed high-quality PAstV4 genomes from a challenging sample matrix type. Moreover, the genome quality supported phylogenetic analysis to reveal a viral population structure that mirrored that of its host. Intrasample genetic diversity was observed, suggesting within- and across-herd viral evolution of currently circulating PAstV4.

Financial Support: NIH NIAID 1R01AI173928, Swine Health Information Center 22-071

Key Words: astrovirus, whole-genome sequencing, molecular assay development

General Abstracts: Session 27—Vaccinology 1

133 - Reduced severity of bovine pinkeye following intranasal vaccination with secreted *Moraxella bovis* and *Moraxella bovoculi* antigens adjuvanted with Carbigen® and Emulsigen®-D.

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To evaluate the efficacy of secreted *M. bovis* and *M. bovoculi* antigens adjuvanted with Carbigen® and Emulsigen®-D for reducing infectious bovine keratoconjunctivitis (IBK; pinkeye) and disease severity, a randomized controlled field trial was conducted in northern California during spring and summer 2025. Crossbred beef steers without active IBK were randomly assigned to receive either a 2-cc dose of an experimental vaccine (secreted *M. bovis* and *M. bovoculi* antigens adjuvanted with Carbigen® and Emulsigen®-D) or a control vaccine (water plus adjuvant) on d 0 and 28. Eye exams were conducted once weekly for 4 mo. Eyes with suspected IBK (as evidenced by the presence of corneal cloudiness) were stained with fluorescein to confirm presence of a corneal ulcer. If present, an ulcer was defined as an IBK event. Ulcerated eyes were photographed for subsequent corneal ulcer surface area measurement and assigned a clinical severity score and corneal ulcer score. Ulcers associated with foreign bodies such as plant awns were not considered as an IBK event unless the ulcer was still present at the next weekly exam. Eyes with IBK events were photographed each week until healed. Oxytetracycline was administered in animals that developed corneal ulcers >0.5 cm in widest diameter. Variables associated with IBK severity and proportions of animals in each group that required antibiotic treatment were evaluated. Nonparametric methods of statistical analysis were used; significance was set at $P < 0.05$. The proportion of steers that developed IBK was not significantly different between groups. The median healing time of corneal ulcers was shorter in the experimental vaccine group, however, the difference from control group animals was not significant. Cumulative ulcer sizes were significantly lower in the experimental vaccine group compared with the control group. Clinical severity scores, ulcer scores, and requirements for antibiotics were all significantly lower in the experimental vaccine group compared with the control group. An experimental intranasal vaccine containing *M. bovis* and *M. bovoculi* antigens adjuvanted with Carbigen® and Emulsigen®-D reduced the clinical severity of IBK as well as the need for antibiotic treatment in IBK-affected cattle. Future studies are planned to determine if additional antigenic components added to the antigens used in this study can reduce occurrence of IBK as well as severity of disease.

Financial Support: This work was supported by USDA National Institute of Food and Agriculture, Animal Health project #7009753; a gift from the California Cattlemen's Association Livestock Memorial Research Fund; and UC Davis School of Veterinary Medicine Dean's Office discretionary funding.

Key Words: pinkeye, infectious bovine keratoconjunctivitis, *Moraxella bovis*, *Moraxella bovoculi*, intranasal vaccination

134 - Ocular immune responses in cattle after intranasal vaccination with secreted *Moraxella bovis* and *Moraxella bovoculi* antigens containing native *Moraxella* spp. cytotoxin.

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This study was done to determine if bovine ocular mucosal immune responses against *Moraxella bovis* cytotoxin could be stimulated following intranasal vaccination with secreted *Moraxella bovis* and *Moraxella bovoculi* antigens enriched in native cytotoxin ("antigen") adjuvanted with either Carbigen® plus Emulsigen®-D or Carbigen® alone. Fifteen Angus steers (370–506 kg) were randomly assigned to receive either: antigen adjuvanted with Carbigen® plus Emulsigen®-D (Carb+Emul group; n = 5); antigen adjuvanted with Carbigen® (Carb group; n = 5); or water plus Carbigen® and Emulsigen®-D; (control; n = 5). Vaccines (2-cc dose) were administered on d 0 and 28 in one nostril using a standard 6-cm plastic intranasal cannula designed for administering intranasal vaccines to calves. Steers were housed in separate pens by group assignment. Tear samples were collected before vaccination (d 0) and on d 14, 28, 42, and 56. Tears were assayed for *M. bovis* cytotoxin-specific tear IgA and tear IgG concentrations and native *M. bovis* cytotoxin neutralizing antibody titers. Fold changes in *M. bovis* cytotoxin-specific tear IgA and tear IgG concentrations and tear *M. bovis* cytotoxin neutralizing antibody titers were calculated from d 0 to 14, d 0 to 28, d 0 to 42, and d 0 to 56. Differences between groups were evaluated using nonparametric statistical methods with significance set at $P < 0.05$. Median fold changes in *M. bovis* cytotoxin-specific tear IgA and tear IgG concentrations were numerically higher in the Carb+Emul or Carb groups, however, these differences were not statistically significant (tear IgA: $P = 0.326$; tear IgG: $P = 0.061$). Overall median fold changes in tear neutralizing titers were significantly different between groups ($P = 0.046$), however, these differences were not statistically significant in pairwise comparisons between groups. Ocular mucosal IgA antibody responses to *M. bovis* cytotoxin were generally more robust in animals that received antigen plus a combination of Carbigen® plus Emulsigen®-D. Use of a longer intranasal cannula for vaccine administration may have improved antibody responses. Further studies are needed to determine if the antigen used in this study adjuvanted with Carbigen® plus Emulsigen®-D can prevent naturally occurring infectious bovine keratoconjunctivitis (IBK; pinkeye) or reduce disease severity when administered intranasally in cattle.

Financial Support: This work was supported by USDA National Institute of Food and Agriculture, Animal Health project #7007281.

Key Words: *Moraxella bovis*, *Moraxella bovoculi*, cytotoxin, intranasal, mucosal immunity

135 - Immunogenicity and protective efficacy of a recombinant *Histomonasmeleagridis* cathepsin L-like protease in turkey poults.

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Histomonosis, a devastating disease that affects gallinaceous birds, is caused by the parabasalid protozoan *Histomonas meleagridis.* Commercial turkey flocks are severely impacted, with mortality exceeding 80% in certain cases. The last remaining tool to control histomonosis in commercial poultry flocks was removed from the market in 2015, leaving the industry without any means of prevention or treatment. Previously, we demonstrated that *H. meleagridis* (PHL 2017) possesses 3 highly transcribed cathepsin L-like proteases (HmCPL1, HmCPL2, and HmCPL3) that could play a critical role in parasite metabolism and virulence. As a result, the immunogenicity and protective efficacy of HmCPL3 were evaluated in turkey poults. For the immunogenicity study, the local and systemic immune response following subcutaneous immunization with recombinant HmCPL3 protein (100 µg/poult) alone or when adjuvanted with Seppic Montanide 71R VG (emulsion) was evaluated using a growing feather-pulp bioassay. To determine the protective efficacy of the HmCPL3 emulsion, an intracloacal challenge model was employed. In summary, vaccination with recombinant HmCPL3 protein alone was more effective at stimulating systemic and tissue cellular immune response in turkey poults compared with the emulsion. The emulsion induced a robust anti-HmCPL3 humoral immune response but did not prevent liver and cecal lesions associated with *H. meleagridis* infection. Research to determine the efficacy of recombinant HmCPL1 and HmCPL2 protein vaccines is in progress.

Key Words: *Histomonas meleagridis*, cathepsin protease, turkey, vaccine

136 - Evaluation of extracellular vesicles as candidates for anti-tick vaccines in cattle.

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Ticks represent an important economic threat to the cattle industry. Two tick species are of special concern, *Rhipicephalus microplus* (cattle fever tick) and *Amblyomma americanum* (lone star tick). Current tick control measures are focused on the use of synthetic acaricides. The emergence of acaricide tolerance and resistance in tick populations has led to a growing interest for the development of anti-tick vaccines. The objective of this study was to evaluate EVs vaccination in cattle. Extracellular vesicles from *R. microplus* were produced from females dissected every day for 7 d of feeding on Angus cattle. In the case of *A. americanum,* extracellular vesicles were produced from females feeding 5 d on 45 kg Holstein calves. Salivary glands (SG) and midguts (MG) were removed and cultured ex vivo in vesicle free-tick medium for 24 h. Extracellular vesicles were isolated by ultracentrifugation. Two different vaccination plans were used. For *R. microplus,* 3 different adjuvants were tested (Saponin, TiterMax, and Montanide ISA 61) with 200 µg SG and 200 µg MG EVs on d 0, 21, and 39. In the case of *A. americanum* vaccinations, three 5-mo-old female heifers were inoculated with 600 µg MG and 600 µg SG EVs at d 0, followed by 2 booster injections with 200 µg MG and 200 µg SG EVs at 28 and 49 d. Control heifers were injected with Saponin and PBS. Reactivity was evaluated by ELISA. After vaccination, animals were infested 7 d after the last booster. Engorged females were recovered to evaluate mortality and oviposition. In the case of *R. microplus,* Saponin and TiterMax adjuvants resulted in the highest level of anti-EV specific IgG antibodies ($P < 0.0001$; 2-way ANOVA). Overall, vaccination with Saponin resulted in the lowest fertility index ($P = 0.0004$; One-way ANOVA). This adjuvant was used for further vaccinations with *A. americanum* EVs, which led to seroconversion. Vaccinated animals also showed a strong skin reaction to tick feeding and a higher on-host tick mortality compared with control animals. We observed higher tick mortalities on vaccinated cattle ($P < 2.2e-16$; Fisher's exact test), lower weight ($P = 2.082e-06$; Fisher's exact test) and lower oviposition ($P = 2.082e-06$; Fisher's exact test). Saponin synergizes successfully with extracellular vesicles and adjustments of the dosage resulted in strong skin reactions and a reduction in tick feeding and oviposition, confirming the effectiveness of EVs.

Financial Support: This project was funded by the USDA National Institute for Food and Agriculture (NIFA) award #2022-67015-42166 and University of Wisconsin, Madison start-up funds to AOC.

Key Words: anti-tick vaccine, adjuvant, extracellular vesicle, tick

137 - West Nile virus vaccine candidates attenuated by dinucleotide enrichment are immunogenic and protective against lethal infection.

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West Nile virus (WNV; *Flaviviridae*) is the most common insect-borne zoonotic virus in the United States. Since 1999, WNV has caused over 10 million human infections in the United States, with thousands of neuroinvasive cases and hundreds of deaths annually. West Nile virus also caused substantial morbidity and mortality in horses. There are 4 vaccines licensed for horses: 3 inactivated virus and a live chimeric. Although WNV veterinary vaccines protect horses against viremia, all require 2 to 4 boost doses and annual reimmunizations, resulting in significant costs. These data underscore the critical need to develop WNV vaccines that provide robust protection after single dose immunization. For CpG and UpA dinucleotide enrichment in the WNV genome, we used the SSE software. Enrichment does not alter the protein code. To test safety, immunogenicity, and protection evoked by vaccine candidates we used immunocompetent C57BL/6J mouse model for WNV infection. Blood, spleen, and brain samples were collected to assess WNV-neutralizing antibody titers, RNA loads, infectious titers, and ELISpot responses. CpG and UpA dinucleotide enrichment in WNV RNA resulted in attenuation of enriched variants. Attenuation was zinc finger antiviral protein 1 (ZAP)-dependent, and ZAP knockout (ZAP-KO) cells were used to generate high-titer stocks. Ten enriched variants and wild-type (WT) viruses, were screened in mice upon intraperitoneal injection. In contrast to lethal WNV-WT, the E-MAX variant, with the RNA region encoding envelope (E) protein enriched both with CpG and UpA, caused no mortality. E-MAX was immunogenic and protective against lethal challenge. Stability of enriched dinucleotides was confirmed upon serial passaging in ZAP-WT and ZAP-KO cells. Evaluation using footpad injection in mice showed E-MAX had a promising safety and immunogenicity profile, although brain infection was occasionally detected. Then, we developed the E-MAX+FR variant by combining CpG/UpA enrichment with 2 amino acid substitutions in functional domains of the WNV E protein. This eliminated neuroinvasion but maintained immunogenicity and protection. CpG/UpA dinucleotide enrichment in the genomic E region in combination with amino acid substitutions in the E protein yields a promising platform for vaccine development against WNV. Further studies to evaluate the dual-safety layer enrichment platform in the natural equine host, using E-MAX+FR as a prototype is warranted.

Financial Support: Department of Veterinary Biosciences, College of Veterinary Medicine, The Ohio State University, Columbus, USA.

138 - Harnessing an enterotoxigenic *Escherichia coli* multiple epitope fusion antigen (MEFA) vaccine candidate (MecVax): Adjuvanticity and thermostability.

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Enterotoxigenic *Escherichia coli* (ETEC) are diverse pathologic variants of *E. coli* characterized by genetic plasticity and production of heat stable (ST) and heal labile (LT) toxins. ETEC remains the leading cause of diarrheal morbidity in children less than 5 yr of age in low- and middle-income countries. It is also the major cause of diarrheal illnesses in travelers (travelers' diarrhea). Enterotoxigenic *E. coli* also causes diarrhea and dehydration in young animals such as calves and piglets, by producing enterotoxin that disrupts intestinal function. The ETEC strains have developed antimicrobial resistance to commonly prescribed antibiotics. Currently, there are no licensed vaccines for ETEC diarrhea. Heterogeneity in virulence factors and difficulty in identifying safe antigens make the development of an ETEC vaccine challenging. By combining computational biology and structural vaccinology tools, our laboratory developed a noble subunit vaccine against ETEC (MecVax), which is composed of 2 tag-less polyvalent proteins: toxoid fusion 3xSTaN12S-mnLTR192G/L211A that targets ETEC enterotoxins and CFA/I/II/IV Multiple Epitope Fusion Antigen (MEFA) that targets ETEC adhesins. Toxoid fusion 3xSTaN12S-mnLTR192G/L211A is a chimeric protein of STa and LT toxoids. We compared the adjuvant effect of double mutant LT (dmLT) and ALOOH in mice model. We also evaluated the thermostability of these recombinant proteins shelved at 37°C for 8 wk in 5% Lactose and/or 0.1% polysorbate, and phosphate buffered saline as vaccine stabilizing buffer formulations. Based on visual examination of SDS-PAGE, toxoid fusion protein and CFA/I/II/IV MEFA that were shelved at 37°C for 3 and 6 wk, respectively, were used to immunize mice. Indirect ELISA was used to determine serum IgG titer and in vitro bacterial adherence inhibition, and neutralization assay was used to evaluate antibody functionality. Two-way ANOVA was employed, and Bonferroni's multiple comparisons test was used to compare differences in immune response between groups. The result indicated that MecVax adjuvanted with dmLT or alum elicits comparable levels of functional antibodies against ETEC adherence and enterotoxicity. Vaccine formulations containing 0.1% polysorbate showed better thermostability and immunogenicity of MecVax in mice model. The findings of this study corroborate toward the development of this protein-based multivalent ETEC vaccine candidate against children's and travelers' diarrhea.

Financial Support: This study was supported by NIH R01 AI177144 and R01 AI175214

Key Words: adjuvant, diarrhea, enterotoxigenic *Escherichia coli*, stabilizing buffer, MecVax, vaccine

General Abstracts: Session 28—Parasitology

139 - Functional characterization of *Ostertagia ostertagi* cathepsin L-like proteases.

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Ostertagia ostertagi is a highly pathogenic parasitic nematode that infects the gastric glands of the bovine abomasum. This infection disrupts the digestive process, resulting in hypoproteinemia, anemia, and eventually diminished cattle productivity. Given the substantial economic impact and the escalating resistance to anthelmintic drugs associated with this parasite, the development of alternative control measures, such as vaccination, is imperative. Parasitic cysteine proteases (CyP) play a pivotal role in nutrient acquisition and immune evasion. Our objective is to produce and characterize functional recombinant CyP. Two CyP from *O. ostertagi*, cathepsin B-like proteins 1 and 2 (OoCBP1/2), were selected for this study. Genes encoding OoCBP1/2 were cloned into the pcDNA3.1+ plasmid and expressed in HEK293F cells. We characterized the purified proteins using fluorogenic cathepsin (cat) substrates, including Z-Arg-Arg-AMC (catB), Z-Phe-Arg-AMC (catB/L), and H-Arg-AMC (catH), and CyP inhibitors such as CA074 (catB), K11777 (catB/L), and E-64 (CyP). The proteolytic activities were also determined using blood protein substrates, including fibrinogen (Fg), bovine serum albumin (BSA), hemoglobin (Hb), and immunoglobulin G (IgG), at pH 5.0 and 7.4. Moreover, the role of OoCBP1/2 in blood coagulation was evaluated using the fibrin polymerization assay and modified recalcification assay (RCT). The findings demonstrated that OoCBP1/2 are present in L3 and adult worms. OoCBP1 partially degraded the host blood proteins at pH 5.0; notably, degradation was decreased at pH 7.4. Conversely, OoCBP2 completely degraded all blood proteins under both pH conditions, except for partial degradation of IgG. Furthermore, OoCBP1 was active on catB/L substrate only at pH 5.0. OoCBP2 was active on catB/L substrate at both pH 5.0 and 7.4. Both proteases had low or undetectable activities with either catB or catH. The activity of OoCBP1/2 on catB/L substrate was inhibited by catB, catB/L, or CyP inhibitors. Moreover, low pH-triggered autoactivation was needed for OoCBP1 but not OoCBP2 in Fg degradation and coagulation. The study shows that OoCBP1/2 are predominantly catL. These proteases are implicated in nutrient acquisition and immune evasion by degrading host blood proteins and IgG, making them promising targets for the development of vaccines and chemotherapeutic agents against *O. ostertagi* infection in cattle.

Financial Support: Research was supported by USDA In-House Appropriated funds (Project Number: 8042-32000-116-000-D)

Key Words: gastrointestinal nematode, *Ostertagia ostertagi,* cathepsin, cysteine protease, fibrinogen, blood clotting

140 - Canine fecal microbiome alterations associated with experimental *Cystoisospora* spp. infection.

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Cystoisospora spp. are one of several primary etiologic agents responsible for diarrhea in young dogs, but have historically been under-studied. The relationship between *Cystoisospora*-associated diarrhea and alterations in the gut microbiome is currently unknown. We hypothesized that infection with *Cystoisospora* spp. leads to alterations in the fecal microbiome of dogs. To evaluate this, we experimentally infected a cohort of 8 research beagles with a mixed inoculum containing *Cystoisospora canis* and *Cystoisospora ohioensis* complex. We collected fecal samples daily in the morning from May 22 through July 5, 2024 (45 consecutive days). A portion of each sample was immediately frozen at −80°C until DNA extraction, whereas the remaining portion was used for diagnostic analyses. We quantified oocyst load using the Mini-FLOTAC technique, with an analytical sensitivity of 5 oocysts per gram. In this study, the prepatent period for *C. ohioensis* was 4 to 5 d, while that for *C. canis* was 8 to 10 d. Dogs were treated with ponazuril either on d 8 (group 1) or d 13 (group 2) postinfection. We extracted DNA from the frozen fecal samples and submitted for 16S region V4 microbiome profiling by Illumina next-generation sequencing. The QIIME2 pipeline was used for bioinformatic analysis. We present the microbiome data spanning the 45-d period. Analysis revealed that microbial composition was largely individualized, with some overlap in community structure across dogs in the same treatment group and of the same sex. Significant variation in dysbiosis-associated bacterial taxa was observed between individuals and treatment groups. Group 2 showed increased *Streptococcus* and decreased *Peptacetobacter hiranonis*, along with significantly higher Shannon diversity (4.15 ± 0.02 SEM vs. 4.00 ± 0.03 SEM; $P = 0.0000087$, $r = 0.23$) compared with Group 1. These findings suggest that *Cystoisospora* spp. infections may contribute to dysbiosis in dogs. Further studies are warranted to elucidate the underlying mechanisms driving these microbial shifts and to assess the impact of therapeutic interventions.

Financial Support: IDEXX Laboratories, Inc.

Key Words: *Cystoisospora*, microbiome, dysbiosis, experimental infection

141 - SNPs associated with praziquantel-resistant *Dipylidium caninum* from US dogs.

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Dipylidium caninum is the most common cestode parasite infecting dogs in the United States. Infections are typically treated with praziquantel. In recent years, praziquantel resistance in clinical isolates of canine *D. caninum* is increasingly being reported in dogs from the United States. There is a critical need to understand the mechanisms of resistance to praziquantel in this common cestode. In a previous study, we had cloned and sequenced voltage gated calcium channels but sequence analysis did not reveal associations with praziquantel resistance. To overcome the limitations of low throughput sequencing, we used the Illumina platform to sequence the whole genomes of *D. caninum* obtained from 20 praziquantel-resistant and 13 praziquantel-susceptible cases in dogs. We confirmed the identity of all resistant samples in this study as the canine genotype (species) of *D. caninum* using whole mitochondrial genomes. We assembled the genomes from each sample with reference guided assembly against a susceptible reference genome using BWA-MEM and DeepVariant to identify SNPs and indels across the genome scaffolds and across ~3,500 protein-coding genes. We then performed association analyses with PLINK. We show the distribution of genome level variations between praziquantel-resistant and susceptible isolates. We further identified 40 candidate protein-coding genes that had a higher number of variants for further analysis. We discuss these resistance-associated genes and prospects for future diagnostic tests to distinguish resistant isolates in clinical cases.

Financial Support: Elanco Animal Health; Kansas State University College of Veterinary Medicine; Auburn University College of Veterinary Medicine

Key Words: cestode, parasite, praziquantel, drug resistance, canine

142 - Examining equine anthelmintic efficacy in multiple parasitic life-stages using modified diagnostic procedures.

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Gastrointestinal parasites are universal in grazing horses. Although macrocyclic lactones were once effective against strongylids, emerging anthelmintic resistance in adult cyathostomins has highlighted the need to assess larvicidal efficacy, particularly considering the pathogenicity of the larval stages, which encyst in the mucosal lining of the colon. Earlier studies showed apparent but nonsignificant larvicidal efficacy of moxidectin, possibly due to low sensitivity from using only a small volume subaliquot of a 5% tissue sample. Our preliminary work found that analyzing the full 5% tissue sample improved detection and precision without adding processing time, offering a better method to evaluate efficacy. The aims of this study were (1) compare cyathostomin larval counts between standard and modified procedures, (2) assess adulticidal and larvicidal efficacy, and (3) evaluate efficacy against other parasite species in a naïve population. Thus, 16 juvenile, anthelmintic-naïve horses naturally infected with all major parasite species were randomized into treatment (moxidectin-praziquantel, n = 8) and control (untreated, n = 8) groups. Adult cyathostomins were recovered from 2% luminal content aliquots with a mean adulticidal efficacy of 99.89%. Encysted larvae were assessed using both the standard 2% and modified whole tissue methods. Larvicidal efficacies by larval stage and method were: 2% aliquot: 40% (EL3), 95% (LL3/L4), 96.67% (total); Modified method: 48.81% (EL3), 74.39% (LL3/L4), and 80.94% (total). These findings suggest that the standard method may slightly overestimate efficacy. Count data were analyzed via generalized mixed models. In the 2% method, only organ affected mucosal larval counts ($P < 0.05$), whereas in the modified method, treatment, organ, larval stage, and the interaction between the latter were all significant ($P < 0.001$). Organ and treatment also significantly influenced adulticidal outcomes ($P < 0.001$). Additionally, there were no differences between *Strongylus vulgaris* adults, tapeworms, ascarids, and botfly larvae counts between treatment groups, suggesting limited efficacy in these species. This is the first demonstration of significant larvicidal efficacy, though variation between larval stages warrants further investigation. Further, the observed disparity between adulticidal and larvicidal efficacy highlights the need for novel drugs targeting pathogenic larval stages as well as increased surveillance on the development of resistance.

Key Words: strongyle, anthelmintic resistance, equine parasite, cyathostomin

143 - Pharmacokinetic analysis of bumped-kinase inhibitor 1553 reveals potential for treatment of equine protozoal myeloencephalitis.

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Equine protozoal myeloencephalitis (EPM) is a common neurologic disease of horses caused by the apicomplexan parasite *Sarcocystis neurona*. The success rate of EPM treatment is estimated to be approximately 70%, with up to 8% of horses relapsing after completion. Bumped-kinase inhibitors (BKI) are antiprotozoal drugs capable of halting apicomplexan growth at the nanomolar concentration. Our previous work with the pre-clinical lead BKI-1708 has shown good systemic exposure, but poor central nervous system (CNS) penetration, suggesting this compound might not be optimal for EPM therapeutics. Considering the need for improved treatment options, BKI-1553 is now under PK investigation in horses. Two horses were dosed orally with 5mg/kg of BKI-1553 once a day for 5 d. To assess systemic concentrations, plasma was collected daily at 0h, 2h, 4h, 6h, 8h, 12h, and then 24/48/72h after the last dose. Additionally, CNS penetration was investigated by performing spinal taps in the lumbosacral space at 54 h and 102 h after the first dose and measuring drug concentration in cerebrospinal fluid (CSF) at steady-state concentration. Horses were monitored daily for adverse effects by physical exams, complete blood cell count and blood biochemistry. Drug quantitation was done by LC-MS/MS with a lower limit of detection of 0.1 µ*M*. Administration of BKI-1553 orally at 5 mg/kg led to peak plasma concentration of approximately 3.5 µ*M* at pre-dose time points. Due to the sampling schedule, the half-life could not be established, but the drug was undetectable 72 h after final dose. The concentration of BKI-1553 in CSF at 54h for horses 1 and 2 was 0.29 µ*M* and 0.21 µ*M*, respectively. At the 102-h time point, CSF could only be obtained from horse 1, and the concentration was 0.30 µ*M*. No adverse effects were observed. Our results demonstrate that BKI-1553 is well-tolerated by horses, sustaining plasma and CSF concentrations sufficient to halt parasite growth following a daily oral dosing regimen. The high concentration pre-dose is likely due to delayed absorption and accumulation over time, as observed in other species. Importantly, therapeutic concentration was found in the CNS as the CSF values are 4 to 6 times above the EC50 to control *S. neurona* growth in vitro (48 n*M*). Next steps include assessment of toxicity over longer periods of administration along with CSF sampling to develop a therapeutic regimen for a non-inferiority clinical trial against the FDA-approved drug ponazuril.

Financial Support: This work was supported by USDA–National Institute of Food and Agriculture Agriculture and Food Research Initiative grant No. 2020–67015-30881. WCVV is the president and TJK is the chief financial officer of ParaTech Inc., a company involved in developing BKIs for use in animal health.

Key Words: *Sarcocystis neurona*, central nervous system, horse

144 - *Eimeria maxima* protein expression in chicken ileum during necrotic enteritis.

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Eimeria maxima infection is a refractory disease and is also the most important predispose factor in chicken necrotic enteritis (NE). Because of its multiple life cycles during its infection in chickens, it remains elusive what *E. maxima* virulence protein antigens are. We hypothesize that the *E. maxima* proteins present in infected birds would be the antigens. The objective of this study was to identify *E. maxima* antigens. Broiler chickens were sequentially infected with *E. maxima* at d 16 and *Clostridium perfringens* at d 20 to 21. Ileal tissue from *E. maxima* and NE birds were lysed and homogenized using a beat beater. The samples were then solubilized, cleaned, and digested with trypsin. The resultant peptides were eluted and fractionated. The HPLC MS/MS was performed for proteomics analysis. Mascot search was executed for raw MS data against *Eimeria* Uniprot database. To assess differential protein expression, the resultant Mascot files were exported to Scaffold Proteome Software and quantitative differences were determined at confidence level of 95%. The biological functions of the *E. maxima* proteins were evaluated by referencing the proteins with the published papers and protein functions database. Total spectrum number of proteins identified from HPLC MS/MS analysis were 143, out of which 111 proteins were related to *Eimeria*. Among the 49 *E. maxima* proteins, the top 10 most abundant proteins were phosphopyruvate hydratase, 82 kDa gametocyte antigen, 56 kDa gametocyte antigen, heat shock protein (HSP70), Immunoglobulin heavy chain binding protein, glyceraldehyde-3-phosphate dehydrogenase, dense granule protein GRA11, tubulin β chain, L-lactate dehydrogenase and 14–3-3 protein. Their expression levels were 18.00%, 10.92%, 8.18%, 7.43%, 5.81%, 5.04%, 4.71%, 4.54%, 2.7%, and 2.16%, respectively. Through searching literature and protein function databases, it showed that gametocyte antigen protein was responsible for oocyte wall formation; heat shock protein was accountable for the survival of pathogens during stress; dense granule protein played crucial role in intracellular survival of pathogen; 14–3-3 protein was the regulator of signaling for invasion and adaptation. We discovered the most expressed *E. maxima* proteins in the intestine of coccidiosis and NE birds. Those proteins had the antigenic potential to be used as vaccine candidates to intervene coccidiosis and NE.

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Key Words: proteomics, protein data analysis, *Eimeria maxima* protein antigenicity, vaccine candidate

Featured Speaker: Session 29—AAVI Symposium

145 - Optimizing innate immunity to protect against bovine respiratory disease.

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Bovine respiratory disease (BRD) remains one of the most economically significant diseases of cattle, driven by complex interactions between multiple pathogens, host factors, and environmental stressors. The diversity of viral and bacterial strains means that adaptive immune responses induced by vaccination or prior infection are not always protective. In contrast, the innate immune system provides immediate, broad-spectrum defense and establishes the context in which adaptive immunity develops. Optimizing innate immune activation can therefore both improve early resistance and ensure the generation of effective, balanced adaptive responses. My research program focuses on defining and harnessing these innate mechanisms to improve respiratory health in cattle. From our earliest studies, we have shown that bovine γδ T cells serve as a critical bridge between innate and adaptive immunity, coordinating inflammation, repair, and immune resolution. We have also explored how innate immune memory, or trained immunity, may be leveraged to enhance protection even in the absence of strong adaptive responses. Building on these findings, we are developing mucosal vaccination and nanoparticle-based platforms designed to engage innate immune-mediated pathways to promote long-term resistance to infection in the respiratory tract. Together, these efforts reframe BRD prevention around the concept of optimizing innate immunity, not only to resist infection, but to calibrate inflammation, support repair, and guide the most effective adaptive immune outcomes.

146 - From concept to commercialization: Using immunogenetics and immunoceuticals to naturally improve animal health.

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Selective breeding for enhanced disease resistance offers a sustainable strategy to improve livestock health while reducing antibiotic dependence. The high immune response (HIR®) technology, developed and patented by Bonnie Mallard and colleagues at the University of Guelph, identifies animals with superior innate and adaptive immune responses. By selecting high immune responders, breeders can produce livestock naturally resistant to diverse pathogens, including virus and bacteria, leading to improved herd health, productivity, and welfare. Commercialized globally as Immunity+® by Semex Alliance and adopted in over 80 countries, HIR® has transformed dairy breeding by reducing disease incidence, veterinary interventions, and production losses. Recent studies demonstrated that Immunity+ dairy cows exposed to highly pathogenic avian influenza (HPAI) A H5N1 required fewer treatments, confirming for the first time that selective breeding can enhance natural resistance to this emerging zoonotic virus. High immune responder cows have greater concentrations of conventional and natural antibodies, as well as antibodies with ultralong CDR3 regions known for their novel therapeutic potential. High responders have also been shown to be more resilient to heat stress helping to mitigate the impact of global warming. The HIR® system has been adapted for pigs and sheep, and is now being adapted for beef cattle and poultry (specifically turkeys), demonstrating broad applicability for improving disease resilience, sustainability, and food security. Beyond genetics, immune function is profoundly influenced by nutritional status and bioactive compounds known as immunoceuticals. Among these, vitamin D plays a central role in regulating immune competence. Research from the Mallard Laboratory indicates that serum 25-hydroxyvitamin D [25(OH)D] levels of 90 to 120 ng/mL are required for optimal immune performance in dairy cattle, well above traditional bone-health recommendations. High immune responder cows often show lower circulating vitamin D, likely reflecting greater metabolic utilization during immune activation. To enable herd-level management, a rapid point-of-care assay for on-farm vitamin D monitoring is under development. Together, HIR®/Immunity+ and targeted immunoceutical supplementation represent complementary, science-based innovations that enhance natural disease resistance, reduce antimicrobial use, and promote sustainable livestock production. Extending these tools to additional species offers transformative potential for global animal health, productivity, and welfare.

General Abstracts: Session 30—Diagnostic Testing

147 - Enhanced metagenomic detection of equine neurotropic viruses with syndrome-based probe capture enrichment.

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Infectious agents pose a major concern for equine neurological disorders, as many cases remain unresolved by routine molecular diagnostics. This highlights the need for advanced molecular tools, such as next-generation sequencing (NGS), to improve pathogen detection and uncover novel or emerging viruses. Traditional metagenomic NGS (mNGS) has lower diagnostic sensitivity, working well only for clinical samples with high viral loads. To enhance mNGS sensitivity, we applied a syndrome-based probe capture method to detect equine neurotropic viruses. Customized capture probes, designed to target over 30 viruses known or potential to cause neurological disease in horses, were validated in a 2-stage process using both laboratory isolates and clinical samples. The probe capture enrichment method effectively enhanced genome coverage for EIV H3N8 (2.53%–77%), EHV-1 (0.58%–21.45%), and EHV-4 (0.09%–30.38%). It also consistently achieved high genome coverage for samples with lower viral loads: over 95% for EIV H3N8 (Ct values up to 33), and over 92% for both EHV-1 (Ct up to 28) and EHV-4 (Ct up to 33). Using the same panel of probes, which also covered equine coronaviruses (ECoV), the enrichment method successfully sequenced a bovine coronavirus (BCoV) isolate despite the relatively low nucleotide identity of the complete genome (≤87%) and spike gene (≤80%) between the 2 viruses. The enrichment yielded a genome coverage enhancement of 0.36% to 32.64% for diluted BCoV samples. Using the same probe panel, 15 EIV-positive clinical samples showed genome coverage enhancement of 3.11% to 97.78%, with average enhancements for the 3 most enhanced segments being NA (48.2%), PB1 (46.9%), and PB2 (43.8%). Over 40% genome coverage for 7 of 8 segments was achieved in clinical samples with Ct values ≤27. Sequencing efforts for clinical samples positive for ECoV, EHV-1, EHV-4, and West Nile virus are currently in progress. Our data support that capture probe enrichment based mNGS could enhance the detection of equine neurotropic viruses. Its lower sequence-matching requirements, compared with primer-based methods, also make it a valuable tool for detecting new equine neurological viral agents.

Key Words: equine, neurotropic virus, metagenomic sequencing, probe capture, enrichment

148 - Development of a rapid, on-site diagnostic test for the detection of mycoplasmosis in bison.

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The North American bison (*Bison bison*) is a keystone species of immense ecological, cultural, and economic importance, yet its long-term stability is threatened by infectious diseases such as mycoplasmosis caused by *Mycoplasma bovis*. First identified in bison in the early 2000s, the disease differs markedly from its presentation in cattle, where *M. bovis* typically acts as a secondary opportunist. In bison, however, mycoplasmosis is more severe, often resulting in high morbidity and mortality. Despite its significance, research on efficient diagnostics and control strategies for this disease in bison has been limited. Given its incurable nature, early detection and continuous surveillance are critical to reducing economic losses and protecting animal welfare. Unfortunately, current diagnostic methods rely on professional diagnostics laboratories, expensive equipment, trained personnel, and lengthy processing times, limiting their use in field settings. To address these limitations, we developed a rapid, on-site assay for *Mycoplasma* detection. This innovative approach combines recombinase polymerase amplification (RPA) for target sequence amplification with clustered regularly interspaced short palindromic repeats (CRISPR)-Cas12a–mediated nucleic acid detection, employing 3 CRISPR RNAs specifically designed according to protospacer adjacent motif constraints to target the *M. bovis* uvrC gene. The process can be completed within 30 min within a temperature of 37°C to 45°C, feasible under field conditions, with Cas12a cleavage activity measured through endpoint fluorescence. The assay showed no cross-reactivity with other *Mycoplasma* species and demonstrated 100% concordance with real-time PCR across more than 100 clinical samples, enabling accurate and rapid pathogen identification without specialized equipment or training. By using simple and affordable resources such as hand warmers for temperature control and smartphones for result visualization, our proposed assay has the potential to revolutionize *Mycoplasma* detection, improve disease management, and support the long-term sustainability of bison populations. Future research will focus on integrating the RPA-CRISPR/Cas12a detection assay with lateral flow strips to enable instrument-free visual readouts, thereby improving on-farm practicality and expanding accessibility for producers and wildlife managers.

Financial Support: This activity was funded by Purdue University as part of AgSEED Crossroads funding to support Indiana's Agriculture and Rural Development.

Key Words: bison, *Mycoplasma bovis,* recombinase polymerase amplification (RPA), CRISPR-Cas12a, on-site detection

149 - Standardizing an antimicrobial susceptibility testing protocol for *Mycoplasma bovis*.

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Mycoplasma bovis is a highly contagious bacterial pathogen affecting cattle, and infections can lead to substantial production losses and disruption in the cattle industry. *Mycoplasma bovis* infections may result in respiratory disease, mastitis, or arthritis. There are many barriers to treatment including inherent resistance to β-lactam antibiotics and the emergence of resistance to multiple classes of antibiotics. Antimicrobial susceptibility testing (AST) against *M. bovis* is not offered by many diagnostic laboratories due to the fastidious nature of the organism and the absence of a standardized AST procedure. We have validated a label-free bacterial enumeration method using an impedance flow cytometer for rapid and accurate counting of *M. bovis* cells, which is a pre-requisite for standardizing an AST procedure. Using the ATCC strain of *M. bovis,* we also validated a broth micro-dilution method of AST with a custom designed 96-well plate. Currently our protocol is being used to plot the MIC value distribution of 100 separate clinical *M. bovis* isolates for commonly used drugs, with 3 replicates per strain. At this time, we have completed screening of 30 isolates with our protocol. Preliminary data shows that MIC values are higher for macrolides and tetracyclines, supporting the findings of similar research. This study will help validate a protocol for *M. bovis* AST, which can be adopted by other laboratories and support informed decision-making regarding the use of antibiotics for *M. bovis* infections.

Key Words: antimicrobial resistance, bovine respiratory disease, antimicrobial susceptibility testing

150 - Uncovering circulating biomarkers for nocardioform placentitis through equine serum proteomics.

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Equine nocardioform placentitis (NP), also known as focal mucoid placentitis, is the second most common form of equine placentitis and one of the leading causes of late-term pregnancy loss in mares. Diagnosis is often attempted via transabdominal ultrasonography, which can reveal placental separation (chorion detachment from the endometrium) and the accumulation of hyperechoic exudate. However, because only a limited portion of the placenta is visualized by ultrasound, the absence of detectable lesions does not rule out disease. Importantly, early diagnosis remains challenging due to the lack of reliable blood-based biomarkers. In this study, we employed serum proteomics to identify and validate candidate biomarkers for NP. Using untargeted LC-MS/MS, we analyzed retrospective serum samples collected 4 wk before conclusion of pregnancy from mares with confirmed NP (n = 5) and healthy controls (n = 5). We identified 100 differentially expressed proteins (DEPs), including 31 upregulated and 69 downregulated in NP. Several DEPs—such as galectin-1, CD74, CD5L, calcyclin, and interferon α-inducible protein 27—were linked to placental biology, inflammation, and immune regulation. Pathway enrichment analysis highlighted oxytocin receptor–mediated signaling, serotonin receptor–mediated signaling, and blood coagulation pathways. The latter suggests activation of the coagulation cascade, potentially leading to a hypercoagulable state, impaired placental perfusion, and fetal hypoxia. To validate these findings, we quantified galectin-1, CD74, and calcyclin using human ELISA kits in an expanded cohort (NP, n = 7; control, n = 5). These assays were optimized and validated in our laboratory: serial dilution curves from equine serum ran parallel to the human standard curves, confirming cross-reactivity. All 3 proteins were significantly elevated in NP compared with controls. Immunohistochemistry further confirmed increased abundance of calcyclin in chorioallantoic tissue from NP lesions. Collectively, these results provide strong evidence that serum proteomics can uncover reliable and biologically relevant biomarkers for NP. Ongoing longitudinal and cross-sectional studies will further expand these findings and support the development of sensitive, noninvasive diagnostic assays aimed at improving early detection, therapeutic intervention, and pregnancy outcomes in mares.

Financial Support: 2024: Equine Research Award – Boehringer Ingelheim Animal Health

Key Words: equine, placentitis, nocardioform, serum proteomics, biomarker

151 - Detection of asymptomatic ocular *Leptospira* colonization in horses: Implications for equine recurrent uveitis.

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Equine recurrent uveitis (ERU) is a significant and economically impactful disease in horses, characterized by repeated episodes of ocular inflammation that can lead to blindness. Affecting up to 25% of horses in the United States, ERU presents diagnostic challenges due to its variable clinical manifestations and multiple proposed etiologies. *Leptospira* infection is a well-established cause of ERU in Europe, and emerging evidence suggests a similar role in the United States. A 2015 study in the southeastern United States reported that 45% of ERU-affected horses tested positive for anti-*Leptospira* antibodies. More recently, a 2022 retrospective study found that 83% of ocular samples from ERU cases contained anti-*Leptospira* antibodies, and 72% had detectable *Leptospira* DNA. To further investigate the role of *Leptospira* in ERU, we hypothesized that some horses with renal colonization may also harbor asymptomatic ocular colonization, regardless of antibody status. We screened ocular fluids and kidney samples from 75 horses using real-time quantitative PCR (RT-qPCR) targeting the *lipl32* and 16S rRNA genes. Sera and ocular fluids were also tested for anti-*Leptospira* antibodies using the microscopic agglutination test (MAT) with a panel of 12 serovars. Of the 75 horses, 31 (41%) were MAT-positive. The most prevalent serovar was Bratislava (33.33%), followed by Grippotyphosa (23%). Among these, 28 horses had antibodies in serum only, 4 in ocular fluids only, and 3 in both serum and ocular fluids. *Leptospira* DNA was detected in 8 aqueous humors, 7 vitreous humors, and 10 kidneys. One horse tested positive in both the kidney and vitreous humor. Eight RT-qPCR-positive samples from 6 horses were confirmed by conventional PCR and Sanger sequencing, with sequences showing the highest sequence identity to *Leptospira kirschneri*. Notably, 4 PCR-positive horses were seronegative by MAT. Our findings suggest that asymptomatic ocular colonization by *Leptospira* may occur in horses, independent of detectable antibody responses. Further research is needed to understand the conditions that facilitate asymptomatic colonization and its potential progression to ERU.

Financial Support: University of Tennessee Graduate Student Faculty Research Award

Key Words: *Leptospira,* leptospirosis, equine, uveitis

152 - A reduced probe set robustly captures antimicrobial resistance genes in metagenomic samples in an enriched long-read workflow.

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Probe enrichment assays provide increased sensitivity to detect rare sequences in metagenomic samples and are used to improve resistome analyses. Probe design is a critical and early step in the probe enrichment workflow, and directly affects the ability of the resulting probe set to capture every possible antimicrobial resistance gene (ARG). Typically, this is accomplished by ensuring that every position of every ARG sequence in the target database is covered by at least one probe (i.e., 100% coverage). In a short-read workflow, a single probe of 120 bp binds to a similarly sized fragment of DNA to generate one sequence read of ~150 bp. Recently, our group published a probe enrichment assay that works with longer fragments of DNA (i.e., >3,000 bp). Our hypothesis was that this long fragment workflow would work comparably with both a “sparse” and “dense” probe design. To test this hypothesis, we designed 2 probe sets: a “sparse” set containing one probe per 1,000 bp of ARG sequence (i.e., 10% coverage), and a “dense” set containing one probe per every ARG position (i.e., 100% coverage). MEGARes v3.0 was used as the target ARG database and Agilent’s in-house software was used for probe design. The sparse and dense probe sets were evaluated using triplicate aliquots of 5 samples obtained from swine manure pits and a positive control. Probe set performance was compared using mixed models with primary outcomes of on-target rate and ARG richness, controlling for nonindependence by replicate. The effect of probe set on resistome composition was assessed using PERMANOVA of β-diversity values. The sparse and dense probe sets contained 4,054 and 45,004 probes, respectively. Average on-target rate was not significantly different between probe sets (mean: 89.9% for dense and 89.5% for sparse, 95% CI: 89%–91% for both). Dense libraries had higher observed richness at the accession level, although this difference was not statistically significant (mean observed ARGs: 698 vs. 658, 95% CI: 511–884 vs. 471–844). Probe set had a smaller impact on resistome composition than sample-to-sample variability (R^2 14% vs. 59%, respectively). These results suggest that a much smaller probe set can be used to perform robust resistome analysis within the context of a long-read workflow. This is beneficial because smaller probe sets are less costly. Additionally, the smaller probe set allows room for more targets to be added at the design stage, expanding possible target sets.

Financial Support: NIH NIAID 1R01AI173928

Key Words: antimicrobial resistance, metagenomics, molecular biology, enrichment, resistome

General Abstracts: Session 31—Highly Pathogenic Avian Influenza 2

153 - H5N1 influenza virus stability and transmission risk in raw milk and cheese.

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Highly pathogenic avian influenza (HPAI) viruses have recently spread to dairy cattle, raising concern about virus stability in unpasteurized dairy products consumed by humans. Here we evaluated the stability of HPAI H5N1 virus in raw milk cheeses using a mini-cheese model prepared with HPAI-spiked raw milk under varying pH levels and in company-made raw milk cheese inadvertently produced with naturally contaminated raw milk. We prepared raw milk cheese with HPAI-spiked milk acidified to pH 6.6, 5.8 and 5.0 before cheesemaking using a mini-cheese model (n = 3/condition) and evaluated the H5N1 virus persistence for up to 120 d of aging. The pH values tested (6.6, 5.8, and 5.0) reflect the natural range encountered in raw milk cheeses, from unacidified milk to semi-hard cheeses and fresh acidified cheeses. The results of the mini-cheese model were validated using company-made raw milk cheeses (n = 4) inadvertently produced with naturally contaminated raw milk. Finally, the infectivity of the H5N1 in aged raw milk cheese was evaluated in ferrets following voluntary oral ingestion. We observed pH-dependent virus survival, with infectious virus surviving the cheesemaking process and up to 120 d of aging in pH 6.6 and 5.8 cheeses. Whereas at pH 5.0, the virus did not survive the cheesemaking process. These findings were validated using the company-made raw milk cheeses. Notably, although ferrets (*Mustela furo*) fed with H5N1 virus-contaminated raw milk became infected, animals fed raw milk cheese or cheese suspension did not. These results demonstrate that H5N1 virus can remain infectious for extended periods in raw milk cheeses under specific conditions, underscoring the potential public health risks of consuming raw milk cheese produced with contaminated milk, and the need for additional mitigation steps in cheese production to prevent human exposure to infectious viruses.

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Key Words: H5N1, food safety, cheese, virus stability, oral susceptibility

154 - Understanding influenza A virus infections in cattle.

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Unexpected outbreaks caused by the H5N1 HPAIV in dairy cows in the United States have raised significant veterinary and public health concerns. When and how the H5N1 HPAIV was introduced into dairy cows, and the broader epidemiology of IAV infections in cattle in the United States remain unclear. We performed a retrospective study to screen more than 1,700 serum samples collected from different bovine breeds in the United States from January 2023 to May 2024 using an ELISA targeting NP to detect IAV infections, and the positive samples were further tested by hemagglutination inhibition (HI) assay. Results showed that 586 of 1,724 samples (33.99%) from 15 US states were seropositive by the NP ELISA assay. Moreover, the HI assay revealed that 45 of these ELISA-positive samples were positive to human seasonal H1N1 and H3N2, and swine H3N2 and H1N2 viruses, and some were positive for 2 or 3 tested IAVs. Surprisingly, none of these ELISA-positive samples were HI positive for the circulating bovine H5N1 strain. Furthermore, we infected cattle respiratory and mammary gland cells with different IAVs and 2 groups of Holstein calves intranasally or orally with a swine H3N2 virus to determine their susceptibility. Naïve calves were comingled with infected animals to investigate virus transmission. Results showed that tested swine and avian IAVs could infect cattle primary respiratory cells and immortalized mammary gland cells. No obvious clinical signs and virus replication were observed in infected and contact calves. However, viral shedding and seroconversion were detected in only nasally infected animals. Our results demonstrate that IAVs other than H5N1 can infect cattle, infections are not limited to dairy cows, and that swine H3N2 virus can infect cattle but does not transmit efficiently among them. All results highlight the value in monitoring IAV epidemiology in cattle, as the viruses might adapt to cattle or reassort with the currently circulating H5N1 HPAIV, increasing risk to humans.

Financial Support: This study was supported by the Centers of Excellence in Influenza Research and Response (CEIRR) funded by NIAID, contract number 75N93021C00016, and by the USDA National Institute of Food and Agriculture, Animal Health project 7007674 and by the USDA NACA #58-3022-3-00.

Key Words: influenza A virus, cattle, infection, serology, pathogenesis

155 - A multispecies livestock H5N1 influenza vaccine.

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In March 2024, highly pathogenic avian influenza H5N1 was identified for the first time in US dairy cattle, with initial outbreaks reported in Kansas and Texas. To mitigate the spread of H5N1 influenza virus in livestock, we developed and evaluated a novel set of human adenovirus-vectored vaccines encoding a centralized consensus hemagglutinin (H5). Given the established role of swine as a key reservoir and mixing vessel for influenza A viruses with zoonotic potential, the vaccine was specifically evaluated in a porcine model with the aim of developing a cross-species immunization strategy applicable to agriculturally significant animals. The vaccine platform uses recombinant human adenoviruses from species C (types 5 and 6) and species D (types 28 and 48), engineered for immunogen delivery. Heterologous boosting was performed using a serotype switch approach that was based on the species of adenovirus. Animals that received species C vaccines were primed with Ad5 and boosted with Ad6. The animals that received species D vaccines were primed with Ad28 and boosted with Ad48. In swine, groups of five 3-wk-old Yorkshire pigs received the vaccine via either intramuscular or intranasal administration. In cattle, four 2-wk-old Holstein dairy calves per group were immunized using a synchronous intramuscular and intranasal approach designed to optimize mucosal and systemic immune responses. For the swine experiments, we included a sham vaccine group that only received Dulbecco's PBS. For the cattle, as animals were limited, we did not include a sham vaccine group. However, sera and PBMCs were collected pre-vaccine to serve as controls for the postvaccine analyses. After vaccination, both cows and pigs generated robust anti-H5 IgG and IgA responses targeting a broad spectrum of H5 antigens, ranging from the ancestral A/goose/Guangdong/1/1996 strain to the contemporary clade 2.3.4.4b strain A/dairy cow/Ohio/B24OSU/2024. Notably, seroconversion was evident in cattle within 2 wk and significantly enhanced by a heterologous serotype-boosting regimen. Furthermore, vaccine-induced T cell responses were observed against both clade 1 (A/Thailand/4(SP-528)/2004) and clade 2.3.4.4b (A/dairy cow/Ohio/B24OSU/2024) strains in both species. Protective efficacy was further evaluated in a murine model, where immune profiles closely mirrored those observed in pigs and cows. Vaccinated mice were fully protected against morbidity and mortality following challenge with lethal doses of H5N1 viruses from clades 1, 2.2, and 2.3.4.4b that represent 20+ years of H5N1 evolution. Statistical analysis was conducted using GraphPad Prism version 10.6. Data are expressed as mean with standard error (mean ± SEM). Murine HI, MNA, ELISA, and ELISpot data were analyzed by one-way ANOVA with Tukey's multiple comparisons. Survival outcomes were analyzed using the Kaplan-Meier log-rank test. A *P*-value below 0.05 was considered statistically significant. These findings demonstrate that the adenovirus-vectored H5 vaccine elicits cross-clade immunity and has potential for use as a multispecies vaccine against a broad range of diverse H5N1 influenza viruses.

Financial Support: This research was supported by the U.S. Department of Agriculture, National Institute of Food and Agriculture, Agriculture and Food Research Initiative.

Key Words: adenovirus vaccine, HPAI, bovine, influenza A virus

156 - Inactivation of H5N1 in raw whole milk through milk acidification.

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Avian influenza virus H5N1 2.3.4.4.b has recently been detected in cattle, with milk from infected animals reported to contain a high viral load, serving as a potential source for shedding and dissemination of this virus. Currently, pasteurization is the only widely recognized method for on-farm inactivation of H5N1 in milk. A current concern is that, according to USDA data, less than 50% of large dairy farms pasteurize nonsaleable milk, with a much lower percentage occurring in medium and small dairy farms. The objective of this pilot study was to evaluate the effect of milk acidification to a pH of ~4.0 to 4.4 on the inactivation of low pathogenic avian influenza (LPAI) and highly pathogenic avian influenza (HPAI) in raw whole milk. Initial trials in our study used the LPAI H6N2 virus as a surrogate for HPAI H5N1. For the acidification trials, citric acid was used to acidify milk. For the evaluation of milk acidification, milk samples were inoculated with LPAI H6N2, and samples were collected before and after treatment at various time points. Evaluation of virus viability was conducted using specific-pathogen-free embryonated chicken eggs, and viral quantification was performed using real-time quantitative PCR. Three acidification experiments were conducted using milk spiked with LPAI H6N2. Given the positive outcome observed in the inactivation of LPAI with citric acid, a fourth trial was conducted with milk containing a high load of H5N1 originating from actively infected cows. Our results indicated that milk acidification with a pH between 4.1 and 4.2 resulted in the inactivation of LPAI H6N2 and HPAI H5N1 virus in milk after 6 h of treatment. This is the first study to report the effectiveness of milk acidification in inactivating HPAI H5N1 in milk from animals infected with H5N1. We have an ongoing project using milk from farms naturally infected with H5N1, evaluating at a larger scale the milk acidification method for deactivating H5N1. We will be presenting the findings from these ongoing trials (data to be available by January 2026).

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Key Words: HPAI, H5N1, dairy cattle, milk acidification, virus inactivation in milk

157 - Infection dynamics and transmission of bovine and human influenza A H5N1 (genotype B3.13) viruses in mammalian models.

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The highly pathogenic avian influenza (HPAI) H5N1 virus clade 2.3.4.4b has become the globally dominant H5 lineage since 2021 causing a growing number of mammalian spillover events across multiple continents. Here, we investigated the comparative pathogenesis and contact transmission of bovine- and human-derived HPAI H5N1 clade 2.3.4.4b genotype B3.13 viruses in mammalian models. Using reverse genetics, we rescued 3 recombinant viruses: rTX2/2 (bovine-derived), rTexas/37, and rMichigan/90 (both human-derived), and compared their infection with the wild-type TX2/24 strain using cell culture studies in vitro and experimental animal studies in vivo. One- or two-way ANOVA with Tukey's multiple comparison tests were used to demonstrate statistically significant differences between viruses. All 4 viruses demonstrated comparable replication kinetics in 4 mammalian cell lines such as bovine uterine epithelial cells (Cal-1), primary bovine mammary epithelial cells (bMEC), Madin-Darby canine kidney (MDCK) and human lung adenocarcinoma (A549) cells. However, the rMichigan/90 strain exhibited significantly ($P \leq 0.01$) smaller plaques in bovine and human cells. In vivo studies showed that mice (n = 6/group) infected with any of the viruses succumbed to infection within 4 to 5 d; however, mice infected with rMichigan/90 virus exhibited slightly ($P \leq 0.001$) lower viral replication and shedding compared with the other strains. Similarly, in hamsters (n = 3/group), all viruses induced body weight loss and oral shedding, with robust virus replication observed in tissues. Nonetheless, rMichigan/90-infected animals showed moderately ($P \leq 0.05$) reduced virus shedding. Contact transmission studies in hamsters (n = 4/group) revealed limited transmissibility for all strains, with only one out of 4 animals inoculated with rMichigan/90 virus transmitting the virus to a naïve contact. These findings indicate that both bovine- and human-derived H5N1 genotype B3.13 viruses retain high pathogenic potential in mammals, though overall transmissibility remains low. Notably, the transmissible rMichigan/90 virus displayed an attenuated disease phenotype in vivo, which may reflect early adaptation of the virus or host-specific attenuation mechanisms.

Financial Support: The work was supported by internal grants from the College of Veterinary Medicine, Cornell University.

Key Words: H5N1, B3.13, pathogenesis, hamster, mouse, transmission

158 - Durable protective immunity to bovine H5N1 influenza virus in dairy cattle following natural infection.

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In March 2024, a genotype B3.13 influenza A (H5N1) virus was introduced into a commercial Ohio dairy herd, resulting in approximately 20% of cattle exhibiting clinical disease over a 3-wk period. Affected cows experienced declines in milk production, reduced rumination, and mastitis. Serologic surveillance revealed that approximately 90% of the herd mounted an antibody response, indicating rapid and predominantly subclinical viral spread. As this epizootic persists into a second year, the durability of naturally acquired immunity in cattle remain undefined. This represents a critical gap in understanding long-term consequences of continued influenza A (H5N1) spread for both herd and human health risk. To evaluate the durability of naturally acquired immunity to influenza A (H5N1) virus, we obtained dairy cows with documented on-farm exposure to the B3.13 genotype. Both previously exposed cows and naïve controls were experimentally challenged via the intramammary route with either the homologous B3.13 strain or the heterologous D1.1 genotype, which emerged in US cattle in January 2025. Infections were administered at 1×10^4 plaque forming units [PFU]) or 1×10^6 PFU infectious doses under controlled conditions in a high containment agricultural BSL-3 facility. Although control animals presented with clinical signs similar to on-farm observations, all homologous re-challenge animals were completely protected against clinical disease at both doses. In contrast, some cows re-infected with the heterologous virus developed transient fever and reductions in feed intake and milk yield but began to recover after a few days. Further, despite detection of viral RNA in milk of experimentally re-infected cows, limited or no infectious virus was recovered throughout the study period. When taken together, these findings indicate that prior influenza A (H5N1) virus exposure restricts viral replication and limits recovery of infectious virus, thereby reducing the potential for onward transmission. However, heterologous challenge can still provoke clinical signs, underscoring the urgency for continued monitoring of viral evolution and immune-mediated protection in dairy cattle herds.

Key Words: influenza A (H5N1), dairy cattle, protective immunity, disease pathogenesis, epidemiology, BSL-3Ag large animal

General Abstracts: Session 32—Epidemiology 3

159 - Highly pathogenic avian influenza A H5N1 surveillance in US horses.

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The unprecedented spread of highly pathogenic avian influenza (HPAI) H5N1 in US dairy cows raises critical questions about the virus's potential to cross-species barriers to infect and cause diseases in other agricultural animals, including horses. To investigate whether horses are susceptible to HPAI H5N1 infection, we conducted a nationwide sero-surveillance study involving a total of 3,156 equine serum samples collected between July 2024 and February 2025. Samples were obtained from diverse geographic locations, with most samples from Kentucky, Minnesota, Nebraska, Kansas, Indiana, and some from 23 other states, where H5N1 has circulated in bovines. Using the IDEXX influenza nucleoprotein (NP) antibody competition ELISA, we found 1,820 samples (57.7%) tested positive for NP antibodies. Five samples tested positive for antibodies against the H5 antigen using the ID screen influenza H5 antibody competition ELISA. Further validation of the 1,820 NP antibody-positive samples with the hemagglutination inhibition (HI) assay showed that they had detectable HI antibody titers against equine H3N8 virus, indicating these horses were previously vaccinated or infected with this subtype. Finally, none of the 1,820 NP antibody-positive equine serum samples were positive in the H5 HI assay whereas bovine H5N1-positive reference sera were successfully detected. Taken together, although horses cohabit with agricultural animals or migratory birds and live in the environment where H5N1 spillover might occur, our data collectively indicate that horses have limited or no susceptibility to H5N1 infection under the current H5N1 infection landscape.

Financial Support: This study was supported by NIH Emory-CEIRR subaward. The authors also thank the support provided by the William Robert Mills Chair Endowment Fund of the University of Kentucky. The members of the Wang-Li lab are particularly appreciated for their great help and suggestions for experiments described in this study. Special thanks to all collaborators who provided samples to this project.

Key Words: H5N1, ELISA, horse, sero-surveillance, species susceptibility, highly pathogenic avian influenza (HPAI)

160 - Equine hepacivirus infection in horse herds over 4 years.

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Equine hepacivirus (EqHV) is an hepatotropic virus that causes chronic hepatitis in horses. Persistent viral infection causes chronic inflammation and scarring which can progress to liver failure. Vertical transmission is documented. Horizontal transmission routes are unknown but might be insect vectored. We hypothesized EqHV transmission is seasonal. Our objective was to monitor infection dynamics over time in horse herds to determine transmission rates, clearance rates, and seasonality of transmission. 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 d, 1 and 2 mo of age. Equine hepacivirus infection status was determined by paired serology using luciferase immunoprecipitation system (LIPS) for anti-NS3 antibodies and RT-qPCR for the 5′UTR. In total, 343 horses were tested at least once and 144 horses were tested over a year. Within each year, 2% to 8% of tested horses were persistently infected and 74% to 83% were seropositive. Acute viremia was detected in 35 horses. Most new infections (28/35, 80%) were identified in June to September. An additional 10 horses seroconverted without detected viremia. Annual summer-fall rise in antibodies was observed in multiple horses. Thirty-two seropositive horses were intermittently seronegative. One plasma lot administered to a foal was EqHV RT-qPCR and LIPS positive and the recipient became transiently infected. EqHV transmission within horse herds containing persistently infected animals is common. An apparent seasonal transmission pattern and rise in antibody concentrations could indicate an insect-mediated transmission route.

Financial Support: Funding provided by National Institutes of Health, National Institute of Allergy and Infectious Diseases (NIAID) under award number K08AI141767 to J.E. Tomlinson; the Agriculture & Food Research Initiative Competitive Grant numbers 2020-67015-31297 to G.R. Van de Walle and 2022-67015-36343 to J.E. Tomlinson from the USDA National Institute of Food and Agriculture.

Key Words: equine, hepatitis, viral, transmission

161 - Molecular survey of equine parvovirus-hepatitis, equine hepacivirus, *Pegivirus caballi*, and *Pegivirus equi*, USA.

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Emerging equine viruses, including equine parvovirus-hepatitis (EqPV-H), equine hepacivirus (EqHV), *Pegivirus caballi*, and *Pegivirus equi*, pose potential risks to equine health. EqPV-H has been linked to hepatitis and acute liver failure, whereas EqHV and pegiviruses are primarily associated with subclinical infections. Despite their clinical relevance, comprehensive epidemiological data in the United States remain limited. In this study, a total of 1,195 equine serum samples collected from diagnostic submissions and surveillance programs across Alabama, Georgia, and Texas were analyzed. Total nucleic acids were extracted, and quantitative PCR assays targeting conserved regions of EqPV-H, EqHV, *P. caballi*, and *P. equi* were performed. All statistical analyses were conducted using RStudio 2023.12.1+402 with the packages *tidyverse*, *gtsummary*, *emmeans*, and *broom*. Between-group comparisons were performed using chi-squared or Fisher's exact test for categorical variables and Student's *t*-test for continuous variables. Univariable and multivariable logistic regression models were constructed to assess associations between demographic variables (age, sex, and breed) and viral detection status for each of the 4 viruses. Associations between viral infection and host factors, including age, sex, breed, and geographic location, were evaluated. Equine parvovirus-hepatitis showed the highest prevalence (19.3%), followed by EqHV (5.6%) and *P. caballi* (1.7%), with only 2 cases of *P. equi* detected. Equine parvovirus-hepatitis infection was significantly associated with breed, age, and sex, whereas EqHV was primarily associated with breed, particularly Thoroughbreds and Quarter Horses. No host-related risk factors were identified for pegiviruses, although *P. caballi*-positive cases were only identified from horses in Georgia. These findings highlight the widespread distribution of EqPV-H and EqHV in US horses, underscoring the importance of continued surveillance, particularly in high-risk breeds and settings. The data provide a foundation for developing preventive strategies and improving understanding of the epidemiology and potential clinical impact of these emerging equine viruses.

Key Words: equine parvovirus-hepatitis, equine hepacivirus, *Pegivirus caballi*, *Pegivirus equi*, molecular survey, risk factor

162 - Factors influencing veterinary student interest in equine practice: A focus group study.

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The number of veterinary graduates entering equine practice continues to decline, and more than half will leave the field within 5 years. The unique perspectives of veterinary students are critical for developing interventions that promote retention and long-term career satisfaction in equine medicine. To explore what factors influence veterinary student interest in and commitment to equine practice, the Conservation of Resources (COR) theory was used as a guiding framework. This theory suggests that resources must be maintained and replenished by individuals to avoid burnout and stress, all while continuing their work. There are 4 types of resources defined by the theory: condition, object, energy, and personal resources. With application of the COR theory, this study aims to assess factors influencing veterinary students' interest and commitment to equine practice. Eighteen veterinary students who had considered or were pursuing equine medicine participated in virtual focus group interviews. Discussions were transcribed and analyzed using Delve, where they were then coded in the context of the COR theory. A card-sorting activity was then used to rank the 4 resource domains in the COR theory. Participants ranked the condition and personal resources as the most important. Students prioritized structured mentorship, supportive work environments, and work-life balance in their future careers. At the same time, students also identified barriers in equine medicine, which included concerns about gender bias, compensation, student debt, and unrealistic client expectations. However, personal passion for equine medicine and positive early experiences with horses were key motivators for continuing to pursue the field. These results suggest that interventions that create structured mentorship programs, increase inclusivity in equine culture, and address workload and compensation concerns may encourage student retention in equine practice and support career satisfaction in the future. A limitation of this study was that we did not collect demographic data such as race, gender, or family status. As a result, the demographic distribution of our sample is unknown. Still, results from our study inform design for future studies that could enroll larger and more representative samples, with attention to demographic factors such as gender, race, and family background.

Financial Support: We thank the study participants for their contributions and extend special appreciation to Talia Wells for facilitating the focus group discussions.

Key Words: equine, veterinary student, mentorship, work-life balance, qualitative, Conservation of Resources theory

163 - Predicting and controlling spillover in multispecies disease transmission networks.

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In multispecies disease systems, pathogen spillover from a reservoir community can enable persistence of infection in a sink community where it would otherwise die out. Prior work has investigated the dynamics of outbreaks in well-mixed sink populations where the basic reproduction number (R_0) is less than 1, but real-world populations often contain substructures represented through contact and transmission networks, whose role in spillover dynamics has received less attention. We analyze models of disease spread in multispecies sink communities, driven by exogenous pathogen spillover, to quantify the roles of transmission network structure on infection in the sink community. Our work is broadly applicable but is motivated by the disease ecology of plant-pollinator networks, with a focus on the spread of parasites among multiple species of bees through their foraging on shared flowers. By analyzing mathematical models of the spread of infection through networks, with a focus on steady-state infection properties, we identify 3 parameter regimens as the value of R_0 in a sink community ranges from 0 to 1. Essentially, the value of R_0 controls what aspects of the network structure are probed by the infection process. For R_0 near 0, overall infection patterns are dominated by direct exogenous infections and one-step subsequent transmission. For R_0 near 1, infection patterns are characterized by dominant eigenvectors of a force-of-infection matrix, indicating which species are most likely to be the ultimate sources and recipients of infection. In between, additional network details can be important; we derive and apply general sensitivity formulas that identify particularly important links and species. Throughout these analyses, we focus on and highlight questions of control: which species or transmission links are the most important to target in order to reduce disease impact on a species of concern? Although steady-state properties in static sink community networks are considered here, future work can extend these approaches to situations where disease prevalence and species interactions are highly dynamic over the time period of interest, and sensitivities can be assigned not only to where management and control should be applied, but also when they should be applied.

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Key Words: spillover, transmission network, multispecies, sink community, plant-pollinator, control

164 - Development and validation of the Veterinary Career Preferences Factors Survey (VCPFS).

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Veterinary workforce shortages, particularly in equine and rural practice, highlight the need to understand the multifaceted drivers of student career decisions. Existing research has identified influences such as debt, work-life balance, and anticipated income, yet no validated instrument has comprehensively assessed these factors in veterinary students. This study describes the development and initial validation of the Veterinary Career Preference Factors Survey (VCPFS), adapted from a tool designed for medical students. The survey was distributed to didactic-year students at 3 veterinary schools in the United States and Canada between July 2024 and April 2025. A total of 183 complete responses were analyzed. Exploratory factor analysis supported a 2-factor solution: work-life balance and stability (10 items; Cronbach's α = 0.87) and field-based or outdoor-oriented (4 items; Cronbach's α = 0.76). Specialty track emerged as the strongest predictor of subscale scores, with companion animal–oriented students placing higher emphasis on work-life balance, and food animal- and equine-oriented students endorsing field-based values. Demographic differences were smaller, though variations by race or ethnicity and sexual orientation warrant further exploration. The VCPFS demonstrated acceptable reliability and validity, offering a novel tool to evaluate veterinary student career motivations. Broader application of this instrument can inform workforce planning, educational strategies, and targeted interventions to support recruitment and retention in shortage areas.

Key Words: workforce, scale development, veterinary student, career preference

General Abstracts: Session 33—Bacteriology 1

165 - Effect of dietary transition between raw and kibble diets on the canine fecal microbiome in a randomized crossover study.

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Diet strongly influences gut microbial composition in dogs, with implications for health, immunity, and metabolism. Raw and kibble diets are known to generate distinct microbial signatures, but their effects on taxa included in the canine dysbiosis index (DI), a validated marker of gut health, remain incompletely understood. Although raw diets often enrich beneficial short chain fatty acid producing bacteria and kibble diets favor opportunistic taxa, the temporal dynamics of these microbial shifts during diet transitions have not been systematically defined. To address this gap, we conducted a randomized crossover feeding trial with 7 purpose-bred, neutered male Beagles (3 yr old; 12 ± 1.5 kg). Dogs were randomized into 2 groups: one received a raw diet followed by kibble, and the other received diets in the reverse order, each separated by a 10-d transition. Fecal samples were collected at baseline and at the start and end of each diet phase, yielding 5 time points per dog. Both diets were independently analyzed for proximate and mineral composition. Fecal microbiomes were profiled using full-length 16S rRNA gene sequencing (PacBio) to achieve species-level resolution and reduce platform bias. All dogs remained clinically healthy, behaviorally normal, and medication-free throughout the study. Diet composition significantly shaped canine gut microbial ecology, altering the balance between normobiosis and dysbiosis. Raw diets enriched *Faecalibacterium*, *Turicibacter*, and *Blautia* and preserved *Clostridium hiranonis*, consistent with a normobiotic state. Conversely, kibble diets promoted *Escherichia coli* and *Streptococcus* while reducing *Faecalibacterium* and *Blautia*, indicative of dysbiosis. Several genera, including *Turicibacter*, *Faecalibacterium*, and *Fusobacterium*, exhibited wave-like fluctuations across dietary transitions, with levels rising under raw feeding and receding with kibble diet. Collectively, these findings confirm that raw diets promote microbial communities closer to normobiosis, whereas kibble diets drive a DI-positive dysbiotic profile. By demonstrating stable diet-driven effects alongside transitional oscillations, this study highlights the importance of dietary formulation in shaping microbial resilience and supports the use of DI-associated taxa as functional biomarkers of diet-induced microbiome health.

Financial Support: This study was supported by Instinct Pet Food/MI Industries, with additional funding provided by the Henthorne Clinical Professorship of Small Animal Medicine.

Key Words: canine gut microbiome, dietary transition, dysbiosis index (DI), 16S rRNA gene sequencing (PacBio), randomized crossover study

166 - *Rhodococcus equi* in horses: Prevalence, multidrug resistance, virulence gene profiles, and survival in macrophages.

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Rhodococcus equi is an important equine pathogen that causes severe pyogranulomatous pneumonia in foals and poses a zoonotic risk to immunocompromised humans. In clinical practice, *R. equi* infections are typically treated using dual antimicrobial therapy using rifampin and macrolide antibiotics. However, the growing emergence of multi-drug-resistant (MDR) strains has intensified treatment challenges and raised significant veterinary and public health concerns. In this study, we investigated the prevalence of antimicrobial resistance and virulence determinants in *R. equi* isolates recovered from necropsied horses in Kentucky. A total of 46 *R. equi* were isolated from a total of 2,182 samples with an overall prevalence of 2.1%, among which the highest prevalence was observed in foals under 3 mo of age (44.7%). Antimicrobial sensitivity testing revealed that all the isolates (100%, 46/46) demonstrated resistance to cefazolin, and penicillin followed by oxacillin + 2% NaCl (97.8%; 45/46), rifampin (34.8%; 16/46), clarithromycin (26.1%, 12/46), and erythromycin (17.4%, 8/46). Similarly, 32.6% (15/46) of the isolates demonstrated multidrug resistance (MDR), with resistance to at least 3 classes of antibiotics tested. Polymerase chain reaction for detecting the virulence genes demonstrated the presence of virulence plasmid encoding genes responsible for intra-macrophagic survival, *vapA* and *vapH*, in 100% (46/46) of the isolates, followed by other virulence plasmid encoding genes, including *vapD* (93.5%, 43/46), *vapC* (89.1%, 41/46), and *vapB* (84.8%, 39/46). Furthermore, phenotypic analysis of the *R. equi* isolates revealed that all the isolates except one were biofilm producers, with 50% (23/46) of the isolates being weak biofilm producers (WBP), 28.261% (13/46) strong biofilm producers (SBP), and 19.565% (9/46) moderate biofilm producers (MBP). All the isolates demonstrated a strong ability to survive inside the murine macrophage (J774A.1) cell lines, demonstrating the enhanced ability of *R. equi* to survive inside the phagocytic cells such as macrophages. This study provides valuable insights into the epidemiology of MDR *R. equi* in necropsied horses in Kentucky, emphasizing the need for vigilant antimicrobial stewardship and development novel therapeutic strategies.

Financial Support: Center for Biomedical Research Excellence (COBRE), and Center for Pharmaceutical Research and Innovation (CPRI, NIH P20 GhdsbM130456) and U.S. Department of Agriculture (USDA) National Institute for Food and Agriculture (NIFA) (grant number 2022-09086)

Key Words: *Rhodococcus equi,* multidrug resistance, virulence, biofilm

167 - Combinatorial *in silico* and phenotypic characterization to identify novel small molecules antimicrobials against ESKAPE pathogens.

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The rise of antimicrobial resistance (AMR) compromises antibiotic effectiveness and poses a serious global health threat, particularly in hospitals and among immunocompromised patients. One of the greatest concerns are the ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species), which are responsible for most hospital-acquired infections and exhibit resistance to conventional antibiotics. Recognizing their importance, the World Health Organization (WHO) included them in its Bacterial Priority Pathogen List (BPPL) from 2017 to guide antibiotic development. In this study, compounds were prioritized using in silico predictions based on physiochemical properties and antimicrobial activity. Leveraging a pre-selected small-molecule library of compounds previously identified as hit molecules-defined by complete growth inhibition or ≥75% attenuation of virulence-screened across 11 high-throughput in vitro assays against diverse bacterial pathogens (n = 9), including foodborne, zoonotic, and phytopathogens, a total of 214 unique compounds were prioritized to screen against ESKAPE pathogens. Forty-three compounds demonstrated activity, including 7 with bactericidal effects. Notably, one compound showed broad-spectrum bactericidal activity across all ESKAPE pathogens, whereas the remaining 6 were species-specific, targeting *S. aureus*, *Enterobacter cloacae*, *Enterococcus faecium*, and *A. baumannii*. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) assays further refined the pool to 29 active hits. Scaffold diversity, confirmed by Tanimoto similarity analysis, underscored the novelty of these chemotypes. Toxicity testing in Caco-2 cells using a lactate dehydrogenase assay confirmed that several hit molecules were nontoxic to mammalian cells. Our next steps include pharmacokinetic and physicochemical profiling to guide lead selection. Collectively, this integrated workflow—combining high-throughput screening, scaffold analysis, and cheminformatic triage—has identified novel heterocyclic and phenolic small molecules with promising efficacy against ESKAPE pathogens, providing a foundation for lead optimization and strengthening the antimicrobial discovery pipeline amid the escalating AMR crisis.

Financial Support: NIFA, IDRC-CRDI, College of Veterinary Medicine-UIUC

Key Words: antimicrobial resistance (AMR), ESKAPE pathogens, small-molecule screening, cheminformatics, drug discovery

168 - Interrogating the clinical outcomes and genetic fitness of coagulase-negative *Staphylococcus* in the udder of range sheep using an experimental model of mastitis.

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Subclinical and clinical mastitis result in substantial economic losses to both dairy and nondairy producers in the United States annually. Subclinical mastitis is particularly troublesome to range ruminant producers, as it results in increased infant mortality, decreased infant growth, and increased dam turnover. It also can lead to the misuse of antimicrobials. Such misuse often facilitates the emergence of antimicrobial resistance, which poses a significant present-day threat to humans, animals, and the environment by jeopardizing both health and sustainability. Research on the genes required for bacteria to survive and exploit the lactating mammary gland in ruminants is lacking, as are cost-effective methods to detect, treat, and prevent subclinical mastitis. Our study aims to address this void. The results of a 2023 survey study conducted by us demonstrated that coagulase-negative *Staphylococcus* (CoNS) species were often associated with subclinical and overt mastitis in intermountain range sheep. Using random transposon mutagenesis, we created a mutant library in *Staphylococcus epidermidis* and challenged the left teat cistern of n = 9 clinically healthy ewes during peak lactation (21 d post-lambing). Clinically evident mastitis was achieved in all ewes, and bacterial mutants were shed in high numbers for 5 to 7 d after infection. Milk samples were further characterized using SCC, California Mastitis Test (CMT), and milk cytology before aliquoting and freezing. These data, along with daily ewe health evaluation data (physical exams, complete blood count, serum chemistry), are being used to select the best post-infection milk samples for Illumina sequencing. Once sequenced, the *S. epidermidis* mutant populations from infected milk samples will be compared with the inoculum to determine the genes critical for colonizing and infecting the lactating ovine mammary gland. Likewise, changes in the microbiome of each ewe's mammary gland after infection will also be evaluated both within and between ewes as only half of the udder (left teat cistern) was infected. Because CoNS is a common cause of subclinical mastitis in all ruminants, any advancements in understanding its bacterial pathogenesis within the udder stands to yield tangible scientific knowledge and tools for food animal producers and veterinarians. Improving the diagnosis and management of mastitis is also likely to improve antimicrobial stewardship.

Financial Support: UAES Seed Grant Award UTA01676

Key Words: One Health, multiomics, mastitis, sheep, *Staphylococcus*

169 - The effect of commensal *Corynebacterium* on BRD-associated *Mannheimia haemolytica* growth *in vitro*.

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Bovine respiratory disease remains the most significant health challenge faced by feedlot calves. Although many etiological agents exist, the opportunistic pathogen *Mannheimia haemolytica* remains one of the most common. Previous studies have examined the association between the bovine respiratory microbiota and bovine respiratory disease (BRD). Members of the *Corynebacterium* genus have been associated with respiratory health. Therefore, the goal of this project is to evaluate commensal *Corynebacterium*'s role in the bovine respiratory tract. Specifically, evaluating its antagonistic interaction with *M. haemolytica* and its potential to colonize the respiratory tract and stimulate the immune system. Supernatant growth curves (n = 4/group) were conducted to determine if synergistic interactions existed between 2 bovine-sourced commensal *Corynebacterium* isolates, jzb023 and jzb029. To assess the interaction between these isolates and *M. haemolytica*, supernatant growth curves (n = 4/group) and live cell coculture with quantitative live-dead cell staining (n = 3/group) were performed. Untargeted metabolomics was used to assess the metabolites produced by jzb023 and jzb029 in vitro. All data were analyzed in R (v4.3.2). Growth curve parameters were calculated with the QURVE R package. Cell count and fluorescence microscopy results were assessed using Welch's t-test. Although no synergistic interactions exist between the *Corynebacterium* isolates, the inclusion of the isolates' filter-sterilized supernatant in growth media significantly reduces *M. haemolytica* maximum growth and growth rate ($P < 0.05$). Live cell coculture with subsequent live-dead cell staining and fluorescence microscopy indicated that coculture of jzb029 with *M. haemolytica* reduced the live-dead cell ratio (Control: 18.02 ± 0.72, jzb029 coculture: 14.10 ± 0.57; $P < 0.05$), although the sample size is small. Our results indicate that antagonistic relationships exist between jzb023 and jzb029 and *M. haemolytica*. Preliminary results illustrate that both isolates suppress *M. haemolytica* growth, and, although the sample size is small, jzb029 reduces the *M. haemolytica* live-dead cell ratio. Untargeted metabolomics analyses are ongoing to determine the potential metabolite responsible for these results. Additionally, the interaction of these isolates with the host is currently being assessed using a bovine turbinate cell culture model.

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.

Key Words: bovine respiratory disease, *Corynebacterium*, probiotics

170 - Resembling epithelial responses to swine mycoplasmas *in vitro*: An organotypic 3D tracheal model captures species- and strain-specific divergences.

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Aggravation of pneumonia lesions by *Mycoplasma hyopneumoniae* (*Mhp*) has been linked with both commensal *M. flocculare* (*Mfloc*) and *Mhp* strain diversity. We previously demonstrated that a porcine-derived airway epithelial cells cultured at the air-liquid interface (ALI-PRECs) supports *Mhp* 232 infection, enabling characterization of cytopathic effect (CPE), ciliostasis, and host gene modulation. Here, we evaluated ALI-PREC susceptibility to *Mfloc* and non-pathogenic *Mhp* J. Tracheal-derived PRECs from 3-wk-old high-health pigs were cultured under ALI conditions for 4 wk. Epithelial integrity was assessed by transepithelial electrical resistance before inoculation. Differentiated ALI-PRECs were inoculated with *Mfloc* 27399 (10^5 CCU/mL) or *Mhp* J (5×10^9 CCU/mL). Positive control included viable *Mhp* 232 (*Mhp* 232v), whereas negative controls included heat-inactivated *Mhp* 232 and medium alone. Cells were monitored every 24 h up to 96 h post-inoculation (hpi) for CPE, cytotoxicity, viability (bacterial culture), and bacterial DNA (qPCR). Cytotoxicity was evaluated in apical washes and subnatants, *Mhp* viability in cell fractions, and qPCR on cells and subnatants to assess epithelial disruption. Graphs and statistical analyses were generated using GraphPad Prism software v10.2.1. Ciliary motility was preserved in *Mfloc*, *Mhp* J, and negative control treatments. Overt CPE was absent in *Mhp* J cultures, whereas *Mfloc* induced localized cell lifting without epithelial discontinuity. In contrast, *Mhp* 232v caused marked CPE, absent in its inactivated counterpart. Viable mycoplasmas were recovered up to 96 hpi in all inoculated ALI-PRECs, with earlier detection in *Mhp* J treatments. *Mycoplasma* DNA was detected by 24 hpi in all inoculated cultures, and epithelial disruption was evidenced by *Mhp* 232v and *Mfloc* DNA detection in subnatants at 24 and 48 hpi, respectively. Consistent with these findings, *Mhp* 232v exceeded 50% of cytotoxicity by 24 hpi, whereas *Mfloc* peaked near 45% at 48 hpi. Importantly, cytotoxicity in subnatants was only detected in *Mhp* 232v treatments. In conclusion, ALI-PRECs reliably mirror epithelial responses to swine mycoplasmas, capturing species- and strain-specific divergences. Notably, our findings suggest that commensal *Mfloc* can induce mild but sustained epithelial disruption, potentially increasing susceptibility to secondary infections with pathogenic mycoplasmas. Future co-infection studies are needed to further investigate these interactions.

Key Words: air-liquid interface (ALI) culture, porcine respiratory epithelial cell, *Mycoplasma hyopneumoniae*, *Mycoplasma flocculare*, epithelial disruption, strain-specific divergence

General Abstracts: Session 34—Salmonella 1

171 - Antibiotic class-dependent evolution of multidrug resistance and fitness in *Salmonella* Kentucky ST198.

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The emergence, persistence, and dissemination of antimicrobial resistance (AMR) are strongly suggested by evolutionary studies to depend on the fitness costs and benefits associated with resistance traits. However, how selective pressures from different antibiotics shape both AMR profiles and bacterial fitness in multidrug-resistant (MDR) pathogens remains unclear. In our previous work, a multidrug-resistant *Salmonella* Kentucky ST198 strain (PU131; MDR-SK198) was serially passaged under subinhibitory concentrations of 5 antibiotics: amoxicillin (Amox), ciprofloxacin (Cip), streptomycin (Str), sulfamethoxazole-trimethoprim (STrim), and tetracycline (Tet), as well as in antibiotic-free conditions (NoAbx), in duplicate lineages (L1 and L2). This experimental evolution revealed antibiotic class-dependent changes in AMR. Lineages exposed to Cip and Str lost resistance to multiple antibiotic classes, whereas Amox-, STrim-, and Tet-exposed lineages retained resistance comparable to the wild type. Interestingly, NoAbx lineages diverged: L1 lost resistance similar to Cip and Str, whereas L2 developed 2 subpopulations, one resembling L1 and the other resembling Amox/STrim/Tet lineages. In the present study, we investigated whether these AMR shifts were associated with differences in bacterial fitness and virulence. We assessed growth kinetics, competition assays with the wild type at 37°C and 42°C, and in vitro infectivity using Caco-2 cells. In parallel, we conducted a retrospective analysis of 52,925 human nontyphoidal *Salmonella* (NTS) isolates (1996–2024) from the US National Antimicrobial Resistance Monitoring System (NARMS). Using Spearman's rank correlation, we evaluated relationships between resistance to specific antibiotic classes and the prevalence of MDR phenotypes. Analysis of the NARMS dataset revealed significant correlations between antibiotic class resistance and MDR status across human NTS isolates. Fitness studies showed that Cip-L2 and NoAbx-L2 (subpopulation 1) were more competitive than the wild type at 42°C, and preliminary assays indicated reduced virulence of Cip-L2 in Caco-2 cells. Together, our findings suggest that the persistence of MDR in *Salmonella* Kentucky is strongly influenced by the class of resistance traits carried, with implications for both fitness and virulence. These results highlight the importance of antibiotic class-specific selection pressures in shaping the evolutionary trajectory of MDR pathogens.

Financial Support: Texas Tech University School of Veterinary Medicine

Key Words: antibiotic use, antimicrobial resistance, evolution, multidrug-resistance

172 - Adopting a metagenomic and quasimetagenomic approach to comparatively investigate the microbial diversity of bovine feces cultured for *Escherichia coli*, *Salmonella*, *Campylobacter*, and *Listeria* spp.

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Advancements have been made in the application of metagenomics, with modifications such as quasimetagenomics, greatly aiding in the rapid detection of pathogens. In this study, the fecal microbiomes of cattle known to be shedding *Salmonella*, *Escherichia coli*, *Listeria*, and *Campylobacter* were investigated using metagenomics and quasimetagenomics to examine the dynamics of microbial populations in infected and uninfected cattle. For this study, DNA extracted directly from fecal samples and culture enrichment samples of 28 apparently healthy cattle kept on grass pastures and a similar feeding regimen were sequenced and analyzed. Cattle farms recorded no use of antibiotics for at least 3 mo before sampling. The fecal shedding statuses of the animal subjects for *Salmonella*, *E. coli*, *Listeria*, and *Campylobacter* were determined in a concurrent study. For bacterial enrichment, standard procedures for selectively enriching the respective pathogens were followed. The compositional abundance of microbial populations in the sequenced data were analyzed using a custom k-mer database and Kraken2. Results showed the bovine fecal microbiome comprised large proportions of diverse microbes dominated by *Bacteroidetes* and *Firmicutes*. A large proportion of *Proteobacteria* in a sample positive for simultaneously shedding *Salmonella*, *E. coli*, and *Campylobacter* revealed potential dysbiosis resulting from the multiple-coinfection. For culture enrichment samples, only *E. coli* was detected in significantly abundant numbers, even to the serovar level. On the other hand, *E. coli* and other anaerobes were significant co-enrichers of *Salmonella* and *Campylobacter* cultures, respectively, masking the detection of these target pathogens. This study shows that co-enrichers can significantly affect the potential application of quasimetagenomics in studying target microbes from bovine feces. Furthermore, the gut microbiome of animals shedding multiple pathogens can offset the bovine gut microbiota. The implications of these findings for microbial culture and public health deserve further study.

Financial Support: This research was funded by grants from USDA/NIFA CBG 2021-38821-34710 and USDA/NIFA/AFRI 2022-67017-36982.

Key Words: metagenomics, *Salmonella*, *Campylobacter*, *Escherichia coli*, *Listeria*, cattle

173 - Genotypic and phenotypic characterization of multidrug resistance in *Salmonella* isolated from cattle.

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Nontyphoidal *Salmonella* is a foodborne pathogen and a global health concern affecting both human and animal health via zoonotic transmission. Although antibiotic therapy remains the standard treatment for salmonellosis, the widespread misuse of antibiotics has contributed to the emergence of multidrug-resistant (MDR) *Salmonella* strains. Our study aimed to comprehensively characterize the genotypic and phenotypic traits of *Salmonella* isolates from necropsied cattle. A total of 1,008 samples were collected from necropsied cattle in Kentucky. Genotyping and serotyping characterization confirmed that 27 isolates belonged to *Salmonella* ssp. *enterica*. *Salmonella* Dublin was the most prevalent serotype (29.6%). All the isolates harbored multiple virulence genes, including invA, hilA, fimA, csgA, and flhD. The biofilm-forming ability of the isolated bacteria was assessed using a crystal violet assay. These isolates produced biofilms at varying intensities. The motile activity of the isolates was assessed on soft-agar plates; 96.3% of the isolates exhibited both swarming and swimming motility. The AMR profile was tested against 19 antibiotics representing 9 classes. The highest resistance was observed to azithromycin (100%), followed by imipenem (92.6%) and chloramphenicol (85.2%). All isolates were MDR, and 5 were extensively drug-resistant (XDR). Pearson correlation analysis revealed several significant genotypic–phenotypic associations. Components of the T1SS (siiA and siiC) exhibited a significant correlation ($r = 1.00$; $P < 0.001$). Strong correlations were also observed between β-lactamase genes and their corresponding phenotypic resistances, such as blaTEM with meropenem and ampicillin, and blaCMY with amoxicillin–clavulanic acid, ampicillin, and ceftiofur ($r = 0.54–0.64$; $P < 0.001$). In conclusion, the emergence of MDR *Salmonella* in cattle poses a significant threat to both the livestock industry and public health. Ongoing whole-genome sequencing (WGS) analyses will provide higher-resolution insights into resistance genes, mobile genetic elements, and virulence determinants compared with conventional methods.

Financial Support: Center for Biomedical Research Excellence (COBRE), and Center for Pharmaceutical Research and Innovation (CPRI, NIH P20 GhdsbM130456) and U.S. Department of Agriculture (USDA) National Institute for Food and Agriculture (NIFA) (grant number 2022-09086)

Key Words: One Health, genomics, multidrug-resistant, salmonellosis

174 - Prevalence, serovar diversity, and antimicrobial resistance of *Salmonella* in mesenteric lymph nodes of dairy-origin steers at harvest.

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Salmonella enterica remains one of the leading foodborne threats worldwide, with livestock serving as a major reservoir. In cattle, the pathogen can persist in mesenteric lymph nodes (MLN), which may inadvertently enter ground beef during processing contaminating the food supply. Despite this risk, *Salmonella* circulation in dairy cattle raised for beef remains poorly understood. This study investigated prevalence, serovar diversity, and antimicrobial resistance in MLN from dairy-origin steers at harvest. Samples (n = 626) were collected at a Midwestern US harvest facility during the summers of 2023 and 2024. Each sample consisted of 3 to 5 pooled MLNs collected along the production line. *Salmonella* was isolated using the One Herd Lab's protocol; colonies were serogrouped, a subset was serotyped by serology, and selected strains underwent antimicrobial susceptibility testing (AST) and whole-genome sequencing for serovar confirmation. Cattle originated from 28 source farms across 7 Midwestern states. *Salmonella* spp. was detected in 41.5% (n = 262) of MLN samples, spanning 82.1% (n = 23) of sources. Among positive samples, serogroups C (34.7%) and E (31.3%) predominated. From 101 serotyped isolates, 25 unique serovars were identified. Whole-genome sequencing confirmed serovars frequently associated with dairy-beef cattle, including Anatum and Montevideo, alongside the clinically significant serovars Dublin, Enteritidis, and Typhimurium. Rare serovars such as Agbeni, Kiambu, and Ruiru were also detected. The AST results from 21 selected isolates showed 66.7% were pan-susceptible. Three exhibited resistance to a single antimicrobial, and 4 were multidrug resistant. Notably, both *Salmonella* Dublin isolates displayed resistance to 7 antimicrobial classes. These findings reveal a high prevalence and broad diversity of *Salmonella* in MLNs from dairy-origin steers, with frequent multidrug resistance. The results emphasize the role of dairy-origin beef in *Salmonella* epidemiology and highlight the need for targeted interventions and continued surveillance at the livestock–food interface. Further studies are warranted to expand the geographical scope to include additional processing plants.

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Key Words: prevalence, *Salmonella*, lymph node, dairy-beef, harvest

175 - Phagocytic uptake and intracellular replication of fluorescently modified *Salmonella* Newport and Lubbock.

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Non-Typhi *Salmonella* enterica serovars are major contributors to foodborne illness in humans, often linked to undercooked ground beef. Despite slaughter-plant interventions, salmonellosis persists due to bacterial colonization of bovine lymph nodes. *Salmonella* Newport has caused multi-state outbreaks in the US, whereas *Salmonella* Lubbock is prevalent in healthy Texas cattle. Both are associated with lymph nodes, posing a contamination risk in ground beef. This study aimed to assess whether fluorescence gene modification affects the invasion and replication behavior of *Salmonella* strains, by comparing fluorescent *Salmonella* Lubbock and *Salmonella* Newport with nonfluorescent *Salmonella*. This approach provides insight into *Salmonella* host–pathogen interactions. J774A.1 murine macrophages were infected with 7 *Salmonella* strains: *Salmonella* Lubbock sfGFP (GL) and its progenitor nonfluorescent *Salmonella* Lubbock (NFL), *Salmonella* Newport mCherry (MN) and its progenitor nonfluorescent *Salmonella* Newport (NFN), *Salmonella* Typhimurium ATCC 14028 (SL29), SL29 ΔinvA (SL1274), SL29 ΔphoPQ (SL1299), plus 3 combinations (GL × MN; NFL × GL; NFN × MN). Colony-forming units (cfu/mL) were measured at 2 h and 24 h. Fold-change was calculated. Data were analyzed using a 2-way full factorial linear regression with marginal means and pairwise comparisons with Bonferroni correction. Macrophage invasion varied among the serovars. Significant differences ($P < 0.05$) at 2 h post-infection were found. Notably, MN and NFN exhibited significantly lower invasion than GL, NFL, combined GL, SL1274, and SL1299. *Salmonella* replication also differed across serovars. Contrastingly, MN was significantly lower ($P < 0.05$) than NFN, GL, NFL, and the combined effects of GL × MN and NFN × MN. As expected, SL1299 showed significantly lower replication ($P < 0.05$) than SL29. *Salmonella* Lubbock exhibited high performance in invasion and replication, even surpassing SL29. When comparing all groups, the strain SL1299 had a 0.2 $\log_{10}$ reduction; MN, its combination GL × MN and SL29 alone had a 0.1 to 0.2 $\log_{10}$ increase, whereas NFN, *Salmonella* Lubbock, and their combinations had >0.4 $\log_{10}$ increases. Our results show that *Salmonella* Lubbock exhibits greater macrophage invasion and replication than *Salmonella* Newport, with its pathogenicity unaffected by fluorescence gene modification, unlike *Salmonella* Newport. Because macrophage invasion is critical for within-host survival and replication, these results highlight key differences in pathogenic potential among *Salmonella enterica* subspecies *enterica* serovars.

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

Key Words: fluorescent *Salmonella*, macrophage, invasion, replication

176 - Targeted shotgun sequencing improves the ability to detect and characterize *Salmonella enterica* in metagenomic samples.

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Salmonella enterica is a major pathogen of humans and animals and causes serious disease in infected individuals. Undetected *Salmonella* can spread to at-risk populations such as the young, old, and immunocompromised. Current detection methods, such as enriched culture, require lengthy testing periods and can alter which organisms or strains are detected. Animals shed *Salmonella enterica* in low concentrations and these diagnostic tools lack the sensitivity to accurately capture strain-level variants. Metagenomic fecal samples present a significant challenge when attempting to identify *Salmonella enterica* that is present in low abundance. More abundant organisms tend to outgrow or obscure the presence of organisms in lower concentrations. To address this challenge, we demonstrate that target-enriched metagenomics shotgun sequencing is an extremely sensitive method to focus characterization of *Salmonella enterica* in metagenomic sequencing libraries without the need for broth enrichment. To do so, we designed a bait set comprised of 179,399 cRNA probes based on the full genome, plasmid, and antimicrobial resistance markers across 39 serovars of *Salmonella enterica* the CDC designates as most important to human and animal health. Results from the targeted shotgun sequencing of fecal samples were compared with tetrathionate broth-enriched samples to evaluate how this method affects strain detection. To confirm the sensitivity and recall of our bioinformatics tools, we performed in silico testing using mock communities alongside our customized reference database composed of all 15,934 *Salmonella enterica* genomes found in RefSeq. This benchmarking laid the foundation for the analysis of our sequenced targeted shotgun samples. Using the VARIANT++ pipeline, we were able to establish multiple serovar detection within individual samples as well as novel genetic variant–level diversity. These data are crucial for characterizing the strain-level ecology of *Salmonella*. Importantly, the target-enriched technique demonstrated here can be applied to future studies of targeted shotgun sequencing in human and animal health. *Salmonella* in beef is highly concerning, and we are eager to test the effectiveness of this method in detecting and characterizing *Salmonella enterica* strain diversity in this different sample type. We conclude that targeted shotgun sequencing is a powerful genomic tool in understanding low-abundance pathogen ecology in metagenomic samples.

Financial Support: Texas A&M University

Key Words: *Salmonella*, targeted sequencing, NGS, shotgun

General Abstracts: Session 35—Coronavirus

177 - Porcine enteroids as a platform for coronavirus viability testing: Initial assessment using porcine deltacoronavirus (PDCoV).

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Porcine enteric coronaviruses continue to cause substantial economic losses to the swine industry. Although molecular assays are highly analytical sensitive, the detection of viral RNA on potentially contaminated surfaces after disinfection does not confirm infectivity. In vivo bioassays can demonstrate viral viability but are often impractical due to cost, logistics, and ethical considerations. Porcine enteroids offer greater physiological relevance than traditional in vitro models and may serve as an ex vivo alternative for viability assessment. This study evaluated the feasibility of porcine enteroids cultured on transwell inserts (PETC) as a diagnostic platform for detecting viable porcine deltacoronavirus (PDCoV). The objectives were to (1) determine the minimum infectious dose of PDCoV required to infect PETC and PDCoV-adapted swine testicular (ST) cells, and (2) assess the infectivity of field samples using ex vivo (PETC), in vitro (ST cells), and in vivo (neonatal piglet) models. First, PDCoV (Michigan/8977/2014; 3.16×10^5 $TCID_{50}$/mL) was 2-fold serially diluted (2^1 to 2^{14}) and inoculated into PETC derived from duodenum, jejunum, and ileum of 7-d-old CDCD piglets (n = 3), as well as into ST cells. Infection was assessed by cytopathic effect (CPE), immunofluorescence assay (IFA) targeting the N protein, and RT-qPCR for detecting the N gene. Second, field samples (feeders, rubber teats, pen floor, and sewage) collected from a PDCoV-affected farm post-disinfection were tested (Cq 30–34). These samples were inoculated into jejunum-derived PETC, ST cells, and piglets. ST cells and PETC were assessed using CPE, IFA, and RT-qPCR, whereas piglets were monitored for clinical signs, histopathology, and viral shedding. In PETC, CPE and IFA were minimal or absent, whereas RT-qPCR detected PDCoV RNA at dilutions between 2^1 to 2^{10} (Cq 24–35). Jejunum-derived PETCs were most susceptible to PDCoV, justifying their use for field sample testing. On the other hand, PDCoV-adapted ST cells displayed more pronounced CPE and IFA signals across all dilutions. No evidence of infection was detected in PETC, ST cells, or piglets following inoculation with field samples. Although culture optimization is ongoing to enhance sensitivity and diagnostic performance, PETC represents a promising ex vivo platform that is consistent with the 3R principles. This system may be extended to evaluate viability for other enteric viruses of clinical importance to the swine industry.

Key Words: porcine enteric coronavirus, porcine deltacoronavirus, enteroid, viability

178 - Effects on viral shedding of bovine coronavirus under stressful conditions.

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Bovine coronavirus (BCoV) is a single-stranded positive-sense RNA virus that causes both enteric and respiratory disease. Bovine coronavirus is transmitted through both the fecal-oral route and the respiratory route. Stressful events such as commingling and transporting have been linked to increased BCoV shedding, sometimes contributing to bovine respiratory disease (BRD) development. The objective of the study was to quantify viral shedding of bovine coronavirus following stressful events, commingling and transporting. Ninety newborn calves were enrolled in this randomized controlled trial upon arrival from a calf raising facility in New Mexico at ~3 d of age. Calves were raised in individual hutches for 2 mo, and then randomly allocated to one of 3 groups: HUTCH calves, which remained in their individual hutches; PEN calves, which were commingled in pens of 150 calves; and PEN-TRUCK calves, which were transported via trailer for 8 h before being commingled in pens of 150. Nasal and fecal swabs were collected before intervention followed by 1, 3, and 7 d after intervention and placed in 1 mL of DNA/RNA shield (Zymo Research Corporation). Air swabs were also collected around hutches and within the pens and will be used for detection of aerosolized virus. Viral RNA extraction was performed using the Zymo Quick-RNA Viral kit and cDNA synthesis was performed using GoScript Reverse transcriptase (Promega). The RT-PCR analysis was performed using the BCoV nucleocapsid protein primers and TaqMan probe, and the 18S rRNA gene was used as a housekeeping control. Bovine coronavirus RNA was detected on nasal swabs for all 3 study groups 3 d after intervention, with higher detection at 7 d for the 2 commingled groups. The PEN-TRUCK group had the highest percentage of positive cases at 7 d. A positive upward trend was seen in the PEN group over the sampling timeline. Under the conditions of this study, commingling appeared to increase the proportion of calves shedding BCoV.

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Key Words: viral shedding, commingling, bovine coronavirus

179 - White-tailed deer as a cryptic reservoir for both ancestral and emerging SARS-CoV-2 variants.

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has infected over 778 million people and caused more than 7 million deaths worldwide. Since its emergence in 2019, several variants of concern (VOCs), from Alpha through Omicron, have emerged and spread globally. The prevailing view has been that when newer variants arise, ancestral lineages are displaced due to the fitness advantages of faster replication or immune evasion. However, accumulating evidence of SARS-CoV-2 infections in animals raises the possibility that older lineages may persist outside human populations. White-tailed deer (WTD) are widespread in North America and have been shown to harbor SARS-CoV-2 following human-to-deer spillover. To investigate ongoing viral circulation, we sampled 135 hunter-harvested WTD in Princeton, New Jersey (February 2025). We detected SARS-CoV-2 RNA in 57% of nasal and oral swabs (n = 77) by N gene qRT-PCR. Whole-genome sequencing revealed both ancient B.1 lineage viruses (n = 38) and Omicron VOCs (n = 32), including BA.2, XBB.1, XBB.1.9, and KP.3.1.1. Phylogenomic analysis indicated multiple independent spillovers as well as sustained deer-to-deer transmission. Notably, B.1 lineage was detected in WTD 2 to 3 yr after detection in humans, suggesting the sustained deer-deer transmission and persistence in the deer population. The SARS-CoV-2 B.1 lineage of WTD was found to be the closest neighbor of the genome detected in humans in 2020. B.1 lineage, BA.2, XBB.1, and XBB.1.9 from WTD have several substitutions across the genome and are highly diverged compared with their counterparts from humans, indicating adaptive evolution in the deer host. Our findings demonstrate that WTD harbor multiple SARS-CoV-2 lineages, including ancestral variants no longer circulating in people. This continued circulation and diversification of ancient lineages in wildlife challenges the assumption that older variants are fully displaced and highlights the potential for nonhuman reservoirs to alter viral evolutionary trajectories. Sustained wildlife surveillance will be essential to monitor these hidden sources of SARS-CoV-2 diversity and to anticipate novel variant emergence with implications for human and animal health.

Financial Support: USDA- NIFA

Key Words: SARS-CoV-2, white-tailed deer, ancestral lineage, cross-species transmission, One Health, viral evolution

180 - Inactivation of dual IFN antagonists in PEDV as a strategy for live-attenuated vaccine design.

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Porcine epidemic diarrhea virus (PEDV), an α-coronavirus, causes high morbidity and mortality in neonatal piglets. The host's innate immune response, particularly type I and III interferons (IFN), plays a critical role in controlling infection. However, PEDV has evolved several IFN antagonists, including nonstructural proteins nsp1, nsp15, and nsp16. This study investigated whether the simultaneous inactivation of 2 antagonists enhances viral attenuation and host immune activation. Using an infectious clone of PEDV-Colorado strain, we engineered a panel of recombinant viruses carrying single and double mutants of nsp1, nsp15, and nsp16. Compared with the isogenic, recombinant wild-type virus (rWT), the single mutants (rNsp1mut, rNsp15mut, rNsp16mut) had similar growth kinetics in Vero-81 cells, whereas double mutants (rNsp1+15mut, rNsp15+16mut, rNsp1+16mut) exhibited impaired replication in Vero-81 and PK-1 cell lines, with rNsp15+16mut showing the most pronounced replication defect. To assess type I IFN response, we generated 2 reporter PK-1 cell lines to measure IFN-β and Interferon-stimulated gene factor 3 (ISGF3) activation. In these reporter cell lines, mutant viruses containing a mutated Nsp15 (rNsp15mut, rNsp1+15mut, rNsp15+16mut) elicited an early and robust activation of IFN-β and ISGF3 pathways, whereas the other mutants (rNsp1mut, rNsp16mut, rNsp1+16mut) induced a higher IFN response than rWT but at relatively later times compared with the Nsp15 mutants. These results suggest that Nsp15 is a major IFN antagonist. Quantitative RT-PCR analysis confirmed this finding and revealed significantly higher expression of type I and III IFNs in PK-1 cells compared with rWT infection, with rNsp15+16mut being the strongest inducer. Moreover, rNsp1+15mut and rNsp15+16mut were more sensitive to exogenous IFN-β treatment, with rNsp15+16mut exhibiting the highest inhibition. In piglet challenge studies, both mutants were attenuated with reduced virus shedding and clinical scores compared with rWT infection, and they elicited comparable PEDV-specific IgG and neutralizing antibodies as rWT infection. Mechanistically, inactivation of nsp15 leads to double-stranded RNA (dsRNA) accumulation and increased stress granule (SG) formation in infected cells. These findings demonstrate that co-inactivation of PEDV IFN antagonists synergistically enhances host innate responses and attenuates viral replication, supporting their potential as live-attenuated vaccine candidates.

Financial Support: This project was supported by the Agriculture and Food Research Initiative Competitive Grant no. 2023-67015-39096 from the USDA National Institute of Food and Agriculture.

Key Words: porcine epidemic diarrhea virus (PEDV), interferon antagonist, Nsp15, vaccine

181 - Characterizing the antiviral role of ZFP36L1 in suppressing RNA viruses associated with gastroenteritis.

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Viral gastroenteritis in pigs causes significant damage to swine worldwide, including the United States. The primary etiological agents are porcine rotaviruses (PRV) and swine enteric coronaviruses (SECDs), including transmissible gastroenteritis virus (TGEV), porcine epidemic diarrhea virus (PEDV), and porcine deltacoronavirus (PDCoV). Identifying host proteins that broadly suppress replication of these viruses could inform antiviral strategies. ZFP36L1, a CCCH-type zinc finger RNA-binding protein involved in mRNA turnover, has demonstrated antiviral properties across RNA viruses. This study investigates the mechanism by which ZFP36L1 suppresses replication using human coronavirus OC43 (HCoV-OC43) and human rotavirus A strain Wa (HRV-Wa) as model systems, which share similarities with SECDs and PRV, and evaluated sodium butyrate as a potential inducer of ZFP36L1. Wild-type (WT), ZFP36L1-overexpressing (OE), and knockdown (KD) HCT-8 cells were infected and analyzed for viral titers and transcripts via qPCR. OE in HCT-8 (HCoV-OC43) and MA104 clone 1 (HRV-Wa) cells significantly reduced titers, whereas KD increased titers versus WT ($P < 0.05$). Experiments were performed in 3 biological replicates with 2 technical repeats (n = 6) for reproducibility. Statistical significance was assessed using paired *t*-test, one-way and 2-way ANOVA ($P < 0.05$). To test poly(A) deadenylation, CNOT1 knockdown was performed in OE cells; titers were not rescued, indicating suppression independent of poly(A) deadenylation. Computational analyses (RPISeq, docking) predicted strongest binding of ZFP36L1 to HCoV nucleocapsid RNA, confirmed by RNA immunoprecipitation and validated by luciferase assays, likely contributing to reduced replication. PyMOL modeling identified residues critical for this interaction. ZFP36L1 also interacted with HRV-Wa segment 6 (VP6), likely contributing to reduced viral titers, under investigation. Sodium butyrate increased ZFP36L1 expression and further reduced HCoV-OC43 titers ($P < 0.05$). Overall, these results indicate ZFP36L1 restricts HCoV-OC43 replication through a poly(A)-independent mechanism involving viral RNA binding and may exert similar effects on other enteric RNA viruses, highlighting its potential as a broad-acting antiviral factor relevant to controlling gastroenteritis in pigs. Future studies will confirm predicted residue via mutagenesis, explore non-deadenylation pathways (PARN, DCP1/2), and assess exogenous nucleocapsid effects on titers.

Financial Support: Gillespie College of Veterinary Medicine at Lincoln Memorial University

Key Words: ZFP36L1, HCoV-OC43, rotavirus, poly(A) deadenylation, nucleocapsid, sodium butyrate

182 - Virulence investigation of a novel porcine epidemic diarrhea virus variant harboring a 32-amino acid deletion in nonstructural protein 3.

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Porcine epidemic diarrhea virus (PEDV) is a highly communicable, enteropathogenic swine coronavirus. Since the global emergence of highly pathogenic genotype 2b (HP-G2b) strains in 2013, HP-G2b PEDV has become endemic in South Korea. During local outbreaks in southern South Korea, we identified novel HP-G2b PEDV variants with a 32-amino acid (aa) deletion (DEL) in nonstructural protein 3 (nsp3), termed nsp3-DEL32, in several affected farms commonly experiencing low neonatal mortality. Porcine epidemic diarrhea virus nsp3-DEL32 variants displayed nearly identical spike barcode profiles and shared 99.6% to 99.9% homology at the genome level. However, the nsp3-DEL32 strains shared 95.0% to 98.9% and 97.6% to 9.3% homology with other South Korean HP-G2b PEDVs in the S gene and whole-genome sequences, respectively. Because the nsp3-DEL32–infected farms had no previous herd history of PEDV outbreak or vaccination, we hypothesized that such a large DEL in nsp3 is associated with PEDV virulence. To test this hypothesis, we generated a recombinant PEDV harboring the same DEL signature in nsp3, designated icnsp3-DEL32, using a reverse genetics system. The cytopathology and growth properties of icnsp3-DEL32 were comparable to the wild-type clone virus, named icKNU-141112. The PEDV nsp3 protein plays a role in negatively regulating interferon (IFN) production to circumvent the host immune responses. Interestingly, transcriptional expression levels of IFN-β, IFN-λ1, and IFN-λ3 were significantly higher in cells infected with icnsp3-DEL32 than those infected with icKNU-141112 ($P = 0.0109$, $P = 0.0009$, and $P = 0.0006$, respectively), suggesting the hindrance of DEL32 in the action of nsp3 as an IFN antagonist. We then performed an experimental infection using suckling piglets with icnsp3-DEL32 or icKNU-141112. The icKNU-141112–inoculated animals presented severe clinical symptoms, including thin, transparent intestinal walls and severe villous atrophy, with 100% mortality. The icnsp3-DEL32–inoculated piglets also suffered from acute, watery diarrhea and finally underwent PEDV-associated mortality. However, 2 piglets (40%) recovered and survived, which exhibited reduced viral loads ($P = 0.0327$) and normal macroscopic and microscopic observations in intestinal tissues, indicating the low enteropathogenicity of icnsp3-DEL32 in neonates. These findings demonstrate that a naturally occurring 32-aa deletion in nsp3 partially attenuates PEDV in piglets, highlighting nsp3 as a contributor to viral pathogenicity.

Key Words: porcine epidemic diarrhea virus, nsp3, IFN antagonist, deletion, reverse genetics, attenuation

Featured Speaker: Session 36—AAVI Symposium

183 - Lifting the veil: Modern approaches to addressing *Salmonella* persistence in porcine macrophages.

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Salmonella enterica is an important foodborne pathogen, and pork products are a source for human disease. Most foodborne isolates of *Salmonella* cause only a self-limiting, transient disease in swine but can cause major disease in humans. Swine can become persistently infected with foodborne *Salmonella*, posing a significant challenge for food safety and public health. A clear understanding on the mechanisms of *Salmonella* transmission and persistence in porcine tissue immune cells is a necessary first step to developing targeted intervention strategies to significantly reduce carriage in swine. Our overall hypothesis is that *Salmonella* resides in mononuclear phagocytic cells (e.g., macrophages) of porcine intestinal and lymphoid tissues, and subsequently modulates the cellular state to facilitate survival. Thus, we used fluorescent immunoprotein and in situ labeling techniques to identify mononuclear phagocyte populations harboring *Salmonella* in the large intestine, tonsil, and lymph node and identified populations of *SIRPA*- and *CSF1R*-expressing cells also positive for *Salmonella* labeling. Single-cell RNA sequencing of tissues from *Salmonella* infected and noninfected pigs has provided insight on the cellular response and nonresponse of specific cell types. Spatial transcriptomics (10x Genomics Xenium), using targeted *Salmonella* and porcine gene probes, was used to first identify *Salmonella* infected cells in porcine tonsil and ileum and subsequently compare transcriptional profiles of infected versus noninfected cells. Given the expression of butyrate receptors by *Salmonella*-positive mononuclear phagocytes, we explored the ability of butyrate to reduce *Salmonella* infectivity and highlight the ability of butyrate to modulate monocyte cell status for limiting *Salmonella* infectivity. Overall, modern technologies are providing the ability for lifting the veil on *Salmonella* mechanisms of persistence in porcine tissue macrophages.

184 - A single-cell level exploration of the pig's immune system in health and disease.

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Unbiased transcriptional profiling of single cells through high-throughput single-cell RNA sequencing (scRNAseq) has transformed the swine immunology field by greatly increasing our understanding of the phenotypic diversity and plasticity of immune cell types and their interactions in complex tissues, in both health and disease. Pairing scRNAseq with T cell receptor (TCR) and B cell receptor (BCR) sequencing provides additional utility for studying the relationship between immune repertoire and different types of immune responses. Here, we developed pig-specific TCR and BCR primers compatible with the droplet-based protocols of the 10x Genomics scRNAseq platform. We describe using this protocol to create a cell atlas of pig hematopoietic development during embryonic, neonatal, and adult life. We highlight the connection between fetal liver, bone marrow, and thymus differentiation with a focus on how the pig's unique TCR and BCR repertoire unfolds. We also link immune cells in the lung with their central lymphoid organ origins. Finally, we characterize the pig's pulmonary immune response to influenza, including the influence of prior vaccination, prior infection, and the genetic ablation of invariant natural killer T cells, a subset of innate-like T cells that bridge the innate and adaptive immune systems. Our data provide new insights into the porcine immune system and contribute a valuable resource for enhancing effective vaccine and therapeutic design to protect swine health.

General Abstracts: Session 37—Diagnostics and Biomarkers

185 - Hapten-mediated signal amplification systems for enhanced immunohistochemical detection of CWD prion protein.

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Early detection of chronic wasting disease (CWD)-positive cervids is key to formulating and implementing mitigation strategies aimed at effectively reducing disease spread. Currently, only immunohistochemistry (IHC) and the enzyme-linked immunosorbent assay (ELISA) are approved for the diagnosis of CWD by the National Animal Health Laboratory Network (NAHLN), with IHC considered the gold standard. Although IHC is a robust method, it has a relatively low sensitivity with suboptimal detection of early preclinical stages of CWD, where the amount of prion antigen is low. Therefore, it is critical to identify alternative amplification methods to enhance CWD detection in support of surveillance and early control strategies for wild and farmed cervids. The objective of this study was to establish hapten-mediated amplification assays for enhancing IHC detection of CWD prion protein. We established 2 hapten-based IHC assays coupled with tyramide signal amplification (TSA): assay 1, based on TSA conjugated to the UT015 hapten (Leica Biosystems), and assay 2, based on a TSA-digoxigenin (DIG) conjugate. We performed checkerboard titrations of all reagents in at least 2 replicates on formalin-fixed, paraffin-embedded sections of obex and retropharyngeal lymph node (RPLN) from 2 CWD strong positive and 2 negative white-tailed deer. We determined that concentrations ranging from 1:400 to 1:5,000 of UT-015-labeled tyramide and 1:400 to 1:800 of DIG-labeled tyramide enhance detection of CWD prion protein on obex and RPLN sections compared with the NAHLN IHC protocol. Signal intensity was computationally assessed (QuPath0.6.0) in at least 3 regions of interest per lymph node. We determined that both assays induced enhanced signal compared with the NAHLN assay, with assay 2 yielding the strongest signal (ANOVA with Tukey's post hoc; *P*-values <0.05). Although both assays showed optimal signal-to-noise ratio in RPLN, both assays induced nonspecific labeling of neuronal bodies on CWD-negative samples. In conclusion, these 2 hapten-mediated signal amplification methods enhance the immunohistochemical detection of CWD prion protein, yielding excellent signal-to-noise ratio in lymphoid tissue. This approach has the potential to improve the assay's sensitivity and enhance the detection of CWD prion protein in antemortem lymphoid tissue. Future work aims to eliminate nonspecific labeling, as well as assess its diagnostic performance compared with the approved NAHLN IHC protocol.

Financial Support: We would like to acknowledge personnel of the Histology and IHC section at the Louisiana Animal Disease Diagnostic Laboratory as well as USDA NAHLN and the Kenneth Burns Endowed Chair in Veterinary Medicine support at LSU Vet Med for funding digital slide scanners. This material was made possible, in part, by Cooperative Agreement AP24VSSPRS00C140 from the United States Department of Agriculture's Animal and Plant Health Inspection Service (APHIS). It may not necessarily express APHIS' views.

Key Words: chronic wasting disease (CWD), immunohistochemistry, tyramide, prion

186 - Reference range and predictors of eye temperature in pre-weaned dairy calves.

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Infrared thermography has been proposed as a noninvasive tool for monitoring calf health. This study aimed to establish a reference range for eye temperature (ET) in dairy calves and to identify factors associated with its variation. Data were collected from 21 preweaning calves under field conditions, including shaded and unshaded hutches. At each visit, ET was recorded 3 times, and rectal temperature (RT) once. Reference intervals were estimated using the *referenceIntervals* R package (parametric method with 90% CI of limits), using the first ET reading from apparently health calves. A linear mixed model with calf ID as a random effect was developed to assess associations between ET and predictors such as repeat (3 ET readings), RT, age, sex, wind speed, shade (yes, no), time of the day (TOD), and temperature-humidity index (THI). The reference interval for ET was 37.3°C to 40.2°C. The mixed model showed that RT (β = 0.54°C, $P < 0.0001$), sex (β = female +0.32°C vs. male, $P < 0.0001$), THI (β = 0.04, $P = 0.0029$), wind speed (β = 0.07, $P = 0.0015$), TOD (β = afternoon +0.24 vs. morning, $P < 0.011$), and shade (β = no +0.79 vs. yes, $P < 0.0001$) were significantly associated with ET. These findings provide the first reference interval for calf ET under farm conditions and demonstrate that environmental and management factors significantly influence measurements. Eye thermography may serve as a useful method for monitoring calf health, though interpretation requires consideration of external influences.

Key Words: calf, eye temperature, infrared thermography, reference interval, mixed model, dairy

187 - Establishment of reference intervals for ocular surface temperature in canines via infrared thermography.

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Rapid, noninvasive temperature screening is a priority in shelter medicine, although the reliability of infrared thermography (IRT) is uncertain. In this study, we evaluated IRT to measure eye temperature (ET) in shelter dogs and established a 95% reference interval (RI). We first assessed camera thermal reading stability by imaging a fixed point on the inner wall of an insulated box every 5 min for 1 h and testing for an effect of time by repeated-measures linear regression. Next, we collected 129 ET readings from 78 apparently healthy, unrestrained shelter dogs (3 measurements every 10 min per visit), alongside rectal temperature (RT), ambient temperature, and humidity. We used linear mixed-effects models to test the effects of factors associated with ET, such as, RT, age, sex, size, ambient temperature, humidity, and repeated measurements, including dog ID as a random intercept. To establish a 95% RI for healthy dogs, we applied a robust bootstrap approach to the first ET reading from each of the 78 unique dogs. In the stability study, there was no significant effect of time on IRT readings ($P > 0.05$). In the canine study, RT ($\beta = 0.7$°C; $P < 0.0001$) and humidity ($\beta = 0.007\%$; $P < 0.0131$) were identified as significant predictors of ET. The 95% RI for ET was calculated as 35.0°C to 38.5°C. These findings suggest that thermal camera performance remains stable over time, ET is associated with RT, and establishes a reference range for ET. Overall, these results support the continued exploration of IRT as a noninvasive tool for canine temperature screening.

Key Words: canine, infrared thermography

188 - Differentiating vaccinated from infected animals (DIVA) using a multiplex Luminex assay for HPAI.

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The widespread circulation of highly pathogenic avian influenza (HPAI) H5N1 virus across multiple animal species, including its unprecedented detection in dairy cattle in 2024, has intensified the demand for effective vaccination strategies. However, due to regulatory complexities surrounding H5N1 vaccination, particularly in livestock, there is a critical need for serological tools capable of differentiating infected from vaccinated animals (DIVA). In response, we developed a novel multispecies, multiplex Luminex assay designed to detect antibodies against 4 key viral proteins: hemagglutinin (HA/H5), neuraminidase (NA/N1), nucleoprotein (NP), and matrix protein (M1). This assay supports DIVA applications for both inactivated and subunit HPAI vaccines. We evaluated the assay using 72 bovine and 43 avian serum samples, all previously characterized by virus neutralization (VN) assays. The bovine cohort included 30 samples from animals vaccinated with a subunit HA-based vaccine and 42 from naturally infected dairy cows. The avian samples were collected from wild birds, with 26 VN-positive and 17 VN-negative specimens. The assay used microspheres coupled with anti-equine IL-4 antibodies, conjugated to recombinant IL-4 fusion proteins representing each viral antigen. Serum samples were incubated with antigen-coated beads, and antigen-specific IgG responses were quantified via median fluorescence intensity (MFI) on the Luminex platform. Vaccinated bovine samples showed antibody responses exclusively to HA-5, whereas naturally infected animals exhibited strong binding to HA1, NA, and NP, with sustained antibody levels post-outbreak. The ROC curve analysis demonstrated high diagnostic accuracy for bovine samples (AUC: HA1 = 0.9909, NP = 0.9964, NA = 0.9347). In avian samples, VN-positive sera showed robust antibody responses to all 4 antigens, with HA1 and NP yielding the highest MFI values. The ROC analysis confirmed excellent diagnostic performance (AUC: HA1 = 1.000, NP = 0.9708, M1 = 0.9152, NA = 0.8187). Overall, the Luminex-based multiplex assay demonstrated good diagnostic performance distinguishing vaccinated animals (HA1-only response) from infected ones (multi-antigen response), validating its utility as a DIVA tool. This platform offers a scalable, efficient solution for serological surveillance and vaccine monitoring in both cattle and poultry, supporting informed decision-making in HPAI control programs.

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Key Words: differentiating infected from vaccinated animals (DIVA), highly pathogenic avian influenza (HPAI), H5N1, influenza A, vaccine, serology

189 - Semi-targeted strand-switching coupled MinION sequencing (ST-PCR-Seq) for avian reovirus.

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Avian orthoreoviruses (ARV) are economically important pathogens of poultry. Avian orthoreoviruses' genetic variability, segmented genomes, and the presence of mixed strains in one sample are factors that complicate control measures. Current genetic typing is valuable for characterization; however, it is labor intensive and time consuming. Additionally, current methods focus on a single gene, failing to address reassortment and the role of the other segments in pathogenicity. Avian orthoreovirus genome segments share a highly conserved region at the 5′ end, with a different conserved sequence at the 3′ end. The project objective is to use a semi-targeted strand-switching approach to target both conserved termini on all segments and thus provide moderate enrichment of ARV sequences as compared with random sequencing. The library preparation was coupled with MinION sequencing. This method reduces specificity at the virus enrichment step (as compared with PCR), which better accounts for genetic variation, will detect multiple segments, and can produce longer sequences. We extracted RNA from 9 cultured avian reovirus samples using Trizol. Reverse transcription and strand switching were performed as previously described, with a single primer specific for either the 5′ or 3′ end. For the PCR step, the reverse primer targets a portion of the 3′ RT primer, whereas the 5′ primer targeted the strand-switching adapter. Random hexamers served as reverse primers controls. Base-calling, trimming, and demultiplexing were performed using in-house scripts using Dorado GPU v1.1.1 (ONT) and Porechop v.0.2.4. Reads were analyzed using Geneious prime v.2023.2.1. and mapped to a custom ARV's sequence reference list. This novel method detected (10 > 20×) and characterized (≥20×) most viral genome in the samples in a time frame of 3 to 4 d. For each sample, ≥6 of the 10 genomic segments were characterized. The rest were identified but suffered from low coverage (<20×). The complete S1 genomic segment was characterized in all samples. This method outperformed random-hexamer strand-switching method, which had <20× coverage. The ST-PCR-Seq was effective for rapid amplification of ARV genomic material. Protocol refinements might improve genome amplification. Nevertheless, this approach shows potential to improve genetic characterization of avian reoviruses 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 National Institute of Food and Agriculture and by USPEA project #730. Graduate student support: Boehringer Ingelheim Veterinary research training program

Key Words: orthoreovirus, semi-targeted, single primer, MinION

190 - Assessing repeatability of ultrasound-based prognostic models for bovine respiratory disease in feedyard cattle.

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Bovine respiratory disease (BRD) is the leading cause of morbidity and mortality in feedyards. Improving prognostic accuracy is critical for guiding antimicrobial use and welfare-focused management. Targeted thoracic point-of-care ultrasound (TT-POCUS) offers real-time visualization of pulmonary abnormalities and may improve prediction of treatment outcomes. Evaluate the repeatability of previously developed TT-POCUS prognostic models for BRD outcomes in feedyard cattle. A cross-sectional study was conducted at a high-plains feedyard during November and December 2024 and May and June 2025. Cattle presented for first (n = 603) or third (n = 66) BRD treatments (total n = 669). Targeted thoracic point-of-care ultrasound of the right caudodorsal lung recorded ultrasound lung score (ULS), A-line counts, B-line counts, and pleural abnormalities. Outcomes were assessed at 60 d: recovered versus did not finish (DNF) and first treatment success versus failure (FTF). Logistic regression models from prior work were applied with adjustments required to resolve multicollinearity and singularity. Among first-treatment cattle, 546 recovered and 57 DNF were identified, whereas 454 were classified as success and 149 as failure. Adjusted models demonstrated that thoracic ultrasound findings, particularly higher ULS, were predictive of poor outcomes. Predictive performance at first treatment was strong, with sensitivity and specificity of 86% each for DNF and 64% and 82% for FTF, respectively. In contrast, among chronically treated cattle at third treatment, 27 recovered and 39 DNF were identified, with predictive performance lower (sensitivity 59%, specificity 78%). TT-POCUS provides a rapid, noninvasive tool for prognostic assessment of BRD in feedyard cattle. Adjusted models demonstrated moderate repeatability, with strongest performance in first-treatment DNF prediction. Findings support TT-POCUS integration into feedyard decision-making and highlight the need for continued refinement of prognostic models.

Financial Support: Foundation for Food and Agriculture Research, Late Day Pulmonary Disease grant.

Key Words: bovine pneumonia, point-of-care, precision veterinary medicine, predictive model, treatment outcome, feedlot

General Abstracts: Session 38—Disease Control and Economics

191 - Evaluation of temperature, mixing frequency, and time on acidified milk quality parameters, physical characteristics, and coliform counts.

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Milk acidification uses low pH to allow storage without the need for refrigeration. Although this practice has been used for feeding calves in group-housed systems, core evidence-based data are missing. Our objective was to evaluate the effect of different laboratory-simulated storage and acidified milk preparation methodologies, including the effect of environmental temperature, mixing frequency, and time after acidification, on milk quality parameters, physical changes, and coliform counts. For this purpose, 3 mixing frequencies (mixing continuously, mixing every 2 h, and mixing every 6 h) and 2 environmental temperatures (23°C and 40.5°C) were evaluated. Citric acid was used to acidify milk to a pH between 4.0 and 4.2. *Escherichia coli* was used to inoculate milk samples, and through serial dilution, we evaluated the log cfu/mL over time. Samples were collected at 0 h (baseline), and 1, 2, 4, 6, 12, 24, 48, 72 (3d), and 96 h (4d) after initial acidification. This study is ongoing, with a completion date expected in mid-autumn 2025. Based on our preliminary data from 3 trials conducted for each milk mixing regimen at 2 temperatures, we have observed that for all mixing frequency regimens, a lower log cfu/mL of *E. coli* is observed in milk for either 23°C or 40.5°C when compared with the control samples starting at 6 h. The milk pH was observed to have minimal variation for the entire study in the continuous mixing trials when compared with mixing every 2 and 6 h, especially for samples collected at 48, 72, and 96 h. Nutritional study evaluation analysis have been collected and are currently being processed. Our findings will generate core knowledge to support recommendations of practices for the acidification of milk, decreasing milk bacterial load, and increasing milk nutritional value. Together, this can reduce the risk of disease originating from milk and the need for antibiotic use, supporting the viability of a cost-effective and sustainable group housing feeding practice.

Financial Support: This project is funded by the California Dairy Research Foundation.

Key Words: milk acidification, calf group housing, disease prevention, diarrhea, antimicrobial stewardship

192 - Benefit-cost analysis of enhanced vaccination program to control brucellosis in cattle in different farming systems in Rwanda.

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Economic assessments can help policymakers decide if allocation of resources is profitable for controlling brucellosis in cattle populations. In Rwanda, the reported prevalence of brucellosis in cattle varies from 1% (zero-grazing and semi-intensive farming systems) to 22% (agro-pastoral farming systems). Interventions to control and prevent brucellosis in cattle in Rwanda are paramount and would require economic assessments and budget allocation. The objectives of this study were to estimate the overall benefit-cost ratio (BCR) from adopting an enhanced vaccination program for brucellosis control in cattle in all 3 farm systems, where all female calves (100%) are vaccinated against *Brucella* spp. infection until disease prevalence was reduced to 1%, as well as estimate the number of years when an enhanced vaccination program becomes profitable (BCR >1). A compartmental stochastic model was divided into 4 compartments: susceptible cattle, infected cattle, protected cattle, and replacements. The change of cattle population from susceptible to infected or from susceptible to protected animals, the number of newly infected animals every year, and the annual prevalence of brucellosis were calculated using Python. The overall BCRs for enhanced vaccination program were 3.2, 2.38, and 2.32 in agropastoral, zero-grazing, and semi-intensive farming systems, respectively. In addition, annual calculated BCRs were >1 after 6 years in agropastoral and 9 years in zero-grazing and semi-intensive farming system. The findings of this study can help animal health policy makers make informed decision on whether or not to adopt an enhanced vaccination program to control brucellosis in cattle in Rwanda.

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Key Words: brucellosis, prevalence, vaccination, benefit-cost ratio, cattle

193 - The relationship between milk compositions around the first service and conception rate in dairy cows.

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In the dairy industry, conception rate (CR) is a critical factor influencing the production cycle and days open, directly affecting production efficiency. Although milk composition serves as an important indicator of individual cow nutritional status for management purposes, the detailed relationship between specific milk composition traits and CR has not been fully elucidated. Therefore, this study aimed to investigate the relationship between milk composition and CR in dairy cows. For this purpose, the present study was conducted on 8 dairy farms in Hokkaido prefecture, Japan. Monthly milk composition data were collected from all lactating cows. We analyzed milk composition and service records from 1,351 cows that calved between 2021 and 2022. For analysis, milk composition data from the test date closest to the service date were used. The milk composition parameters included: de novo fatty acids (FA) per 100 g of raw milk (DnM, %), proportion of de novo FA to total FA (DnF, %), and proportion of preformed FA to total FA (PrF, %). A logistic regression model was used for statistical analysis, with whether a cow was conceived at first service (1 or 0), represented as CR, as the dependent variable. The independent variables were each milk composition trait, parity and service season. Farm was included as random effect. The result showed the CR in this study was 42.1%. Statistical analysis revealed that CR was significantly associated with DnM, DnF, and PrF in both primiparous and multiparous cows ($P < 0.05$). In DnM, cows with DnM <0.9% had a lower CR than those with DnM ≥0.9% (odds ratio [OR] and 95% CI: 0.61 [0.41–0.90] for primiparous cows; 0.73 [0.57–0.93] for multiparous cows). In DnF, cows with DnF <21% had a lower CR than those with DnF ≥21% (OR [95% CI]: 0.46 [0.26–0.81] for primiparous cows; 0.63 [0.42–0.96] for multiparous cows). In PnF, cows with PrF >50% had a lower CR than those with PrF ≤50% (OR [95% CI]: 0.55 [0.34–0.87] for primiparous cows; 0.73 [0.56–0.96] for multiparous cows). In conclusion, this study indicates that postpartum milk composition significantly affects subsequent CR. These findings suggest that specific milk composition traits, particularly DnM, DnF, and PrF could serve as valuable indicators for dairy herd reproductive management.

Key Words: fatty acid, conception rate, milk composition

194 - Factors associated with veterinarian longevity in rural practice.

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The objective of this study was to determine factors associated with veterinarian longevity in rural practice, longevity defined as employed in a rural practice for 5 years or more. An anonymous, electronic survey was distributed to alumni of Kansas State University and Iowa State University's Colleges of Veterinary Medicine, and to members of the American Association of Bovine Practitioners, the Academy of Veterinary Consultants, and state veterinary medical associations in Kansas, Nebraska, and Iowa. Of 841 responding veterinarians, 338 were included in the analysis based on consent, employment in private practice in the United States, self-reporting as rural, completion of any part of the survey after the inclusion questions, and being out of veterinary school for more than 5 years. A multivariable logistic regression model was fitted via manual forward and backward selection of independent variables with longevity as the dependent variable. An α of 0.05 was used as a cutoff for significance and inclusion in the final model. Factors positively associated with longevity included ownership in a practice, spending the majority of one's career in rural practice, having a brick-and-mortar practice, and being involved in livestock organizations. There was a negative association between longevity and loan forgiveness. The model showed that ownership, business structure, and organizational involvement are important factors in retaining veterinarians in rural communities. Programs intended to increase and retain veterinary services in this sector should consider these factors.

Financial Support: Hodes Family Business Management Fund

Key Words: survey, retention, professional organization

195 - Assessment of the economic impact of porcine reproductive and respiratory syndrome (PRRS) on Japanese swine commercial farms.

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Porcine reproductive and respiratory syndrome (PRRS) is one of the most economically significant endemic diseases in the global swine industry. It causes reproductive failure in sows and respiratory disease in pre- and postweaning pigs, leading to decreased productivity and substantial financial losses. In Japan, updated economic assessments of PRRS impacts are needed. Therefore, this study aimed to quantify the economic impact of PRRS infection on Japanese swine commercial farms using a stochastic simulation model. For this propose, this study collected data from 105 farrow-to-finish commercial farms in Japan. Farms were classified according to their PRRS status based on diagnostic test results, with 34 farms identified as PRRS-negative and 71 farms as PRRS-positive. In this study, the economic impact is expressed in annual margin over feed cost per sow (MOFC), which was estimated by subtracting annual feed cost of marketed pigs per sow from annual revenue of marketed pigs per sow. A Monte Carlo stochastic simulation modeling with the @RISK software was used to estimate MOFC under 2 scenarios: PRRS-negative and PRRS-positive farms. For each PRRS status scenario, probability distributions were fitted to the observed data using @RISK's distribution fitting functions. Each scenario was simulated using 10,000 Monte Carlo iterations to generate the distribution of MOFC. The economic unit used in this analysis was the Japanese Yen (JPY). In July 2025, US$1 = 150 JPY and €1 = 170 JPY. The results showed mean ± SD and associated 90% CI (5% to 95%) of MOFC under the PRRS-negative scenario was 628,017 ± 121,363 JPY and 459,580 to 846,657 JPY, whereas those under the PRRS-positive scenario were 541,793 ± 109,279 JPY and 376,586 to 731,173 JPY. In conclusion, results from the model indicate that PRRS infection drastically affects the profitability of Japanese commercial pig farms. The model serves as a valuable decision-support tool for farmers and veterinarians when a farm is proven to be affected by PRRS. Its output can help stakeholders understand the necessity of interventions in cases where the disease significantly affects their enterprise's profitability.

Key Words: swine, porcine reproductive and respiratory syndrome (PRRS), stochastic model, economic

196 - Impact of porcine reproductive and respiratory syndrome (PRRS) on greenhouse gas emissions on commercial swine farms.

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Reducing greenhouse gas (GHG) emissions is a critical social goal in achieving a sustainable society. Pig production contributes to GHG emissions through various processes, primarily from manure management, animal housing, and feed production and transportation. GHG emissions of pig production are notably influenced by farm productivity. Porcine reproductive and respiratory syndrome (PRRS) is a significant disease affecting farm productivity, leading to increased mortality and decreased farm productivity within the global swine industry. Therefore, this study aimed to investigate the influence of PRRS status on GHG emissions on commercial swine farms. This study was conducted on 117 commercial farrow-to-finish farms in Japan. Greenhouse gas emissions were computationally modeled by 4 processes: manure management, animal housing, and feed production and transportation. To assess the GHG emissions in each process, annual productivity data in 2023 were collected from the 117 farms. Data used for calculation of GHG emissions were age at slaughter, the number of market pigs, the number of breeding pigs, the feed amount purchased per breeding pig, carcass weight per pig, and feed conversion ratio. The functional units in the breeding section and finishing section were one weaned piglet and 1 kg of carcass weight of fattening pig, respectively. In addition, PRRS status was examined by PCR using blood samples and classified into PRRS-negative (n = 39) and PRRS-positive farms (n = 78). In addition, a *t*-test was used to statistically compare GHG emissions between the PRRS-positive and PRRS-negative groups. For the breeding section, PRRS-positive farms tended to have higher GHG emissions than PRRS-negative farms (83.2 ± 8.2 g vs. 80.1 ± 7.6 CO_2e/weaned piglet; $P = 0.05$). For finishing section, PRRS-positive farms had higher GHG emissions than PRRS-negative farms (4,584 ± 467 g vs. 4,199 ± 361 CO_2e/kg carcass weight; $P < 0.05$). Additionally, GHG emissions for breeding sections were positively associated with those for finishing sections in both PRRS-negative and PRRS-positive farms ($P < 0.05$). This study investigated the impact of PRRS on GHG emissions across 117 swine farms in Japan. We confirmed that GHG emissions were higher in PRRS-positive farms compared with PRRS-negative farms for both breeding and finishing sections, indicating that the spread of PRRS significantly affects GHG emissions.

Key Words: swine, fattening pig, greenhouse gas, porcine reproductive and respiratory syndrome (PRRS)

General Abstracts: Session 39—Epidemiology 4

197 - Characterization of swine interstate transportation networks using multi-source records.

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Certificates of veterinary inspection (CVI) are required for most state-to-state animal transportations. Alternatively, livestock production sites under the same ownership may establish commuter herd agreement (CHA) with states to facilitate frequent transportations. This study aims to characterize the swine transportation networks associated with Ohio using data extracted from CVIs and CHAs. We extracted county-to-county swine transportation data using CVIs and CHAs submitted to the Ohio Department of Agriculture between 2018 and 2023. Origin county, destination county, date of transportation, and number of animals were collected for the construction of directed county-level networks using igraph package in Python. County-level measures were reported as (1) out-/in- degree as the number of unique counties that received/sent pigs from/to the given county, and (2) out-/in-degree weighted by the number of transported pigs. Networks were characterized by (1) giant strongly connected component (GSCC) as the largest set of counties with mutual directed reachability, (2) assortativity as the correlation of a county's total degree with that of its partners, (3) transitivity as the ratio of triangles to connected triples, and (4) reciprocity as the proportion of county pairs with bidirectional shipments. Across 2018–2023, we identified 29,834 transportations with 15,598,156 pigs moved in total (522.8 pigs per transportation). The CHA records (2021–2023) represented 1.8% of transportations and 4.3% of transported animals in the dataset. In Ohio, Fulton and Licking counties were the most frequent shippers, whereas Clark and Auglaize counties shipped out the most number of pigs. Madison county imported pigs most frequently, and Mercer and Auglaize counties imported the most number of pigs. The GSCC contained 20% to 23% of counties each year, indicating a highly connected subnetwork. Degree assortativity was negative, suggesting that counties with a high degree tend to connect to those with a low degree. A low reciprocity and transitivity indicated predominantly one-way movement with comparatively few counties that both send to and receive from one another. Swine transportation patterns by counties were consistent. A highly connected subnetwork was identified, which may facilitate disease transmission within. Finally, counties with high and low degrees behaved like hubs and spokes rather than tight local clusters. This information could help resource allocation during disease outbreaks.

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Key Words: network analysis, swine transportation

198 - First characterization of feeding patterns and habitat preferences of mosquito vectors on commercial swine farms in the United States.

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This study characterizes the feeding patterns and habitat preferences of mosquito species on US commercial swine farms. Between June and October of 2024, biweekly mosquito sampling was conducted on 10 farms (5 sow farms and 5 wean-to-market [WTM] farms) in southeast Iowa. Biogents sentinel light traps and aspirators were used outdoors (near sewage lagoons, ponds, and surrounding vegetation) to capture host-seeking and resting mosquitoes, respectively, whereas only traps were used indoors (barns). Captured mosquitoes were identified, sexed, and counted. Blood meals were Sanger sequenced to confirm both mosquito species and vertebrate host identity. In total, 21,762 female mosquitoes were captured, of which 2.1% (444/21,762) were blood-fed. Of these, 25.7% (114/444) were captured on sow farms and 74.3% (330/444) on WTM farms. Although no significant differences were observed on sow farms, blood-fed mosquitoes were significantly more abundant indoors than outdoors on WTM farms ($P < 0.01$). Across production systems, the predominant blood-fed mosquito species were *Anopheles quadrimaculatus* (n = 157), *Culex pipiens* (n = 105), *Aedes vexans* (n = 65), *Culex salinarius* (n = 46), and *Anopheles punctipennis* (n = 45). Pigs accounted for the majority of blood meals (n = 384), followed by white-tailed deer (n = 38), cattle (n = 11), and birds (n = 11). The majority of the domestic pig blood meals came from mosquitoes trapped indoors; however, 15.6% (60/384) were from mosquitoes captured outdoors. Conversely, 25.0% (15/60) of non-pig blood meals were extracted from mosquitoes trapped indoors. The probability of a mosquito species feeding on pigs compared with other hosts was significantly higher in late June through to mid-September ($P < 0.01$). Vegetation habitats consistently harbored more resting mosquitoes than water bodies ($P \leq 0.02$), on both sow and WTM farms. The detection of pig blood meals in mosquito species known to transmit arboviruses, captured both indoors and outdoors, is a critical biosecurity vulnerability. Therefore, routine mosquito surveillance and targeted control measures, both indoors and in surrounding habitats, should be incorporated into herd biosecurity programs to reduce mosquito-related health risks in commercial swine operations.

Financial Support: This work was funded by the United States Department of Agriculture – Agricultural Research Services (USDA ARS) Non-Assistance Cooperative Agreement No. 58-3020-9-022, the Center for Outcomes Research and Epidemiology, and the College of Veterinary Medicine, Kansas State University.

Key Words: mosquito, host-feeding pattern, habitat preference, swine farm, arbovirus

199 - Dynamics of PEDV infection across wean-to-finish herds in the United States Midwest.

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Porcine epidemic diarrhea virus (PEDV) is a highly contagious pathogen that causes severe gastrointestinal disease in pigs. Limited information is available on the epidemiology of PEDV in growing pigs. Understanding PEDV status in these populations is essential to guide control and eradication strategies. This study aimed to assess PEDV status and infection patterns in wean-to-finish (WTF) herds across the US Midwest. Sixty-three WTF herds from 10 production companies located in the Midwestern US were enrolled in a prospective cohort study (September 2017–December 2018). Herds were conveniently selected based on logistics for sample submission and producer willingness to participate. At each site, 8 oral fluid samples were collected every 4 wk, at ~3, 8, 12, 16, 20, and 25 wk post-placement using cotton ropes. We detected PEDV RNA by RT-qPCR, with Ct = 36 considered positive. Up to 3 Ct <30 positives per sampling event were sequenced for S1 spike and genetically characterized. A total of 2,585 oral fluid samples were tested, with 139 positives (5.4%) from 8 herds (13%) across 3 companies (A = 4, B = 3, C = 1). Positives occurred mainly at 3 wk (33.8%), 8 wk (30.2%), and 12 wk (25.9%), declining at 16 (5.0%), 20 (1.4%), and 25 wk (3.6%). Most herds were positive from 3 to 20 wk; one herd tested positive at 25 wk. Six herds received piglets from one sow farm and 2 from 2 farms. Six herds were classified as PEDV-negative (status 4), one as positive stable (2fvi), and one as unknown according to data from the Morrison Swine Health Monitoring Project (MSHMP). Thirty out of 33 samples were successfully sequenced, classified as US PEDV prototype strains, and shared between 98.8% and 100% nucleotide identity. Sequences clustered by herd and sampling event were distinct among herds. This study provides valuable insights into the infection dynamics of PEDV in WTF in the Midwestern US. Most detections occurred early in the growing phase, suggesting that piglets may be weaned PEDV-positive, acquire infection during transport, or become infected shortly after entering the WTF site, with PEDV detection persisting for at least 8 wk. These findings highlight the need for early monitoring and strategies to ensure the placement of PEDV-negative piglets into WTF sites, thereby reducing transmission and supporting long-term control and eradication efforts in growing pigs.

Financial Support: the University of Minnesota Swine Disease Eradication Center (SDEC)

Key Words: porcine epidemic diarrhea virus (PEDV), epidemiology, phylogeny, oral fluid, RT-qPCR

200 - Beyond the flock: Tracking H5N1 in wild mammals.

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The goose/Guandong (Gs/GD) lineage highly pathogenic avian influenza (HPAI) virus [A/goose/Guangdong/1/1996(H5N1)] is the progenitor of the currently circulating Eurasian-(EA) lineage HPAI H5N1 2.3.4.4b (EA HP H5) and has been the most consequential HPAI lineage globally. Despite increased reports of infections, the extent of exposure and role of wild mammals in the ecology and transmission dynamics of the virus remains poorly understood. We surveyed wild mammals in Ohio to investigate potential spillover of EA HP H5. Although no active infections were detected by swab-based molecular tests, antibodies to avian influenza virus (AIV) nucleoprotein (NP; 54.9%, n = 61), EA HP H5 (43.2%, n = 48), North American (NAm.) low pathogenic avian influenza (LPAI) H5 (22.5%, n = 25), and AIV neuraminidase (N1) (44.1%, n = 49) were detected in raccoons and opossum. All seropositive animals were sampled at Ohio marshes with previously confirmed EA HP H5 detections in waterfowl. These findings suggest prior exposure and recovery of wild mammals to these viruses. Wild mammals may play an intermediary role in the mammalian adaptation of avian influenza A viruses. Therefore, ongoing surveillance of wild mammals is crucial for assessing the risk to public health.

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Key Words: avian influenza, wildlife, surveillance, influenza epidemiology, Eurasian highly pathogenic avian influenza (EA HPAI) H5N1 2.3.4.4b

201 - Targeted metagenomic characterization of the resistome in free-ranging white-tailed deer in New York State.

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Antimicrobial resistance (AMR) is an increasing threat to human and animal health, with wildlife playing an important role. White-tailed deer (*Odocoileus virginianus*) are highly abundant and frequently interact with both natural habitats and human-modified landscapes, increasing opportunities for exposure and transmission. Studies of antimicrobial resistance genes (ARGs) in deer remain scarce, and no prior work has described the resistome of free-ranging deer in the northeastern United States. This study provides a descriptive characterization of the resistome of free-ranging white-tailed deer (WTD) in New York using targeted hybrid-capture metagenomics. Total genomic DNA from 93 deer nasal swabs was assessed for ARG composition using the Qiagen xHYB AMR hybrid-capture panel, followed by sequencing on an Illumina NextSeq 2000. Reads were analyzed with NCBI AMRFinderPlus, and subtyping verified in the Comprehensive Antibiotic Resistance Database. Stress response genes, efflux-only genes, and partial sequences were filtered out before analysis and are not included in the results. Analyses emphasized genes linked to anthropogenic exposure and clinically significant resistance. A total of 637 ARGs were detected in these samples, representing 147 unique genes in 16 antibiotic classes. The ARG richness ranged from 0 to 40 per sample, with a mean of 7.2 and a median of 3.5. Genes conferring resistance to aminoglycosides were most common (191 detections), followed by tetracyclines (109 detections), β-lactams (62 detections), and sulfonamides (24 detections). Anthropogenic markers included *tet(W;* 20 detections), *tet(Q)* (19 detections), *sul2* (16 detections), and *sul1* (8 detections). Genes of higher clinical concern were rare but included *tet(X2)* (9 detections), *blaDHA* (3 detections), plasmid-mediated quinolone resistance determinants (*qnr*, *aac[6']-Ib-cr*), and a single detection of *mcr-3*. The most frequent β-lactam family gene was *blaOXA* (42 detections), spanning environmental and clinically relevant subfamilies. The WTD respiratory resistome comprised 147 unique genes. Tetracycline and sulfonamide markers were detected frequently. Clinically important determinants such as *mcr-3*, *tet(X2)*, and plasmid-mediated *AmpC* were rare, whereas *blaOXA* genes were common and distributed across diverse subfamilies. These findings establish a baseline and underscore the importance of WTD in the ecology of AMR.

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Key Words: antimicrobial resistance, wildlife, white-tailed deer, resistome, targeted metagenomics, surveillance

202 - Diverse avian influenza in waterfowl: Eurasian highly pathogenic and North American low pathogen avian influenza in the Mississippi Flyway.

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The Eurasian (EA) lineage highly pathogenic avian influenza (HPAI) H5N1 clade 2.3.4.4b was detected in North America in late 2021, marking its first transcontinental spread from Europe via migratory birds. Reassortment between EA HPAI H5N1 clade 2.3.4.4b (EA HP H5) and North American (NAm) low pathogenic avian influenza (LPAI) viruses endemic in wild birds has generated novel genotypes (e.g., B3.13, D1.1). Several of these reassortants exhibited enhanced potential to cross species barriers, spreading from wild birds into poultry, wildlife, and livestock, with occasional human infections linked to poultry and livestock exposure. This highlights the role of wild birds in spread, evolution, and spillover of influenza viruses with potential public health implications and underscores the need for sustained targeted surveillance in wild bird populations. To address this need, active influenza A virus (IAV) surveillance was conducted in the Mississippi Flyway in Ohio and Wisconsin. From Autumn 2023 to Autumn 2025, a total of 4,331 cloacal swab samples were collected from 126 wild bird species and tested for IAV via rRT-PCR. Samples included waterfowl (n = 2,584), passerines (n = 1,649), raptors (n = 44), seabirds (n = 19), shorebirds (n = 7), and others (n = 28). Overall, 14.5% of samples were IAV-positive (n = 583 waterfowl; n = 1 passerine), with 1.19% (n = 48) representing diverse EA HP H5 genotypes (A2, B3.6, D1.1, and 2 unassigned). Preliminary findings confirm co-circulation of LPAI viruses, exhibiting subtype diversity from H1 to H11 and N1 to N9, alongside EA HP H5 viruses in wild birds. This co-circulation creates opportunities for viral reassortment and the emergence of novel strains, potentially elevating risks to both animal and human health. Ongoing phylogenetic analyses aims to elucidate the dynamics of viral spread, spillover, and reassortment. Sustained surveillance in wild bird reservoirs remains critical for understanding viral evolution, spread, and spillover risk.

Financial Support: This work was funded, in part, with federal funds from the Centers of Excellence for Influenza Research and Response (CEIRR), National Institute of Allergy and Infectious Diseases, National Institutes of Health, Department of Health and Human Services, under Contract no. HHSN272201400006C.

Key Words: Eurasian highly pathogenic avian influenza (HPAI) H5N1 clade 2.3.4.4b, North American low pathogenic avian influenza (LPAI), waterfowl, evolution, spread, public health

General Abstracts: Session 40—Bacteriology 2

203 - Quorum sensing–mediated regulation of RaS-RiPP biosynthesis across diverse *Streptococcus* species.

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Streptococci are abundant members of the human and animal microbiome that colonize diverse host niches and act as commensals or opportunistic pathogens. Species such as *Streptococcus suis*—a major pathogen of pigs—and *S. agalactiae*—a cause of bovine mastitis—underscore their relevance to animal health. Their adaptability and interactions with hosts and other microbes are orchestrated in part by quorum sensing (QS) systems that regulate gene expression through signaling peptides. Among these, the Rgg–SHP QS pathway has recently been linked to the regulation of radical S-adenosylmethionine–associated ribosomally synthesized and post-translationally modified peptides (RaS-RiPPs)—a newly emerging family of natural products with roles in interbacterial communication, competition, and virulence. Despite their prevalence, the regulatory mechanisms and environmental triggers controlling RaS-RiPP biosynthesis remain poorly defined. To dissect these mechanisms, we engineered *S. suis* and *S. oralis* strains with targeted mutations in key QS regulators (*rggT* and *rggG*) and promoter-reporter fusions in luciferase vectors. Luciferase assays (3 biological replicates per strain, each with 3 technical replicates) were performed in rich and chemically defined media, with and without synthetic SHP peptides (e.g., *S. suis* TQQ SHP V1 C9, HYYRFN-FTY), to monitor transcriptional responses of RaS-RiPP–associated promoters (*PtggA*, *PgrcA*, *PshpG*). Data were analyzed using one-way ANOVA followed by Tukey's multiple comparisons test. These assays revealed distinct activation profiles between species and growth conditions, indicating that RaS-RiPP expression is tightly modulated by specific peptide signals and environmental cues. Complementary work using Δ*rggT* and Δ*rggG* mutants supports a direct role for Rgg–SHP-mediated regulation of RaS-RiPP biosynthesis. Current efforts are expanding this framework to additional streptococcal species, including *S. thermophilus* and *S. agalactiae*, to explore evolutionary conservation and functional diversity of RaS-RiPP regulation across host-associated lineages. Collectively, these findings define a complex regulatory network integrating quorum and environmental sensing to modulate natural product biosynthesis in *Streptococcus*. This work establishes a foundation for understanding how RaS-RiPP influence colonization, virulence, and microbial ecology and informs their potential development as modulatory therapeutics against streptococcal infections.

Financial Support: Roy J Carver Charitable Trust/ Grant# 25-6022

Key Words: RaS-RiPP, quorum sensing, Rgg–SHP signaling, *Streptococcus*, virulence

204 - Characterization of the cell envelope of *Coxiella burnetii* propagated in ovine trophectoderm cells.

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Coxiella burnetii is a gram-negative intracellular bacterial pathogen of significant importance to both animal and human health. Small ruminants such as sheep, goats, and cattle serve as the main reservoir hosts for *C. burnetii*. The bacterium replicates within the placental trophoblast cells of these animals, leading to abortion. *Coxiella burnetii* can persist in both the host and the environment for prolonged periods due to its unique ability to remodel its cell envelope. However, no studies have yet been conducted to characterize the *C. burnetii* cell envelope using biologically relevant host cells. Here, using high-resolution glycoproteome analysis, we compared the cell envelope architecture of *C. burnetii* grown in ovine trophectoderm (oTr1) cells and in axenic ACCM-D broth outside of host cells. *Coxiella burnetii* forms large *Coxiella*-containing vacuoles of heterogeneous sizes inside oTr1 cells. Analyses via HPLC, LC-MS/MS, and gene expression showed that *C. burnetii* from oTr1 cells exhibit significantly elevated LD-transpeptidase activity, with increased 3–3 cross-linking and tethering of β-barrel outer membrane proteins (BbpA, BbpB, BbpC) to the peptidoglycan layer, compared with *C. burnetii* grown in ACCM-D broth. Interestingly, tethering of the unique lipoprotein LimB to the peptidoglycan layer was absent in *C. burnetii* grown in oTr1 cells. This study provides the first molecular-level insights into *C. burnetii* cell envelope remodeling in biologically relevant host cells, which could aid in the development of treatments for chronic human infections and in designing rational methods to decontaminate environmental reservoirs during outbreaks on animal farms.

Financial Support: This work is supported by funds from the Department of Population Medicine and Diagnostic Sciences at the College of Veterinary Medicine, Cornell University, and the Cornell Institute of Biotechnology seed grant program.

Key Words: *Coxiella burnetii*, cell envelope, peptidoglycan, trophoblast cell, zoonotic disease, mass spectrometry

205 - Characterization of unannotated transcripts in *Leptospira* reveals mechanisms of host adaptation and environmental persistence.

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Pathogenic *Leptospira* species cause leptospirosis, a globally important zoonotic disease. Despite its impact on animal and human health, the contribution of unannotated regions of the transcriptome to virulence, environmental persistence, and host adaptation remains poorly understood. We aimed to identify and functionally characterize novel transcripts in *Leptospira* species to elucidate molecular pathways relevant to survival in diverse ecological niches. Using Oxford Nanopore RNA sequencing, we analyzed transcriptomes from nonpathogenic *L. biflexa* Patoc (LBP) and pathogenic *L. interrogans* serovars Copenhageni (LIC) and Icterohemorrhagiae (LII). Over 1,700 unannotated transcripts were detected, and statistical analyses (Shapiro–Wilk, Kolmogorov–Smirnov, Z-score filtering) were used to define transcript size distributions and remove potential DNA contamination. Open reading frames were predicted, and functional annotation was conducted using RefSeq, Rapid Annotations using Subsystems Technology (RAST), Gene Ontology (GO), and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways. We identified 701 unannotated transcripts containing open reading frames, representing 39.4% of the dataset. Functional analysis revealed distinct transcriptional strategies among *Leptospira* species. We found that LBP exhibited broad functional potential with low-to-moderate transcription, consistent with environmental adaptability; LIC demonstrated high transcriptional and translational activity focused on stress adaptation and protein synthesis, aligning with host-associated survival; and LII showed low overall transcriptional activity, suggesting niche-limited persistence. Enrichment analyses highlighted pathways related to ribosomal function, motility, stress response, toxin production, and antibiotic resistance. This study highlights the complexity of the *Leptospira* transcriptome, revealing previously unrecognized RNA molecules with potential roles in adaptation and disease. These discoveries enhance our understanding of *Leptospira* biology and underscore the importance of exploring uncharted genomic regions in microbial pathogens to address global health challenges.

Key Words: *Leptospira*, transcriptome, annotation, host adaptation, environmental persistence

206 - *Mycobacterium bovis* shows increased virulence compared to *Mycobacterium tuberculosis* in a susceptible mouse model.

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Mycobacterium tuberculosis (*M. tb*), the primary cause of human tuberculosis (TB), and *Mycobacterium bovis* (*M. bovis*), the main agent of animal TB, share 99.9% genetic similarity yet generally cause different disease manifestations—pulmonary versus extrapulmonary TB. The biological basis for their differing pathogenesis and virulence remains unclear. We used a mouse strain predisposed to extrapulmonary TB and infected animals with either *M. tb* or *M. bovis* via oral gavage (OG) or intratracheal (INT) routes. Mice were monitored over 30 d, with samples collected on d 15 and 30 to assess bacterial burden, cytokine profiles, and weight changes. *Mycobacterium bovis* caused more severe disease and disseminated faster than *M. tb*, especially following INT infection. Mice infected INT with *M. bovis* exhibited rapid, progressive weight loss by d 15, with mortality by d 21 (n = 3), alongside extensive lung inflammation. In contrast, mice infected via OG with *M. bovis* showed no weight loss, and all mice infected with *M. tb* (via either route) maintained or gained weight. Cytokine analysis revealed a strong IFN-γ response in the INT *M. bovis* group, accompanied by increased IL-10 from regulatory T cells, suggesting immune dysregulation. Oral gavage *M. bovis* infection triggered IL-1β production, suggesting inflammasome activation in response to bacterial translocation through the gut. This immune response correlated with lower bacterial loads and better disease control. *Mycobacterium tuberculosis* infections induced more balanced immune responses with features of immune suppression regardless of the route. *Mycobacterium bovis* elicits severe lung inflammation and weight loss via INT infection, whereas oral exposure results in a more controlled infection. *Mycobacterium tuberculosis* appears to evade immune responses more effectively, promoting chronic persistence. These findings highlight important differences in host–pathogen interactions that influence disease outcomes, despite the close genetic relatedness between the 2 pathogens.

Key Words: zoonotic tuberculosis, animal model, virulence, intratracheal, oral gavage

207 - Optimization of an *in vitro Leptospira* growth inhibition assay to evaluate the protective efficacy of anti-*Leptospira* antibodies.

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Evaluation of the protective antibody response is crucial for confirming vaccine efficacy and establishing protection against microbes. In the case of leptospirosis, the hamster potency test has limitations due to ethical concerns. Although serological alternatives such as ELISA and microscopic agglutination test certainly enable detection of antibody response to vaccines, they fail to assess the protective efficacy of the antibody. Thus, considering the 3Rs of animal research, it is ideal to develop an *in vitro Leptospira* growth inhibition assay as a preliminary screening tool for evaluating protective potential of vaccine induced antibodies before moving to *in vivo* models. In this study, we attempted to optimize an *in vitro Leptospira* growth inhibition assay (LGIA) to conduct a preliminary evaluation of antibody efficacy. Previous studies on *Leptospira* growth inhibition lack methodological details. Thus, we sought to optimize the combination of incubation period, bacterial numbers, and antibody concentrations that function efficiently in inhibiting bacterial growth. We incubated *Leptospira interrogans* serovar Manilae in a dose range from to 10^7 per milliliter with anti-*Leptospira* polyclonal antibodies generated in rabbits against this serovar. *Leptospira* treated with PBS and negative rabbit serum were used as controls. Cultures were examined for the presence of *Leptospira* growth using dark field microscopy on d 2, 4, 7, 14, and 28. We observed that 10^4 was the ideal bacterial count for effective growth inhibition. Upon conducting this assay for 28 d, we found that bacterial growth was inhibited until d 14 compared with the controls. In response to bacterial treatment with various antiserum concentrations, we observed 1:10 as a highly effective antiserum concentration for preventing bacterial growth. Thus, we conclude that 10^4 bacteria, 1:10 antibody, and up to 14-d incubation would be an optimal combination to conduct this assay for the effective growth inhibition of the bacterial strain tested. This LGIA can be used as a tool for the early assessment of antibody response in vaccinated animals before challenging them to analyze the protective response to the vaccine. However, optimization of this assay is needed when working with different *Leptospira* strains.

Key Words: *Leptospira* growth inhibition assay (LGIA), protective antibody responses, vaccine efficacy, *in vitro* optimization, ethical alternatives (3Rs), serovar Manilae

208 - Neurophysiological and morphological signatures of *Clostridioides difficile* toxin exposure in a brain-on-a-chip model.

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Clostridioides difficile is a leading cause of antibiotic-associated diarrhea, and emerging data suggest that toxin-mediated effects on the nervous system may occur via the gut–brain axis. We sought to define the direct neurotoxic actions of TcdA and TcdB on neuronal networks using a microengineered brain-on-a-chip model. Primary cortical cultures from ICR mice were cultured on microelectrode arrays (MEAs). Spontaneous network activity was recorded before and after exposure to TcdA (100 ng/mL), TcdB (100 ng/mL), and both. Spike rate, active units, firing pattern, and synchrony (cross-correlogram asymmetry) were quantified. Cytoskeletal integrity was assessed by immunocytochemistry (F-actin; MAP2) with confocal imaging. TcdA produced 2 distinct electrophysiological patterns: early suppression, followed by brief recovery and then gradual decline. TcdB caused a progressive, irreversible decrease in spike rate and active units, accompanied by a persistent disruption of temporal coordination, or firing order, across the network. Combined TcdA and TcdB led to a more pronounced, time-dependent suppression of firing than TcdB alone, without acute collapse. Morphologically, TcdB induced marked F-actin loss, neurite retraction, and reduced distal MAP2; TcdA caused modest F-actin attenuation with preserved architecture. The combination preserved gross dendritic structure but reduced F-actin intensity, consistent with early cytoskeletal remodeling. In this brain-on-a-chip model, TcdA and TcdB directly disrupt neuronal network function with distinct temporal firing patterns and synchrony signatures and provoke cytoskeletal injury (pronounced with TcdB). These patterns suggest differing mechanisms of neurotoxicity, potentially reflecting receptor-specific or glial-mediated pathways. Characterizing toxin-specific disruptions involves elucidating pathways such as receptor-mediated or other specific mechanisms, thereby advancing our understanding of how *Clostridioides difficile* toxins impair neural network function.

Key Words: *Clostridioides difficile*, TcdA, TcdB, microelectrode array (MEA), brain-on-a-chip, neurotoxicity, gut–brain axis

General Abstracts: Session 41—Salmonella 2

209 - Rapid, field-ready multiplex qPCR for same-day detection of priority *Salmonella* serovars in poultry production.

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Salmonella enterica remains a major foodborne pathogen linked to poultry and presents persistent food safety and public health challenges. Traditional culture-based methods, although highly sensitive, require multiple days to generate results, delaying decision-making at the preharvest stage. Rapid, sensitive, and field-ready molecular assays are urgently needed to support earlier surveillance and intervention strategies. The GenoPATHX multiplex qPCR assay was developed to provide same-day detection of high-priority *Salmonella* serovars considered key performance indicators (KPIs) for poultry production systems. The assay simultaneously targets *Salmonella* Typhimurium, *Salmonella* Enteritidis, *Salmonella* Monophasic, *Salmonella* Muenchen, *Salmonella* Hadar, and a genus-level marker. Analytical sensitivity was evaluated using inoculated chicken and turkey rinsates to determine limits of detection (LODs). Field applicability was assessed using 36 environmental samples including poultry litter (n = 16), feces (n = 16), and boot socks (n = 4), processed via a centrifugation workflow and PrepMan/Proteinase K lysis. The DNA extracts were analyzed on serovar-specific channels corresponding to KPI panels, and results were benchmarked against the USDA FSIS-MLG culture method to estimate diagnostic sensitivity and specificity. The LODs for the chicken KPI panel ranged from 6.5 to 52 cfu/mL, whereas the turkey panel exhibited 3.4 to 231 cfu/mL; the genus-level marker demonstrated an LOD of 37 cfu/mL. Of the environmental samples, 13/36 (36.1%) tested positive at the genus level, with detection across all matrices: boot socks 4/4 (100%), feces 8/16 (50%), and litter 1/16 (6.3%). All KPI targets were detected, with *Salmonella* Typhimurium and *Salmonella* Enteritidis being most frequent. Several samples showed multiple serovar-specific signals, pending confirmation by whole-genome sequencing–based serotyping. Relative to culture, the assay demonstrated strong diagnostic sensitivity and specificity, supporting its potential for early risk flagging without sacrificing analytical performance. GenoPATHX enables same-day, preharvest detection of priority *Salmonella* serovars with low LODs, streamlined DNA extraction, and compatibility with routine workflows. These results highlight the assay's ability to deliver actionable data within a standard workday, enhancing surveillance and informing targeted interventions in commercial poultry operations. Future work will expand inclusivity and exclusivity testing, refine diagnostic accuracy, and validate robustness.

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Key Words: *Salmonella*, qPCR, GenoPATHX, poultry, preharvest

210 - FTIR spectroscopy combined with machine learning for rapid serotype-independent differential detection of biofilm-positive and biofilm-negative *Salmonella*.

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Foodborne illnesses caused by nontyphoidal *Salmonella* (NTS) 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. Although 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. Previously, our laboratory demonstrated the potential of Fourier transform infrared (FTIR) spectroscopy to differentiate biofilm-producing and non-producing strains with unweighted pair group method with arithmetic mean (UPGMA) clustering and spectral splicing, achieving a maximum sensitivity of 93% and specificity of 83%. Here, we present an enhanced machine learning prediction of biofilm-positive (BFP) from biofilm-negative (BFN) isolates. A total of 514 spectra (500–3,996 nm^{-1}) data were acquired for 152 NTS strains from poultry (77.2%) and human (22.8%) sources, and 12 different serotypes, which have been previously classified into biofilm-positive or biofilm-negative phenotype using conventional biofilm assays (CRCBB agar, calcofluor test, and tube test). Spectral data were split into training (56%) and test and holdout (44%) sets, and 4 different machine learning classification algorithms were selected: random forest (RF), logistic regression (LR), light gradient boosting model (Light GBM), and support vector machine (SVM). The Shapley additive explanations (SHAP) feature selection method was used to rank spectral features contributing to an initial 98% of model prediction and 767 of 3,497 (21.9%) features were used in final model training and testing. Model evaluation based on testing and holdout datasets gave sensitivity, specificity, and ROC-AUC for RF (97%, 97.8%, 99.3%), LR (92.4%, 82.6%, 92.3%), Light GBM (98.5%, 97.8%, 99.1%), SVM (92.4%, 89.1%, 94.5%), respectively, outperforming previous classification. Machine learning method combined with FTIR spectroscopy spectra processing provided enhanced classification of biofilm phenotype. This approach provides a promising rapid, reliable, and cost-effective method for distinguishing biofilm-forming *Salmonella* strains, addressing a critical gap in current surveillance methods.

Financial Support: Texas Tech University

Key Words: biofilm, machine learning, *Salmonella*, Fourier transform infrared (FTIR)

211 - Tracking *Salmonella enterica* within the gastrointestinal tract, lymph nodes, and liver abscesses of feedlot cattle.

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Liver abscesses, a major health and economic concern in feedlot cattle, are a bacterial infection attributed to *Fusobacterium necrophorum* and *Trueperella pyogenes*. In addition, *Salmonella enterica* is frequently isolated from abscessed livers. Although these livers are condemned at slaughter, posing no direct food safety risk, the presence of *Salmonella* raises questions about its role in abscess development and relatedness to gastrointestinal- and lymph node–derived *Salmonella.* This study investigated the population dynamics and genomic relatedness of *Salmonella* isolated from the gastrointestinal tract (rumen, colon, and feces), subiliac lymph nodes, and liver abscesses of the same cattle cohort. Whole-genome sequencing was performed on 91 isolates recovered from the rumen (n = 22), colon (n = 33), and liver abscesses (n = 36), with analyses for serotyping, multilocus sequence types (MLST), plasmids, and antimicrobial resistance genes. Phylogenetic analysis compared these genomes with *Salmonella* (n = 23) from feces and lymph nodes of the same cohort (unpublished). The predominant serotypes among the 91 isolates and their respective MLST were Anatum ST64 (n = 27), Kentucky ST198 (n = 25), Montevideo ST138 (n = 21), and Lubbock ST413 (n = 8). Less common serotypes included Agona ST13 (n = 6), Altona ST1549 (n = 3), and Lille ST297 (n = 1). Notably, liver abscesses yielded multiple isolates of Montevideo (n = 8), Anatum (n = 10), Kentucky (n = 10), and Lubbock (n = 4), all of which were also detected in feces, lymph nodes, rumen, or colon of the same animals, as well as Agona (n = 2), and Altona (n = 2), which were not found in lymph nodes or feces. All isolates carried the cryptic *aac(6')-Iaa* gene, while fos*A7* was uniquely detected in Agona. Identified IncI plasmids (n = 9) were not serotype- or sample-specific. Phylogenetic analysis containing strains from diverse sample types of the same animal or across animals in the same pen showed ≤10 SNP differences for the predominant serotypes. Importantly, MLST revealed a single lineage per serotype and was consistent across isolates from the gastrointestinal tract, liver abscesses, feces, and lymph nodes, indicating clonal stability independent of sample type. These patterns support dissemination of shared clones from the gut to systemic sites, including the liver. Future studies will extend beyond matched animal comparisons to evaluate *Salmonella* population dynamics across different animals within the same cohort.

Key Words: *Salmonella*, liver abscess, gastrointestinal tract, bovine, lymph node

212 - Navigating complexity: *Salmonella* Dublin risk factors in a Midwestern US calf movement system.

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Movement of live cattle is a known risk for pathogen spread. Understanding movement patterns, particularly at high-risk commingling facilities, is essential for targeted pathogen surveillance and disease mitigation strategies. This repeated cross-sectional study investigated calf movement patterns and detection of *Salmonella* spp. (including *Salmonella* Dublin) within a Midwestern livestock dealer facility, a critical yet underexplored component of the US cattle supply chain. From May to October 2023, records of 1,184 calf movements were analyzed alongside identification of *Salmonella* spp. by culture and *Salmonella* Dublin via PCR from environmental sampling. Weekly network metrics were assessed, including number of incoming sources, density, and source stability (Jaccard similarity indexes [JSI]). Mixed-effects logistic regression models were fit using the lme4 package in R to evaluate the association between movement patterns and pen-level pathogen detection. Statistical significance was set at $P < 0.05$, and odds ratios (95% CI) were calculated for each predictor. Spearman's rank correlation was calculated to evaluate the association between JSI and pathogen detection, respectively, for *Salmonella* spp. and *Salmonella* Dublin. Results revealed a high number of sources and animals passing through the facility, with fluctuating connectivity over time. Environmental sampling confirmed variable *Salmonella* Dublin presence, with a significant decreasing trend over time ($P < 0.01$), despite peak *Salmonella* spp. detection in late summer. Weekly movement networks showed variability in source stability, with lower JSI values (i.e., more variation in source zip codes) typically preceding increased detection of *Salmonella* Dublin ($P = 0.04$), suggesting that introduction of new or shifting sources may be risk factors. Our findings highlighted the need for further research into which characteristics of these new sources, such as geography, management practices, or biosecurity, might contribute to *Salmonella* Dublin presence and potential persistence. Results from this study underscored the role of livestock dealer networks in pathogen occurrence and reinforced the need for targeted mitigation strategies at such facilities. Strengthening external biosecurity through accurate and accessible intra- and interstate movement records and environmental monitoring is vital for mitigating the risks of *Salmonella* Dublin, a pathogen of critical concern for public and animal health.

Financial Support: This study was funded by the Agriculture and Food Research Initiative (AFRI) of the National Institute of Food and Agriculture (NIFA-USDA, Washington, DC; competitive grant no. 2022- 68015-36628).

Key Words: calf movement, biosecurity, *Salmonella* Dublin, sorting facility, market chain

213 - Prevalence and predictors of tetracycline resistance in *Salmonella* and *Campylobacter* spp. isolated from cecal samples of minor meat-producing animals at slaughters in the United States, 2020–2022.

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This study aimed to investigate the prevalence of antimicrobial resistance (AMR) and factors associated with tetracycline resistance in *Salmonella* and *Campylobacter* spp. isolated from the cecal samples of minor meat-producing animals (goats, sheep, and lambs) at US slaughterhouses. A retrospective analysis was conducted using AMR profiles of *Salmonella* and *Campylobacter* isolates collected between 2020 and 2022 from the NARMS database. The Cochran–Armitage trend test was used to assess trends in AMR proportions, and multivariable logistic regression models were fitted to evaluate the association between predictors (host species, season, and year) and outcomes. Overall, 9.9% (46/466) of *Salmonella* and 69.4% (209/301) of *Campylobacter* spp. isolates were resistant to tetracycline. The highest proportion of tetracycline resistance was observed in *Salmonella* spp. isolates from goat cecum (11.6%), followed by lambs (10.7%) and sheep (7.5%). Likewise, the highest proportion of tetracycline resistance was observed among *Campylobacter* spp. isolates in goat cecum (71.7%), followed by lambs (67.1%) and sheep (65.5%). Monthly, tetracycline resistance in *Salmonella* spp. isolates peaked in July (18%; 9/50) and was lowest in February (3%; 1/34). Seasonally, tetracycline resistance was highest in the fall (12.1%; 14/116) and lowest in the spring (4.9%; 5/102) in *Salmonella* spp. isolates. In *Campylobacter* spp. isolates, tetracycline resistance was highest in October (85.7%; 6/7) and lowest in May (50%; 11/22). Seasonally, tetracycline resistance was highest in the fall (73.8%; 31/42) and lowest in spring (65.3%; 77/118) in *Campylobacter* spp. isolates. No significant temporal trend was observed in tetracycline resistance among *Salmonella* spp. ($P = 0.06$) and *Campylobacter* spp. ($P = 0.53$) isolates. Multivariable logistic models showed no significant association between host species, season, year, and tetracycline resistance in *Salmonella* and *Campylobacter* spp. isolates. Tetracycline resistance was more prevalent in *Campylobacter* than in *Salmonella* spp. isolates, with goat cecal samples exhibiting the highest tetracycline resistance levels across both pathogens. These findings emphasize the need for continued surveillance, targeted epidemiologic research, and antimicrobial stewardship in minor livestock to combat AMR.

Financial Support: This research received no external funding

Key Words: surveillance, antimicrobial resistance, tetracycline, *Salmonella*, *Campylobacter*, small ruminant

214 - Design and construction of genetically modified *Salmonella* strains for *in ovo* administration.

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We describe our recent efforts in the design and construction of novel self-destructing protective immunity–enhanced *Salmonella* vaccines (sd-PIESV) and self-destructing attenuated adjuvant *Salmonella* strains (SDAA) that aim to induce strong protective immune responses in chickens against *S. enterica*, *Campylobacter jejuni*, and *Clostridium perfringens* to eliminate their prevalence in live poultry and enhance food safety. Growth kinetics in vitro using un-purple and Luria–Bertani (LB) broth, and safety following *in ovo* inoculation at 18 d of incubation were assessed. We have also determined the ability of sd-PIESV strains to synthesize multiple *C. jejuni* and *Cl. perfringens* antigens, as well as the potential of SDAAS strains to activate NOD1, NOD2, TLR4, TLR5, TLR8, and TLR9 in human embryonic kidney (HEK)-Blue cells. Last, we have determined the ability of these strains to colonize and invade different tissues in the inoculated embryo. The SDAAS and sd-PIESV strains tested have demonstrated strict requirement of DAP, D-alanine and glucosamine or L-arabinose to grow in either un-purple or LB broth. We also demonstrate how these strains can be safely inoculated *in ovo* without compromising hatchability or chick quality in a dose-dependent manner. The SDAAS strains demonstrated improved activation of all PRR evaluated when compared with the wild-type strain. The sd-PIESV strains tested successfully synthesize all *Cl. perfringens* and *C. jejuni* antigens encoded in plasmids pG8R256 and pG8R410, respectively. Lastly, SDAAS and sd-PIESV strains were recovered from different organs of embryos and day-of-hatch chicks following *in ovo* inoculation. Data were analyzed using one-way ANOVA test and no significant difference was found at $P < 0.05$. The SDAAS and sd-PIESV strains described in this work were designed to induce protective immune responses in chickens against pathogens of public health importance. Mutations in genes responsible for the synthesis of essential components of the bacterial cell wall result in the regulated delayed lysis phenotype, enabling delivery to chicken embryos *in ovo*. The SDAAS strains tested strongly activated different PRR in vitro and the sd-PIESV strains successfully synthesized multiple *Cl. perfringens* and *C. jejuni* antigens. Together, these platforms should reduce pathogen prevalence in live poultry, minimizing transmission to humans through the food chain to ensure consumers have access to a safer food supply.

Financial Support: This study was supported by the United States Department of Agriculture – National Institute of Food and Agriculture, grant number 2020-67017-33237 and 2025-67017-45107.

Key Words: *Salmonella*, *Campylobacter jejuni*, *Clostridium perfringens*, in ovo, innate, adaptative

General Abstracts: Session 42—Statistical Methods and Mathematical Modeling

215 - NMAforest: An R package for generating forest plots in network meta-analysis with decomposition of direct, indirect, and study-level evidence contributions.

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Network meta-analysis (NMA) allows simultaneous comparison of multiple treatments, even when direct head-to-head studies are lacking. However, commonly used software reports only overall and pairwise effect estimates and CI, leaving unclear how much of the evidence comes from studies for indirect comparisons and the NMA comparison. This contrasts with pairwise meta-analysis, where the contribution of each study is readily reported and visualized as weights. In network meta-analysis, the information each study contributes to estimates is best understood as evidence paths, which may include more than one study. It has been proposed that evidence flows, or evidence paths, can convey how study information contributes to the estimates from NMA. Using an approach to visually assess the contribution of evidence paths to indirect and NMA estimates, we developed NMAforest, an R package that decomposes treatment-effect estimates into their contributing components and visualizes those contributions. NMAforest links each estimate to the network's structure and uses the "Hat matrix" framework to quantify how much each individual study path (direct comparison or indirect path) contributes to the overall NMA estimate. In practice, the forest plot displays the overall NMA estimate, the direct comparison estimate, and distinct indirect paths side by side, and further breaks down each indirect path into study combinations, with colored proportion bars indicating their relative contributions. This approach enhances transparency and enables researchers to judge the robustness and source of evidence supporting treatment effects. Our methodological examples highlight the package's features, which can be applied to treatment comparisons in animal health studies, showing how certain direct and indirect paths dominated the estimate in some comparisons. NMAforest is available on CRAN and supports better evidence interpretation for disease control and therapeutic decision making in animal health.

Key Words: network meta-analysis, forest plot, indirect path, study contribution, evidence decomposition

217 - Predicting influenza incidence in Arizona, USA: Comparative evaluation of statistical and machine learning approaches.

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Influenza remains a major public health burden globally, including in Arizona, and its transmission is affected by a variety of environmental, demographic, and geographic variables. This study aimed to compare the predictive performance of conventional statistical models and supervised machine learning algorithms to predict the incidence rate of influenza infections in Arizona. Data were obtained from various sources, including Arizona Department of Health Services and the Environmental Protection Agency. The data included influenza incidence rate, climate variables and age/gender distribution with county-level information in Arizona. The dependent variable was the rate of influenza incidence (per 100,000 people). Supervised learning models—random forest, gradient boosting, support vector regression (SVR), and k-nearest neighbors (KNN)—were trained and compared with a negative binomial regression model. Model performance was assessed using mean squared error (MSE), root mean squared error (RMSE), mean absolute error (MAE), and R^2 score. The results showed that the gradient boosting model achieved the highest predictive performance (R^2 = 0.987, RMSE = 19.79), followed by random forest (R^2 = 0.969, RMSE = 30.11). The negative binomial regression model demonstrated strong fit and interpretability (R^2 = 0.912, RMSE = 47.72). In contrast, KNN and SVR underperformed, with SVR showing a poor fit (R^2 = −0.195). This study concluded that gradient boosting and random forest models provided better and more accurate predictions of influenza incidence rate in Arizona. These results demonstrate that machine learning and statistical approaches are complementary and together enable accurate predictions and support public health decisions.

Key Words: Arizona, incidence, machine learning, statistical model, prediction

218 - Time series forecasting of valley fever in Arizona, USA, using machine learning and deep learning algorithms.

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Valley fever (VF) has been a significant public health concern in the southwestern United States, including Arizona. Maricopa County, Arizona, in particular, records the highest incidence rate annually. This study was aimed at modeling the temporal dynamics and identifying predictors of VF incidence using time series forecasting models and machine and deep learning algorithms. Data on VF, climatic or environmental, and demographic variables were collected from various state, local, and federal organizations. Time series analysis using a decomposition method and seasonal autoregressive integrated moving average (SARIMA) models was used to analyze trends in the data and predict future disease incidence until 2050. Negative binomial (NB) and random forest regression were used to determine the effects of predictors on annual VF incidence rates. Time series decomposition identified a strong seasonal gradient in the VF incidence rate, highest in late fall and early winter. The SARIMA model provided good short-term predictions with an RMSE of approximately 221 cases and was employed to forecast future trends through the year 2050. The NB model performed slightly better (R^2 = 0.91) compared with the random forest model (R^2 = 0.90). The high overall explanatory power of the NB model could suggest that the combination of environmental and demographic factors contributes meaningfully to predicting VF trends and can be leveraged for future scenario modeling through 2050. In conclusion, this multimodel study verified seasonality of VF in Maricopa County, Arizona, and identified important environmental and demographic factors associated with the risk of VF.

Key Words: Arizona, deep learning, machine learning, time series forecasting, valley fever

219 - Enhancing disease risk analyses: Country variation in predicted contaminated meat supply.

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Preserving livestock health relies on effectively predicting foreign animal disease introduction risk to support pathogen detection and reduce outbreak severity. Such predictions are challenged by shifting outbreaks, varied introduction pathways, and data gaps, so we need an adaptive analytical framework. As a foundational step, we predicted the proportion of contaminated meat in disease-positive countries. We developed a mechanistic model to simulate the proportion of African swine fever virus (ASFv)-contaminated pork in a country. We used World Organization for Animal Health outbreak data and UN Food and Agriculture Organization livestock and slaughter data. We focused on 73 countries with ASFv outbreaks between 2005 and 2024: Africa (31), Asia (20), Europe (19), North America (2), and Oceania (1). For each country and year, we simulated 100 iterations. We calculated ASFv diagnostic sensitivity and specificity and used these alongside infected swine counts to estimate apparent and true prevalence. Assuming 5% of outbreak control zone swine were not contained, we estimated the number of infected swine in the supply. Using this value, reported slaughters, and average carcass weight, we estimated the proportion of ASFv-contaminated pork. To identify influential parameters, we performed a sensitivity analysis using partial rank correlation coefficients for individual effects and percent influence for pairwise interactions. Country influence was assessed via global and regional leave-one-out analyses. The proportion of ASFv-contaminated pork ranged from 0 to 0.26. Higher mean proportions since 2020 in North America, Europe, and Oceania were consistent with first detections and growing outbreaks. Wide interannual uncertainty in Africa reflected regional variation. Sensitivity analyses showed greatest influence from the proportion of infected swine (34%), infected-not-contained swine (28%), and infected-slaughtered swine (21%). Among pairwise interactions, country variation in swine stock accounted for 92% of the influence. Leave-one-out analyses showed 7 countries positively influenced the mean; others had mild negative influence. Globally influential countries similarly affected their regional means. We developed a mechanistic framework for predicting country-level contaminated meat supply over time. Although demonstrated for ASFv, this framework is adaptable across species and pathogens. These data-driven insights support disease risk assessments critical for policymaking.

Key Words: disease risk modeling, simulation, African swine fever virus (ASFv), swine

220 - Fitness learning of evolutionary variants over time for surveillance of emerging PRRS viral strains.

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With more than $1.2 billion annual losses in the US swine industry, PRRSV continues to challenge vaccine design efforts due to the high mutational variability in GP5 glycoprotein, which undermines protection from neutralizing immune responses. Supervised machine learning models can identify antibody escape-prone residues and critical functional domains by analyzing sequence patterns. We trained a Bayesian variational autoencoder inspired by the EVE model, on ESM-2 protein language model embeddings of 33,717 sequences of PRRSV GP5 glycoprotein to assess the fitness landscape of all possible single-point mutants. Our escape score integrates structural avidity and local charge distribution changes upon mutation with evolutionary fitness predictions, enabling interpretable mapping of fitness to the structural features. By aggregating individual mutation effects, our model not only predicts escape propensities but also enables real-time surveillance of emerging/re-emerging PRRSV variants and antigenic design constructs. Incorporating protein language models that capture richer structural and evolutionary context helps in correlating antibody escape tendencies with porcine receptor binding affinity predictions (Pearson correlation coefficient r = 0.74), while detecting seasonal patterns in escape tendencies of sequences obtained over the past decade. This study aimed to develop a dynamic surveillance tool for monitoring antibody escape tendencies in PRRSV variants, enabling early prediction of outbreak risks. By tracking progressive shifts in critical epitopes of high-escape variants, the tool supports rational antibody design and informs timely intervention strategies.

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Key Words: machine learning, PRRSV surveillance, antibody escape, sequence analysis, viral persistence

Featured Speaker: Session 43—AVEPM Schwabe Symposium

221 - Opening remarks. “Science’s social contract: Epidemiology in the real world.”

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In a world where expert opinion and evidence-based decision making are increasingly challenged and where the balance between proactive and reactive science is tempered by the vagaries of societal need, how do we justify our existence as researchers? Ensuring our continued relevance and ability to make a difference require us to demonstrate the quality and impact of our science at the same time as we continue to advance our discipline. Within the context of technical, behavioral, and institutional level solutions, as epidemiologists we have both the privilege of being creative as well as the responsibility to be pragmatic—our contract is to deliver across this wonderful spectrum.

222 - Through the looking glass: Epidemiology, policy, and practice in reverse.

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Facts and arguments comprise the near-eternal interplay between data and their interpretation by humans. This dynamic occurs at all social scales ranging from individuals through governments and beyond. Traditionally, science has been relied on to provide unbiased information—whether directly solicited or not—and society has thereafter debated its meaning, leading first to policy enactment and later (presumably) to adoption and implementation of consistent practices. In the realm of public health, whether human or veterinary, the epidemiological approach has provided population-level data upon which to generate policies and foster practices. The process itself tends to be cyclical, rather than linear, as further improvements and updates to the scientific input are sought. But what if health practices—and, by further extension, policies—were to come detached from scientific inputs? What predisposing factors might lead to such a situation? Does the blame (if any) fall on the scientific community and their approaches and methods? In this presentation, examples from both animal and human health will be used to illustrate the potential pitfalls in relying strictly on science to develop and implement policies and to encourage the adoption of aligned practices. The longstanding and “wicked” problem of antimicrobial resistance and the related concept of antibiotic stewardship help illustrate the concepts of a moral economy and of social marginalization in the ongoing debate over the use of antimicrobials in food animals and in the optimization of risk(s) across the realms of animal health and food safety. Examples from the COVID-19 pandemic will be used to explore the downside of a singular focus on one public health metric, as opposed to adopting a broader and more holistic multivariate approach, in establishing public health policy. Several paths forward will be explored, with emphasis on those in which epidemiology, as a discipline, is capable of flourishing and in which the data themselves are allowed to speak.

General Abstracts: Session 44—Swine Influenza 2

223 - A multivalent mRNA-lipid nanoparticle vaccine against influenza A virus circulating in swine.

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Preventing infection and transmission of influenza A virus (IAV) in swine is difficult due to evolution and subsequent evasion of the host immune response. The traditional approach for effective IAV control has been the development of inactivated whole virus vaccines that reflect the antigenic diversity of circulating swine IAV strains. This method depends on comparing vaccine antigens to genomic diversity detected in surveillance activities. It is hampered by the 15 to 20 distinct hemagglutinin (HA) clades of IAV that have been regularly detected in the United States. In this study, we addressed the challenge of cocirculating genetic clades of swine IAV and constant evolution through a multivalent mRNA-lipid nanoparticle (LNP) vaccine that maximized genetic coverage. We applied computational approaches through PARNAS to select 8 HA vaccine antigens representing more than 90% of observed swine HA genetic diversity detected between 2022 and 2025 in the United States. The components included 3 HA genes from the 1A classical swine lineage (1A.1.1.3, 1A.3.3.2, 1A.3.3.3-c3), 2 HA genes from the 1B human seasonal lineage (1B.2.1, 1B.2.2.2), and 3 H3 HA genes (1990.4.a, 2010.1, 2010.2). Pigs were vaccinated and boosted intramuscularly with the individual HA mRNA-LNP or the 8-HA mRNA-LNP. Multivalent as well as matched H1 and mismatched H1 monovalent groups were challenged with an H1 1A.3.3.3-c3 IAV strain, representative of 20% of H1 detected between 2022 and 2025 in the United States. This study was designed to assess characterization of the mRNA-LNP antibody response and protection from challenge. These data will be used to determine whether there are cross-reactive or HA-specific antibodies that react to the 8 encoded antigens. Moreover, the data will provide evidence on whether multivalent mRNA-LNP vaccines can be deployed to improve animal health and aid pandemic preparedness by minimizing the transmission and diversity of IAV in swine.

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Key Words: influenza, multivalent, mRNA vaccine, lipid nanoparticle, swine

224 - A versatile noninfectious platform for the rapid development and updating of vaccines against influenza A virus of swine.

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We aimed to develop a “plug-and-play” platform for rapid vaccine development and updates against influenza viruses. This platform uses a DNA plasmid to deliver genes encoding vaccine immunogens. To enhance DNA vaccine immunogenicity, we developed lipid nanoparticle (LNP) formulations to encapsulate the plasmid DNA. Six groups of piglets were included: 3 with maternally derived antibodies (MDA) and 3 without. Piglets were immunized intramuscularly with a single dose of the LNP-DNA vaccine or 2 doses of the whole-inactivated virus (WIV) vaccine on d 0 and 21 or served as nonvaccinated (NV) controls. All piglets were challenged with H1N2 virus via intranasal and intratracheal routes. In MDA-negative pigs, both vaccines induced strong hemagglutination inhibition (HI) antibody responses, with the LNP-DNA vaccine producing significantly higher titers than the WIV vaccine at d 41 (1:900 vs. 1:320, $P < 0.05$). In MDA-positive pigs, the LNP-DNA vaccine also induced significantly higher HI titers compared with the NV group (1:140 vs. 1:22, $P < 0.05$), while the WIV vaccine did not differ significantly from the NV group. The WIV vaccine induced weak cellular immune responses regardless of MDA status. The mean numbers of IFN-γ-secreting cells (IFN-γ-SC) in PBMC of MDA-negative and MDA-positive pigs receiving the WIV vaccine were 17 and 29, respectively. In contrast, the LNP-DNA vaccine elicited strong cellular immune responses in both MDA-negative and MDA-positive pigs, with mean IFN-γ-SC counts of 130 and 82, respectively. After challenge, infectious virus was not detected in MDA-negative pigs immunized with either vaccine. In MDA-positive pigs, the mean infectious virus titer in nasal secretions on d 5 was significantly lower in the LNP-DNA group ($10^{1.6}$) compared with the WIV and NV groups ($10^{5.3}$ and $10^{4.1}$, respectively). On d 5 postchallenge, lung consolidation typical of swine IAV infection was observed in MDA-negative pigs in the NV group (3.4% of lung surface affected). Lung consolidation was not observed in MDA-negative pigs immunized with either vaccine. In MDA-positive pigs, the NV and WIV groups showed similar lung consolidation (~1%), while the LNP-DNA group did not show any visible consolidation. These results demonstrate that LNP-encapsulated DNA can serve as a versatile vaccine platform for swine IAV.

Financial Support: This study was funded by the Agriculture and Food Research Initiative competitive grants 2025-39601-44627 and the Multi-state Hatch project 1020750 of the USDA National Institute for Food and Agriculture.

Key Words: DNA vaccine, lipid nanoparticle, swine, influenza, maternally derived antibody

225 - Human H3 3a.1 clade influenza A virus detected in swine.

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Influenza A virus is a key component of the swine respiratory disease complex. New viral genetic diversity is frequently introduced through human to swine influenza transmission. Since 2022, a notable number of H3 human-to-swine spillover events have been detected, through passive surveillance at the Iowa State University Veterinary Diagnostic Laboratory (ISU VDL). The 3a.1 clade has circulated in humans since 2023 and became globally dominant in humans in the 2023–2024 influenza A season. Since 2024, repeated spillovers of the contemporary human H3 3a.1 clade into swine have been observed, raising concern about the potential establishment of a novel genetic lineage. The objective of this study was to determine if detections of 3a.1 in swine in 2025 were swine-to-swine transmissions, and to determine whether these strains had reassorted with swine-derived genes. The first detection of this clade in swine occurred on January 30, 2024, through 2 unrelated cases that occurred in Oklahoma and Pennsylvania. Over this study period, 15 sequences from 12 cases were submitted to the ISU VDL, with the most recent detected on May 28, 2025. Additional spillovers have been identified in Indiana, Mississippi, Nebraska, North Carolina, Ohio, and Canada. No epidemiological links between locations have been established. All sequences have high similarity to the contemporary human strains, suggesting recent spillover. A cluster of cases in Nebraska was noted, but transmission was not readily inferred due to high similarity to human-derived viruses. All swine-derived strains carry a T3A mutation in the signal peptide, a pervasive mutation specific to the human 3a.1 clade. Other nucleotide mutations were observed, but were not persistent between strains, supporting the hypothesis of recent repeated introduction into swine. The maximum nucleotide difference between the hemagglutinin genes was 20 nucleotides, with an average difference of 13 nucleotides. Seven whole genomes have been recovered related to these cases. At present, no evidence of onward transmission in swine has been identified, but repeated spillovers raise concerns about potential establishment of a new H3 lineage. Risk of the establishment of a new swine lineage remains high and continued monitoring is needed.

Key Words: influenza, spillover, zoonosis

226 - Modeling influenza virus host adaptation and tropism using precision-cut tissue slices.

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Since its emergence in US wild bird populations in 2021, H5N1 clade 2.3.4.4b influenza virus has continued to spread rapidly, recently spilling over into dairy cattle, with more than 1,000 herds affected across 17 states. This outbreak highlights the virus's expanding mammalian host range and raises concern about its potential impact on the swine industry, particularly given pigs' role as "mixing vessels" for influenza viruses that may adapt to humans. To prepare for potential spillover scenarios, there is an urgent need for relevant models to evaluate H5N1 replication in swine and human tissues. Here, we developed a physiologically relevant, high-throughput ex vivo model using precision-cut lung slices (PCLS) from donor swine and human lung tissue. The PCLS cultures remained viable for at least 5 d and supported robust replication of influenza A viruses, demonstrating the utility of this system for studying viral infection dynamics in both species. Hematoxylin and eosin staining showed intact lung architecture before infection but marked epithelial disruption and inflammation following influenza virus exposure. Immunohistochemistry further revealed increasing viral nucleoprotein (NP) expression over time, along with localized immune cell infiltration ($CD45^+$). Together, these results establish swine and human PCLS as a translational model for evaluating influenza virus pathogenesis and cross-species transmission and will be leveraged to study H5N1 spillover risk in follow-up high-containment studies.

Financial Support: This work was supported by the National Institute of Health and the USDA National Institute of Food and Agriculture (NIFA)

Key Words: precision-cut lung slice, ex vivo tissue culture, influenza A virus, viral replication dynamics, host tropism, spillover

227 - Characterization of influenza A virus lineages in cohorts of pigs from weaning to near market.

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Influenza A virus (IAV) is endemic in swine, where co-circulation of hemagglutinin (HA) and neuraminidase (NA) lineages creates opportunities for reassortment and novel genotypes. The extent to which genotypes persist through the pig production cycle is unclear. Understanding IAV dynamics helps identify stages where diversity and reassortment are most likely, guiding control strategies. We evaluated the diversity and persistence of IAV genotypes in cohorts of pigs from weaning to near market. A total of 720 pigs in 3 cohorts was sampled at weaning, in the nursery, and in finishing. Pigs originated from 3 breeding herds, were commingled at weaning, and remained as cohorts. Samples were tested by RT-qPCR targeting the matrix gene; positives were subtyped for HA (H1/H3) and NA (N1/N2), and 91 samples were plaque purified in MDCK cells to identify individual virions. Whole genomes were sequenced, and genotypes were defined by segment origin and lineage diversity using PhyloPart. Genotypes were determined by cohort and production stage, and Shannon diversity index was calculated. From 173 complete genomes, 20 unique genotypes were identified. Most (75%) appeared at a single stage, while 25% persisted across stages. Despite shared origins, cohorts differed in the genotypes present. One breeding herd contributed most genotypes at weaning, and only one genotype was found at finishing. Internal segments were dominated by TRIG or pdm lineages, whereas HA and NA segments represented multiple lineages. H1A.1.1.3, H1A.3.3.3-c3, H3.2010.1, H3.1990.4.a, N1.C.3.2, N2.1998BM, and N2.2002B were detected, often co-circulating at weaning and early nursery but sporadic in finishers. On some occasions, more than one genotype was identified in the same pig. Shannon diversity was highest at weaning and nursery and decreased toward finishing (Kruskal–Wallis, $P < 0.05$). These findings highlight the dynamic nature of IAV diversity in swine. Although reassortment among internal genes was evident, HA and NA segments were more stable. Diversity was highest in young pigs and declined with age, suggesting that lineage-specific immunity and management shape IAV dynamics. Tracking lineage distribution within and between cohorts improves understanding of IAV evolution, with implications for surveillance and control.

Key Words: influenza, swine, evolution, diversity

228 - Establishment of human H3 2a.1 clade influenza A virus in swine.

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Influenza A virus (IAV) is a major component of the swine respiratory disease complex. Human-to-swine transmission events introduce new viral genetic diversity, which can subsequently reassort with endemic swine lineage IAV. Since 2022, an increased number of H3 human-to-swine spillover cases were detected through passive surveillance at the Iowa State University Veterinary Diagnostic Laboratory (ISU VDL). The human seasonal H3 2a.1 clade circulated in humans between 2021 and 2023. This lineage has been implicated in multiple reverse zoonotic spillover events, with the first detection in swine submitted to the ISU VDL in cases occurring in December of 2022. Repeat spillovers of this lineage into swine were documented between 2022 and 2024, with a marked decrease in 2024. Since 2023, this clade is no longer detected in the human population. However, there have been at least 6 detections of the 2a.1 lineage in US swine between July and September 2025 based on hemagglutinin (HA) sequences. This study establishes evidence that this human-origin clade is now endemically spreading within US swine because it continues to be detected in swine after its disappearance from human seasonal detections. In 2024 and prior years, 11 2a.1 HA sequences were associated with cases collected in the states of Colorado, Illinois, Ohio, and Missouri. Six additional positive detections were identified since July of 2025, collected in Illinois and Iowa. These new swine cases form a divergent monophyletic clade aligning with Illinois cases collected from swine before 2025, suggesting a direct genetic relationship between these HA sequences. These most recent HA sequences are on average 25 nucleotides different from their nearest human counterpart. Six whole genomes have been recovered related to H3 2a.1 cases in swine. These recent detections are reassortant viruses that carry the swine N2.2002B neuraminidase. All polymerase genes are from the swine triple reassortant lineage, with the nucleoprotein and matrix genes from the 2009 pandemic lineage. The NS1gene from 2024 cases was from the pandemic 2009 lineage, while recent cases detected in 2025 were from the triple reassortant lineage, suggesting further reassortment. At present, this lineage appears to be transmitting swine-to-swine, with newest cases in Iowa and Illinois and would represent an emerging lineage with minimal population immunity in swine.

Financial Support: Iowa State University Veterinary Diagnostic Laboratory

Key Words: influenza, swine, human reverse zoonosis

General Abstracts: Session 45—Bovine Respiratory Disease 2

229 - Nonspecific immune stimulation induces innate tolerance in bovine monocyte-derived macrophages.

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The innate immune response has classically been considered nonspecific, without the capacity for memory. Recent advancements have demonstrated that chromatin binding changes can mediate lasting alterations to innate immune responses, termed “training.” Nonspecific innate immune stimulation has been explored as a way to mitigate respiratory disease in cattle; however, the duration of increased cellular responses following stimulation has not been determined. Therefore, the objective of this study was to evaluate the capacity of a nonspecific TLR agonists (TLR4, TLR3/TLR9) to induce a “trained” innate phenotype in bovine monocyte-derived macrophages (MDM). Peripheral blood was collected from healthy preweaning dairy calves (n = 12) via jugular venipuncture, and peripheral blood mononuclear cells (PBMC) were derived through Ficoll density centrifugation. The MDM were derived, and after 7 d of differentiation and proliferation, were stimulated with LPS or a nonspecific liposomal TLR3/TLR9 complex (LTC) for 8 h. Media was removed, and cells remained undisturbed in culture for 72 h. Cells were stimulated a second time with LPS alone for 4 h, and then supernatants were collected 24 h later. Cytokine levels were assessed via ELISA. All statistical tests were performed in Prism8 (GraphPad), using a one-way ANOVA with Tukey’s multiple comparisons test. The MDM stimulated twice with LPS 3 d apart displayed a 47.5-fold reduction in IL-6 production ($P < 0.001$) compared with MDM that were only stimulated once at the second time point. Stimulation with LTC resulted in a 19-fold reduction in IL-6 production ($P < 0.001$) and was not significantly different from stimulation twice with LPS ($P = 0.64$). In this study, stimulation of bovine MDM with nonspecific TLR agonists resulted in reduced inflammatory cytokine production in response to LPS stimulation. This tolerant phenotype persisted in cells stimulated with either TLR4 or TLR3/9 agonists, demonstrating that regardless of viral or bacterial-associated TLR stimulation, prior stimulus can induce a tolerant phenotype in innate immune cells that persists days after initial exposure. These findings have important implications for the study of innate immune stimulants in cattle and provide insight into phenotypic effects of multidose immune stimulation on MDM.

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Key Words: immunology, bovine respiratory disease, innate training

230 - Immune stimulation prior to vaccination to prevent respiratory disease attenuates *ex vivo* alveolar macrophage cytokine production in preweaning calves.

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Optimizing vaccine responses in cattle has the potential to mitigate respiratory disease occurrence, yet more work is needed to understand how to best regulate calf immune responses to vaccination. Innate training, or the induction of altered innate responses to a secondary stimulus, has been explored as a way to modulate host immune responses across a variety of disease states. Vaccination activates both the innate and adaptive arms of the immune system, but less research has addressed how to improve innate responses to vaccination. Therefore, the objective of this study was to evaluate the capacity of nonspecific immune stimulation with a toll-like receptor (TLR) 3/9 agonist to increase innate immune functionality after vaccination. Calves were enrolled in a randomized controlled trial to evaluate in vivo stimulation on innate training phenotypes. Preweaning calves were intramuscularly administered 1 mL of liposomal TLR3/TLR9 complex (LTC; n = 7), or an equal amount of a buffered sucrose diluent (CON; n = 6). Calves were vaccinated 10 d later with a multivalent vaccine against bovine respiratory syncytial virus, bovine herpes virus 1, bovine viral diarrhea virus types 1 and 2, and bovine parainfluenza virus type 3 (Bovi-Shield GOLD®, Zoetis). Approximately 18 to 24 h after vaccination, bronchoalveolar lavage was collected from all calves and alveolar macrophages were derived for cell culture. Cells were left unstimulated or stimulated for 48 h with LPS. Supernatants were collected and evaluated for IL-6 cytokine production via ELISA. All statistical tests were performed in Prism8 (GraphPad), using the Mann–Whitney U Test, with significance set to $P < 0.05$, and tendency set to $P = 0.05–0.1$. No calf developed respiratory disease signs through 8 wk of age during the study. Unstimulated alveolar macrophages from LTC calves produced 7.4-fold less IL-6 than CON calves ($P = 0.045$). Upon LPS stimulation, there was a tendency for alveolar macrophages from LTC calves to produce less IL-6 than CON calves (3.1-fold mean difference, $P = 0.073$). In this study, administration of a liposomal TLR3/TLR9 agonist to calves 10 d before vaccination attenuated baseline alveolar macrophage IL-6 production and tended to reduce LPS-stimulated IL-6 production. These moderate immune changes may reflect altered macrophage responses due to innate stimulation, and more work is needed to understand relevance for calf health outcomes.

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Key Words: immunology, bovine respiratory disease, innate training

231 - Bovine cathelicidins expression in respiratory tissues during bovine respiratory syncytial virus infection.

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Bovine respiratory disease (BRD) significantly affects cattle worldwide, with imperfect prevention and treatment options. Host defense peptides called cathelicidins are promising candidates for targeted therapeutics against BRD, as these small, cationic peptides have antimicrobial and immune-modulating activities shown to be critical in airway epithelial defense. One cause of BRD as a single pathogen disease is bovine respiratory syncytial virus (BRSV), a common pathogen of both bovine and humans. Little is known about cathelicidin expression during BRD. This study characterized the endogenous expression of mRNA for all bovine cathelicidins (1–7) in respiratory tissues of BRSV-infected calves using SYBR Green RT-qPCR analysis. Seven 2-mo-old Holstein bull calves were challenged with nebulized 5×10^5 TCID50 units/mL of BRSV. Daily clinical scores were recorded, and necropsy occurred on d 8 postinfection; 2 healthy 2-mo-old Holstein bull calves were necropsied separately. At necropsy, lungs were evaluated for pneumonia by scoring of percent grossly affected lung. Samples of nasal epithelium, trachea, and normal and abnormal appearing lung tissues were snap frozen and stored at −80°C until RNA extraction. RT-qPCR was conducted for the 7 cathelicidins and 2 reference genes, *UBE2Q1* and *CRNKL1*. All primers were designed using Primer3 and BLAST. Relative gene expression was determined using the $2^{-\Delta\Delta CT}$ method. Challenged calves demonstrated mild clinical signs with fevers (>.5°F) starting on d 3. Necropsy revealed lung scores of 7% to 50%. Cathelicidin expression in the nasal epithelium, based on median values, indicated upregulation of cathelicidins 4, 5, 6, and 7 in calves with detectable levels. In the trachea, all cathelicidins were upregulated in detectable samples. Both normal and abnormal lung tissues showed downregulation or baseline expression of all cathelicidins except cathelicidin 5. Of all calves, the calf with the most extensive lung lesions had highest cathelicidin expression in the upper respiratory tract. Statistical and correlation analyses are pending. To our knowledge this is the first description of cathelicidin gene expression in BRSV-infected cattle. Expression was higher in the upper respiratory tract versus the lower; this may have been due to different kinetics of infection in these 2 regions. Future research will identify expressing cell types, and develop approaches to manipulate expression to prevent disease.

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Key Words: gene expression, innate immunology, cathelicidin, bovine, BRSV, RT-qPCR

232 - Associations of antibiotic treatment for preweaning bovine respiratory disease on the periparturient peripheral leukocyte transcriptome of primiparous Holstein dairy cows.

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Preweaning bovine respiratory disease is a driver of antimicrobial use in dairy calves. Use of antibiotics can result in negative downstream effects on the host (e.g., immune and metabolic dysfunction). This is an underexplored area within the physiologically demanding periparturient period. The ability of cattle to adapt to these demands dictates their productive life. This study aimed to explore differential expression within the peripheral leukocyte transcriptome of periparturient primiparous cows previously diagnosed and treated for chronic preweaning lobar lung consolidation. Calves enrolled on 2 dairies in central Washington within the first 10 d of life (May 2021: n = 61; May 2022: n = 60) were evaluated weekly for 12 wk via thoracic ultrasonography and followed to their first lactation. Fifteen calves were diagnosed with chronic lobar consolidation (≥1 lung lobe consolidated for ≥3 wk in duration; age at onset = 32–56 d), of which 7 were treated with antibiotics by farm personnel and 8 were left untreated during the period of consolidation. Calves in each group were otherwise found to have similar clinical and hematological characteristics. All 15 animals were evaluated during the first 2 wk postpartum and deemed healthy based on clinical and hematological assessments for common disease states (e.g., metritis, mastitis, lameness, hyperketonemia). Leukocytes were isolated from whole blood samples collected during the first and second week of lactation via modified Ficoll–Paque separation followed by RNA extraction and sequencing. The RNaseq (n = 30) expression levels were quantified using GEMmaker with a Kallisto pseudoaligner against the *Bos taurus* genome (ARS-UCD1.3). The edgeR software package identified 31 upregulated and 62 downregulated differentially expressed genes (DEG) among cows previously treated for preweaning lung consolidation compared with those left untreated. Although preliminary results did not show differences in upregulated enrichment pathways, there were differences ($P \leq 0.01$) in downregulated pathways associated with suppression of antiviral response genes (e.g., response to type 1 interferon). Further exploration of these immunomodulatory changes in the transcriptome may provide complementary insight into disease-associated phenotypes and postpartum cow management. Additionally, this can further inform management strategies related to antibiotic stewardship as well as genetic-selection strategies aimed at improving animal robustness.

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Key Words: transcriptomics, dairy calf, transition period, respiratory disease, antibiotic treatment

233 - Bovine response to outer membrane vesicles and biofilm matrix antigens of *Histophilus somni* when grown to simulate the *in vivo* environment.

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Current vaccines to prevent bovine respiratory and systemic diseases due to *Histophilus somni* are inadequate because antigens expressed during host infection may be absent in bacteria grown in culture. *Histophilus somni* forms a biofilm during chronic infections, and half of the bacterial genome is differentially expressed during biofilm formation. Iron-binding proteins are expressed by *H. somni* in the host, but not in culture medium, and *H. somni* persists within phagocytic cells through inhibition of phagosome-lysosome fusion. Our objectives were to combine in vivo-like outer membrane vesicles ($_{iv}$OMV) with biofilm matrix (BM), immunize calves with the $_{iv}$OMV-BM vaccine combined with a novel adjuvant, and assess the humoral and cellular immune response. The $_{iv}$OMV expressing iron-binding proteins were obtained by ultracentrifugation of cell-free culture in medium containing ethylenediamine-N,N′-bis(2-hydroxyphenyl)acetic acid to sequester free iron. The BM was obtained from biofilms grown under static conditions for 5 d. The proteins in $_{iv}$OMV and BM were characterized by proteomics and bioinformatics. The modified lipid A adjuvant BECC438 was added. Two groups of calves were immunized intramuscularly twice 3 wk apart with identical doses of $_{iv}$OMV containing a low dose of BM or a high dose of BM. Sera and plasma were collected weekly for 8 wk to determine IgM, IgG1, IgG2 titers (ELISA), cytokine concentrations (Bio-Plex), and bactericidal activity (viable plate count). An early IgM response was recorded, which was followed by an increasing IgG response, particularly IgG2. The antibody response to $_{iv}$OMV was greater than to BM. The antibody response to $_{iv}$OMV was weaker in calves immunized with larger doses of BM. However, the responses to IFN-γ and several other cytokines were greater in calves immunized with the high dose of BM. Bactericidal assays indicated that calves immunized with the low dose of BM made a stronger bactericidal response earlier than calves immunized with the higher dose of BM; both groups made strong bactericidal responses by 42 d postinoculation. A strong humoral and cellular immune response was made to the $_{iv}$OMV-BM vaccine, although a stronger response to OMV than BM was noted. The BM may inhibit the immune response to surface protein antigens. Additional immunogenicity tests are ongoing to confirm the optimal correlates of immunity and vaccine dose before challenge and efficacy studies.

Financial Support: This work was supported primarily by USDA-NIFA grant 2023-67015-39655

Key Words: *Histophilus somni*, vaccine, biofilm, outer membrane vesicles, *in vivo*, immune response

234 - Bovine alphaherpesvirus 1 (BoHV-1) promotes neuron survival and ribosomal remodeling in trigeminal ganglia of latently infected calves.

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Bovine alphaherpesvirus 1 (BoHV-1) causes upper respiratory tract disease, conjunctivitis, is a cofactor of bovine respiratory disease, and can induce abortions. During acute infection, BoHV-1 virions are transported to trigeminal ganglia (TG) via synaptic connections, and lifelong latency is established. The latency related (LR) gene is the only viral gene abundantly expressed in latently infected neurons. The objectives of this study were to identify how BoHV-1, including LR gene products, affects cellular signaling pathways that promote maintaining latency in TG sensory neurons. RNA from TG of 3 uninfected and latently infected calves were prepared. An Illumina TruSeq stranded RNA library preparation kit that contained 100 ng of RNA was used to construct cDNA libraries, which were sequenced as 75-bp paired-end reads on an Illumina NextSeq 500 (Illumina, San Diego, CA). Upregulated or downregulated genes were separately imported using NCBI gene ID as the identifier (*P*-value ≤0.05 were considered statistically significant). More than 300 differentially expressed genes (DEGs) were identified in latently infected TG). The bioinformatics program, Database for Annotation, Visualization, and Integrated Discovery (DAVID) identified biological processes associated with the DEG. Upregulated DEG were enriched for pathways that regulate neuronal synapses and survival. For example, G-protein subunit gamma 12 (GNG12), G protein subunit alpha Q (GNAQ), and phospholipase C (PLC) promote neuronal synaptic growth and are upstream of the phosphoinositide 3-kinases (PI3K)-AKT signaling axis. Notably, downregulated DEG were involved with ribosomal functions or promoted cell death, oxidative phosphorylation, and neurodegeneration, including the pro-apoptotic gene keratin 18 (KRT18). Furthermore, genes encoding cytochrome c oxidase subunits are known to regulate oxidative phosphorylation and neurodegeneration. Collectively, this study suggests that maintaining a latent infection in TG neurons is an activate process, and LR gene products directly or indirectly promote neuronal survival via upregulating prosurvival signals and impairing programmed cell death.

Financial Support: USDA NIFA 2020-06435 and 2023-07864

Key Words: bovine alphaherpesvirus 1 (BoHV-1), latent infection in sensory neurons, transcriptomics, differentially expressed genes that promote maintaining latency

General Abstracts: Session 46—Immunology 3

235 - Structural and antigenic characterization of *Babesia bovis* HAP2 domains.

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The tick-borne apicomplexan parasite *Babesia bovis* causes bovine babesiosis, which leads to enormous food and economic losses around the world. The existing resources to manage this disease are limited and have pitfalls; therefore, introduction of new strategies is urgently needed. *Babesia bovis* reproduces sexually in the midgut of its tick vector. The well-conserved ancient protein HAP2 plays a crucial role in the gamete fusion of this parasite and is a strong candidate for developing transmission-blocking vaccines. We previously demonstrated that immunization of cattle with full size *B. bovis* HAP2 blocks transmission of the parasite by *Rhipicephalus microplus*. Understanding the conserved structural features and antigenicity of HAP2 protein and its domains will facilitate developing effective methods to control pathogen transmission. In this study, we analyzed and compared AlphaFold2-predicted 3D structure of *B. bovis* HAP2 with the well-characterized crystal structures of HAP2 of *Chlamydomonas reinhardtii* and *Arabidopsis thaliana*. The comparisons and structural analysis resulted in the definition of 3 domains' sequences, fusion loops, and disulfide bonds in the *B. bovis* HAP2. In addition, recombinant versions of each 3 predicted HAP2 domains were recognized by antibodies from HAP2 immunized and transmission-protected cattle, confirming their antigenicity. Remarkably, domain II was highly recognized compared with the other 2 domains. This study introduces new directions in designing novel functional assays and improved vaccine design through targeting the HAP2 protein.

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Key Words: bovine babesiosis, *Babesia bovis*, HAP2, HAP2 domains, transmission-blocking vaccine

236 - Effect of vaccinating cows once at pregnancy check on clostridial antibody titers in calves at branding age.

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Clostridium spp. cause disease in cattle around the world, often affecting the young with rapidly fatal disease. Vaccination can mitigate the effects of clostridial infection. For other agents, vaccinating gestating cows with a 2-dose regimen has increased antibody titers in their calves. However, gathering and vaccinating cows twice is time consuming. Thus, producers often administer only single-dose vaccination. The objective of this study was to determine whether vaccinating gestating cows with a single dose of multivalent clostridial vaccine increases their calves' antibody titers against *C. septicum*, *C. novyi*, and *C. sordelli* at branding (45–90 d of age). A second objective was to evaluate the effect of cow titer at vaccination on calf titer. One hundred-eleven beef cows from a single ranch in 3 pasture groups were randomly assigned to receive either a multivalent clostridial vaccine or a saline injection at fall pregnancy check (vaccinates, n = 56; controls, n = 55). Antibody titers in cows before vaccination and calves at branding were measured using a microfluidic ELISA assay; titers were expressed as percent of negative control. The relationship between cow vaccination status and calf titer, and between cow titer at vaccination and calf titer, were assessed with linear mixed models, with significance set at $P \leq 0.05$. Cows had higher antibody titers than calves. In cows, *C. novyi* titers were 602.8 ± 123.9 (mean ± S.D.), *C. septicum* titers were 305.1 ± 58.8, and *C. sordellii* titers were 458.2 ± 77.0. In calves, *C. novyi* titers were 182.0 ± 50.4, *C. septicum* titers were 136.0 ± 39.6, and *C. sordellii* titers were 163.9 ± 41.8. A significant effect of cow vaccination on calf titer was not identified for *C. novyi* ($P = 0.200$), *C. septicum* ($P = 0.260$), or *C. sordellii* ($P = 0.994$). Regardless of cow vaccination status, the cow's titer at the time she was vaccinated had a significant effect on her calf's titer to *C. novyi* ($P = 0.008$), with higher cow titers associated with higher calf titers. In contrast, a significant effect was not seen for *C. septicum* ($P = 0.882$) or *C. sordellii* ($P = 0.197$). A single dose of multivalent clostridial vaccine given to beef cows at fall pregnancy diagnosis was not associated with significantly greater titers in their calves at 45 to 90 d of age. A significant positive association of cow titer with calf titer for *C. novyi* supports the concept that antibody concentrations in cows can influence passive immunity in their calves.

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Key Words: ELISA, *Clostridium sordellii, Clostridium septicum, Clostridium novyi*

237 - Repeated endotoxic challenges in lactating dairy cows under negative energy balance: Trained immunity or endotoxin tolerance?

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Postpartum dairy cows experience negative energy balance (NEB), often with endotoxemia, increasing their risk of metabolic and infectious diseases. Early health events can predispose cows to subsequent illnesses later in lactation. Although the metabolic effects of NEB are recognized, the impact of repeated inflammatory challenges during NEB is less clear. It is unknown whether repeated endotoxemia induces trained immunity (heightened response) or endotoxin tolerance (blunted response). This study aimed to determine how repeated endotoxemia during NEB affect inflammatory responses in dairy cows. Thirty-two multiparous lactating Holstein cows underwent a 4-period (P) study. Period 1 (7 d) collected baseline data with ad libitum feeding. In P2 (4 d), cows were feed-restricted to induce a NEB (−14 Mcal/d) and received an i.v. infusion of saline (SAL; n = 16) or LPS (1 µg/kg of BW; n = 16) on d 3. Period 3 (7 d) was a washout with full feeding. In P4 (4 d), the NEB protocol was repeated, and cows were rerandomized within their original groups SAL (S-) or LPS (L-) to receive a second infusion of SAL or LPS, resulting in 4 treatment groups: S-SAL, S-LPS, L-SAL, and L-LPS (n = 8/group). A mixed model using repeated measures over time, treatment, period, and their interaction as fixed effects and cow as a random effect was employed. The LPS increased vaginal temperature (1.0°C) in the first 6h. The response was more severe in L-LPS compared with S-LPS (1.82°C vs. 1.0°C). The LPS increased plasma LPS-binding protein (LBP) and haptoglobin 5.8- and 2.3-fold, respectively, compared with SAL. The L-LPS presented a faster increment (6 h) and quicker reduction (72 h) of LBP compared with S-LPS. Haptoglobin was 1.88-fold higher in S-LPS compared with L-LPS. The LPS increased plasma concentrations of IFN-γ, lPyes-10, MCP-1, IL-6, IL-10, IL-4, MIP-1α, MIP-1β, and TNFα. The L-LPS exhibited elevated concentrations of IP-10, MCP-1, MIP-1α, and MIP-1β compared with S-LPS and saline groups. Repeated endotoxemic events elicited an exacerbated febrile and cytokine response consistent with trained immunity. In contrast cows under 2 events of feed restriction and a single inflammatory challenge (S-LPS) exhibited attenuated inflammation and a blunted cytokine response. These findings suggest that NEB affects how the immune system adjusts to repeated endotoxin exposure, which can affect disease risk in dairy cows. The findings indicate that priming cows during the transition period may boost their defense against future endotoxemia.

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 and by the Michigan Alliance for Animal Agriculture (AA-24-034; Lansing, MI)

Key Words: trained immunity, endotoxin tolerance, negative energy balance

238 - ZFP36L1 negatively regulates autophagy to suppress murine norovirus replication.

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Norovirus is a leading cause of acute gastroenteritis worldwide, with the highest burden in children, and remains a major concern due to the absence of vaccines or specific therapies. Identifying a host antiviral factor that controls viral replication will be critical for advancing our understanding of norovirus pathogenesis and for guiding the development of novel therapeutic strategies. ZFP36L1, a member of the tristetraprolin family of RNA-binding proteins, has demonstrated antiviral activity against multiple RNA viruses including influenza A virus, flaviviruses, and coronavirus. However, its role in norovirus infection has not been previously evaluated. The present study employed murine norovirus-1 (MNV-1) as a surrogate model to investigate the antiviral role of ZFP36L1. RAW 264.7 and HEK293T cells were overexpressed or knocked down for ZFP36L1 using lentiviral transduction. Virus infection assays showed that overexpression of ZFP36L1 significantly suppressed MNV-1, whereas knockdown enhanced replication compared with control cells. Data were analyzed using paired *t*-tests, one-way ANOVA, and 2-way ANOVA, with $P < 0.05$ considered significant. These results demonstrate that ZFP36L1 is an important host restriction factor against norovirus. Mechanistic studies revealed that MNV-1 infection strongly induced autophagy, as indicated by Atg13 and LC3B upregulation, LC3-I to LC3-II conversion, and reduced p62 turnover. Pharmacologic induction of autophagy by rapamycin significantly increased MNV-1 titers, whereas inhibition with 3-methyladenine reduced viral replication, confirming that norovirus exploits the autophagic machinery for efficient replication. In contrast, ZFP36L1 overexpression suppressed basal, virus-induced, and rapamycin-induced autophagy, reducing Atg13/LC3 expression and increasing p62 accumulation. Modulation of autophagy did not alter ZFP36L1 expression, suggesting that ZFP36L1 functions upstream of autophagy to mediate its effect. Collectively, these findings establish that ZFP36L1 exerts antiviral activity against MNV-1 by inhibiting autophagy, a host process exploited by the virus for replication. This work highlights the role of ZFP36L1 in norovirus infection and expands its profile as a broad-spectrum antiviral factor. Future studies will focus on identifying the direct RNA or protein targets of
ZFP36L1, validating these findings in human intestinal models, and evaluating the therapeutic potential of ZFP36L1 modulation.

Financial Support: Lincoln Memorial University

Key Words: ZFP36L1, tristetraprolin, murine norovirus, autophagy, RNA-binding protein, host–virus interaction

239 - Of mice and cattle: Toward the development of mouse models for ruminant respiratory mycoplasmoses.

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Pneumonia due to *Mycoplasma mycoides* cluster agents and *Mycoplasma bovis* (*Mb*) constitutes a major threat to livestock health and agricultural economies worldwide. In vivo exploration of host-pathogen interactions for these bacteria has so far been hampered by challenges inherent to large animal models, including exorbitant costs, availability of immunological reagents, and access to appropriate large animal facilities. Early efforts were made to develop small animal models to study ruminant mycoplasmoses but were abandoned due to poorly reproducing characteristic disease seen in the natural host. However, these studies did not use appropriate routes of exposure. Here, using intranasal inoculation of mice with *Mb* or *Mycoplasma mycoides* ssp. *capri* (*Mmc*), we show that mice develop acute disease that is reminiscent of what is observed in ruminants. Infected mice display clinical signs such as piloerection, dyspnea, and lethargy, as well as lose significant body weight compared with sterile growth medium–instilled controls. Instillation with heat-killed *Mb* or *Mmc* does not recapitulate clinical disease. Histopathologic evaluation and immunophenotyping reveals a suppurative, neutrophil-rich bronchopneumonia for *Mb,* but primarily monocytic pneumonia and pleuritis for *Mmc*, mimicking similar presentation to those produced in the natural hosts. Pleuritis due to *Mmc* instillation was histopathologically observable even at 24 d post initial infection. Viable *Mycoplasma* can be recovered from lungs of infected animals, fulfilling Koch's postulates. Specific depletion of neutrophils, as well as broad depletion of Ly6C+ cells using monoclonal antibodies differentially affect bacterial burdens and disease severity, showing utility of the model for the study of host-pathogen interactions.

Financial Support: Funds supporting this research were made available by the Center of Excellence for Vaccine Research

Key Words: bovine respiratory disease, *Mycoplasma bovis*, *Mycoplasma mycoides*, mycoplasmoses, animal model

240 - Anti-inflammatory fecal microbiota transplant in neonates, but not adults, prevented lung damage in asthmatic mice.

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Dysbiotic gut microbiota can significantly influence the lungs through the bidirectional gut-lung axis. Previously, we showed that offspring of mice given human-derived fecal microbiota transplants (FMT) from allergic infants (INF-B) had decreased lung function and increased airway hyperresponsiveness (AHR) compared with control mice of the same genotype with anti-inflammatory commensal mouse microbiota (CM). Baseline lung mechanics in mice with INF-B microbiota was significantly impaired compared with mice with mouse microbiota with or without exposure to an allergen. We hypothesized that transfer of protective microbiota (CM) to INF-B-microbiota mice in the neonatal period, but not during young adulthood, would prevent lung damage and prevent AHR. In study 1, we cross-fostered C57BL6/J pups within 24 h after birth as follows: CM to INF-B (10 mice), INF-B to CM (19 mice), and CM to CM (14 mice), and compared them to age-matched mice reared by their own dams (12 mice/group). In study 2, we treated 7-wk-old mice carrying CM (11 mice) or INF-B microbiota (10 mice) with cefoperazone for 7 d and then orally gavaged them with the opposite microbiota. Control groups CM, INF-B, CM + antibiotics, INF-B + antibiotics had 10 mice per group. In both studies, lung function was evaluated at 11–12 wks of age by flexiVent phenotyping to measure AHR via challenge with increasing doses of the cholinergic agonist methacholine. Illumina sequencing of the 16S V4 region of the rRNA gene was used to analyze gut microbiota taxonomic composition in all mice to assess the efficacy of fecal transplants. We compared bacterial taxa between groups using principal component analysis (PCA), Shannon diversity indices, and similarity percentage (SIMPER) analysis. For SIMPER analysis, we identified the mean relative abundance of taxa that contributed 90% of the average dissimilarity between groups. Data are presented as medians with minimum and maximum values. For airway function, data represents mean ± SE from 9 to 21 mice/group for microbiota and 9 to 14 for lung function; data were analyzed with Kruskal–Wallis with Mann–Whitney for pairwise comparisons and Bonferroni-corrected *P*-values. In study 1, microbiota of cross-fostered mice had a microbiota composition similar to that of the foster dam, not the birth dam, based on PCA. Statistical significance for microbiome analyses were, PCA, $P < 0.05$, Shannon 0.005, SIMPER $P < 0.00005$. The CM pups reared by INF-B dams had increased AHR compared with CM pups reared by a different CM dam, $P < 0.05$. The INF-B pups reared by CM dams had AHR and lung function values similar to CM mice, and a trend to lower AHR compared with INF-B controls ($P = 0.078$). In contrast, in study 2, young adult CM mice transplanted with INF-B microbiota at 8 wk showed no difference in AHR compared with siblings given sham transplants. Study 2 mice retained their original respiratory phenotype with decreased lung function and elevated AHR despite effective transplant. Cross-fostering was successful as a noninvasive method of FMT at birth in this mouse model. Lung function impairment due to gut microbiota allergy agonists was ameliorated by early, but not late, transplant of protective taxa.

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Key Words: gut-lung axis, fecal microbiota transplant, lung function impairment, asthma, protective microbiota

General Abstracts: Session 47—Vaccinology 2

241 - Recombinant bacterin vaccines targeting class 1 viral fusion protein highly conserved regions.

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Developing inexpensive, easily manufactured, thermostable, variant-resistant vaccines for viruses with class 1 viral fusion proteins that can induce DIVA-friendly immune responses is challenging. We developed a new vaccine platform, killed whole cell (KWC) genome-reduced bacteria (GRB), in which vaccines are produced by expressing class 1 viral fusion protein (e.g., coronavirus spike, influenza virus HA) subunits on the surface of bacteria using a gram-negative autotransporter. Using GRB versus wild-type bacteria enhances the visibility of recombinant antigens expressed on the bacterial surface. *Hemophilus influenzae* has a trimeric autotransporter, Hia, with a structure that closely resembles the coiled-coil structures of the stem/stalk regions of class 1 viral fusion proteins, highly conserved regions that bind broadly neutralizing monoclonal antibodies and have been the target of efforts to develop universal vaccines, including universal influenza vaccines. We synthesized a plasmid with an expression cassette that has an inducible version of Hia. We designed antigens that include the stalk region, with added surface expression–promoting sequences, trimerization domains to stabilize the trimeric coiled-coil stalk, and immunomodulators (TLR agonists, noncognate universal T-cell antigens) to enhance and shape the immune response. We synthesized DNAs encoding those antigens and cloned them into the plasmid's Hia expression cassette. We transformed the plasmids into GRB, and then made candidate vaccines against the stem/stalk regions of coronavirus (porcine epidemic diarrhea virus and SARS-CoV-2) and H1N1 and H5N1 influenza viruses by growing the transformed GRB, inducing antigen expression, and inactivating the bacteria. The resulting recombinant bacterin vaccines specifically bound antibodies in flow cytometry experiments. Vaccinating mice with the vaccines induced antibodies that bound stalk peptides in ELISA. An H1N1 influenza recombinant stalk vaccine induced immune responses that neutralized both H1N1 and H5N1 virus in a hemagglutinin inhibition assay, suggesting that our new KWC/GRB vaccine platform offers a pathway to the development of a variant-resistant or potentially universal bacterin influenza vaccine. The platform has the potential to rapidly and inexpensively produce additional vaccines against other viruses with class 1 viral fusion proteins and, with additional engineering strategies, against other important agricultural and human pathogens.

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Key Words: vaccine, bacterin, influenza, coronavirus, variant-resistant, stem/stalk

242 - Immunogenicity characterization of SecVax, a combined acellular vaccine against *Shigella*, *Vibrio cholerae*, and enterotoxigenic *Escherichia coli*.

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Diarrheal diseases caused by *Shigella*, *Vibrio cholerae*, and enterotoxigenic *Escherichia coli* (ETEC) remain major public health burdens, causing more than 500 million diarrhea episodes and approximately 400,000 deaths annually. Despite the significant global disease burden, there are currently no licensed vaccines for *Shigella* or ETEC. Although oral cholera vaccines (OCV) are available, their protective efficacy remains limited among adults and little or none in very young children in endemic countries. Virulence heterogeneity and stimulation of strong immunity, particularly in young children, are the key obstacles to developing vaccines against *Shigella*, *Vibrio cholerae*, and ETEC. Therefore, innovative and broadly protective vaccine strategies remain urgently needed to reduce global morbidity and mortality caused by bacterial enteric pathogens. By applying a multiepitope fusion antigen (MEFA) vaccinology platform, we recently constructed epitope- and structure-based polyvalent proteins to induce cross-protective antibodies against heterogeneous *Shigella*, *Vibrio cholerae*, or ETEC strains. In this study, we combined a polyvalent *Shigella* MEFA protein and a cholera MEFA protein with 2 polyvalent ETEC MEFA proteins, examined antigen compatibility and broad antibody responses to the target virulence factors and toxins in mouse immunization, and then assessed mouse anti-adhesin and antitoxin antibody functions against the adherence or invasion from the adhesins or invasives and toxin enterotoxicity, thus assessing the potential application of a broadly protective vaccine against the 3 groups of bacteria. Data showed that mice intramuscularly immunized with the combined vaccine candidate (SecVax) developed antibodies to all the target virulence factors of 3 groups of bacteria and neutralized the enterotoxicity of ETEC toxins STa and LT. SecVax-induced antibodies significantly inhibited the invasion of all *Shigella* species and prevented the adherence of all important *Vibrio cholerae* serotypes and ETEC pathotypes. These results indicated that SecVax is broadly immunogenic and cross-protective against *Shigella*, *Vibrio cholerae*, or ETEC, suggesting SecVax can be a *Shigella*/cholera/ETEC combined vaccine against diarrheal diseases, and the MEFA platform demonstrates strong potential for vaccine development against heterogeneous pathogens or different diseases.

Key Words: *Shigella*, *Vibrio cholerae*, enterotoxigenic *Escherichia coli* (ETEC), combined vaccine, vaccinology platform, diarrhea

243 - Functional epitope mapping of *Vibrio cholerae* TcpA protein and cholera toxin.

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Cholera is a severe diarrheal disease of global importance, causing millions of cases and significant mortality each year. *Vibrio cholerae*, its etiological agent, initiates infection by colonizing the intestinal mucosa, a process critically dependent on the toxin-coregulated pilus (TCP). TcpA, the major TCP subunit, is essential for bacterial adherence and is conserved across O1 and O139 serogroups, making it a broadly relevant antigen. Due to its surface exposure and central role in pathogenesis, TcpA is a promising cholera vaccine target. Antibodies targeting TcpA have been shown to reduce colonization and protect animal models. In addition to TcpA, cholera toxin (CT), composed of an enzymatically active A subunit and a GM1-binding pentameric B subunit, is another major virulence factor. Using in silico prediction, we identified 11 B-cell epitopes from the A subunit and 6 from the B subunit. For TcpA, 12 continuous B cell epitopes, 6 each from Classical and El Tor variants, were identified. These epitopes were fused to the carrier protein CsaB to generate recombinant fusion proteins, which were expressed, purified, and validated by SDS-PAGE and western blotting. Mice were immunized intramuscularly with each construct, and sera were evaluated for anti-TcpA IgG levels and functional activity using bacterial adherence inhibition assays with Caco-2 intestinal epithelial cells. One-way ANOVA was used to assess statistical significance across groups. All tested TcpA epitopes elicited anti-TcpA IgG responses. Notably, antibodies against epitopes 2 and 6 of TcpA Classical and epitopes 4 and 6 of TcpA El Tor significantly inhibited adherence of *V. cholerae* Classical, El Tor, and O139 Bengal strains, respectively, with inhibition levels comparable to full-length TcpA proteins. Although all epitopes were immunogenic, only these 4 showed strong adherence-inhibitory activity. All 6 CT B subunit epitopes also elicited robust IgG responses in mice, while A subunit epitope characterization is ongoing. Functional validation of B subunit epitopes will be performed using a GM1 receptor-blocking ELISA, and A subunit epitopes will be tested via neutralization assays. These findings enhance our understanding of TcpA and CT-specific immune responses and identify functional epitopes that can be directly used in designing a cross-protective, epitope-based, multivalent cholera immunogen, which supports the advancement of protein-based cholera vaccine development.

Key Words: *Vibrio cholerae*, TcpA, cholera toxin, B-cell epitopes, protein vaccine

244 - Self-destructing attenuated adjuvant *Salmonella* (SDAAS) strains function as safe live adjuvants to enhance mucosal and systemic vaccine immunity.

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Safe and potent adjuvants are essential for next-generation vaccines, particularly for mucosal immunization, where conventional adjuvants are limited. We developed self-destructing attenuated adjuvant *Salmonella* (SDAAS), a live bacterial platform engineered for arabinose-dependent lysis, lipid A detoxification, truncated phase-locked flagellin, and disrupted *sifA* to ensure biosafety while engaging innate immune sensors. Three candidate strains (χ12612, χ12621, χ12626) were evaluated for containment, immune stimulation, and adjuvant potential using ovalbumin (OVA) as a model antigen. In vitro assays confirmed strict auxotrophy and significantly reduced endotoxin release compared with wild type ($P < 0.0001$, one-way ANOVA). Human embryonic kidney-Blue assays demonstrated robust activation of TLR4 and TLR5 pathways. In vivo, intranasal delivery was completely safe up to 108 cfu, and subcutaneous co-immunization with OVA plus SDAAS strains significantly increased serum anti-OVA IgG compared with OVA alone ($P < 0.0001$, 2-way ANOVA), reaching levels comparable to alum and Sigma Adjuvant System. Ex vivo assays showed that peripheral blood mononuclear cells from SDAAS-immunized mice secreted elevated IL-6 and TNF-α but minimal IL-10, supporting a proinflammatory bias. Transcriptomic profiling revealed that χ12621 induced the strongest response, with >1,900 differentially expressed genes enriched in ribosome, proteasome, oxidative phosphorylation, and antigen presentation pathways. These signatures, combined with cytokine and metabolic reprogramming, indicate induction of trained immunity, innate memory that amplifies secondary protection beyond the delivered antigen. Collectively, SDAAS strains are safe, self-limiting, and programmable adjuvants that integrate pattern recognition receptor signaling with trained immunity features, offering a versatile platform for next-generation mucosal and systemic vaccines in both human and veterinary medicine.

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Key Words: self-destructing attenuated adjuvant *Salmonella* (SDAAS), *Salmonella enterica*, vaccine adjuvant, innate immune activation, transcriptomics, ovalbumin

245 - Impact of an intranasal L-DBF vaccine on the gut microbiota in young and elderly mice.

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Sh*igella* spp. cause bacillary dysentery (shigellosis) with high morbidity and mortality in low- and middle-income countries. Infection occurs through the fecal-oral route and can be devastating for vulnerable populations, including infants and the elderly. These bacteria invade host cells using a type III secretion system (T3SS). No licensed vaccine yet exists for shigellosis, but we have generated a recombinant fusion protein, L-DBF, combining the T3SS needle tip protein (IpaD), translocator protein (IpaB), and the LTA1 subunit of enterotoxigenic *Escherichia coli* labile toxin, which offers broad protection in a mouse model of lethal pulmonary infection. The L-DBF vaccine protects high-risk groups, including young and elderly mice. Here, we investigated how the gut microbiota of young and elderly mice respond to intranasal L-DBF vaccination formulated in an oil-in-water emulsion (ME). Samples from lungs, small intestines, and feces were collected on d 14 after 2 or 3 doses of L-DBF in ME (n = 10/group). Elderly mice showed much lower gut microbial richness compared with younger mice, with age having a strong effect ($P < 0.001$), while treatment did not affect richness. For β-diversity, age again had the biggest effect ($P < 0.0001$), but treatment and its interaction with age also showed smaller, yet significant, influences. Specifically, elderly mice given 2 or 3 vaccine doses had notable shifts in gut microbiome composition, unlike young mice or elderly mice compared with each other within vaccinated groups. These findings provide insight into the communication between the intranasal mucosal surface following subunit vaccination and the microbiota at a distant mucosal site, thereby highlighting the effect of vaccination and the host's microbiome.

Financial Support: NIAID grants R01AI138970 to WLP

Key Words: shigellosis, intranasal vaccine, IpaB, IpaD, vaccine, gut microbiota

246 - Safety and immunogenicity of a novel planktonic and biofilm-targeting vaccine to prevent *Mesomycoplasma ovipneumoniae* infection in domestic lambs.

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Me*somycoplasma ovipneumoniae (M. ovi)* is a facultative pathogenic bacterium that contributes to economically significant respiratory diseases in domestic and wild sheep. There are currently no preventive strategies or treatments to combat *M. ovi.* We here developed an autogenic, multistrain *M. ovi* vaccine and tested the safety, immunogenicity, and efficacy in a vaccine-challenge experiment. Three genetically distinct *M. ovi* strains isolated from the respiratory tracts of local sheep flocks in Montana were grown as planktonic cultures or adherent biofilms and were formulated into 2 formalin-killed, Montanide-adjuvanted vaccines, termed the planktonic vaccine and the biofilm vaccine. Twenty-one 3-mo-old specific pathogen-free (SPF) lambs were randomly assigned to 3 groups of 7 animals and received 2 doses of saline, the planktonic vaccine, or the biofilm vaccine subcutaneously. Three weeks after the second dose, all 21 lambs were experimentally infected intrabronchially with the 3 *M. ovi* strains using an endoscope. Five weeks after the challenge infection, all animals were euthanized and necropsied. Vaccine administration resulted in mild swelling at the injection site and occasional elevated temperatures, but no significant adverse effects. Significant antibody responses were detected by ELISA in the vaccinated animals at 4 and 6 wk (2-way ANOVA, $P < 0.01$) post vaccination, with a trend for higher antibody levels with the biofilm vaccine. Animals in the saline group developed significant antibody responses (2-way ANOVA, $P < 0.01$) within 3 wk post challenge that remained lower than in the biofilm vaccine group. Five weeks after challenge, *M. ovi* antigen triggered significant proliferative responses (2-way ANOVA, $P < 0.01$) in blood lymphocytes from all animals, with no clear differences between the groups. None of the animals showed signs of clinical respiratory disease throughout the experiments, and at necropsy, no respiratory tract pathology was found. In summary, our study demonstrated that both planktonic and biofilm-based killed vaccines to *M. ovi* are safe and induce significant serum antibody responses in domestic lambs. In ongoing work, we are analyzing the efficacy of the vaccine by measuring the *M. ovi* colonization in the nasal cavity, other respiratory sites, and the lungs in all the lambs after infection using qPCR and culture methods.

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Key Words: *Mesomycoplasma ovipneumoniae*, vaccine, planktonic, biofilms, antibody

General Abstracts: Session 48—Virology 3

247 - Phosphotidylethanolamine modulates genotype 3 hepatitis E virus replication during pregnancy in rabbits.

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According to the World Health Organization, hepatitis E virus (HEV) infects approximately 20 million people globally each year with 70,000 deaths and 3,000 stillbirths. Approximately 30% pregnancy-associated mortality has been reported during the second and third trimesters in HEV-infected pregnant woman. Hepatitis E virus is known to replicate in liver and placental cells, but the role of the complex lipid metabolism in the liver or placenta in modulating HEV replication is unknown. By utilizing a rabbit strain of HEV (HEV-3ra), we defined the lipid profile of HEV-infected liver and placenta of pregnant rabbits recapitulating HEV infection during pregnancy. Liver and placenta tissues of both HEV-infected and control animals were stained to assess differences in lipid droplets. Free fatty acids (FFA) were quantified in the liver and placenta tissues. The FFA kinetics in serum was performed on d 0, 7, 14, 21, and 28 post-infection (dpi). Targeted lipidomics was performed for the lipidomic profile of liver, placenta, and sera collected from HEV-infected and control rabbits at 28 dpi. The PISD/PCYT2 gene, which is responsible for phosphotidylethanolamine (PE) expression in huh7-S10–3 human liver cells, was knocked out by using a CRISPR/Cas9 gene knockout system to determine the effect on genotype 1 (HEV-1) and genotype 3 HEV (HEV-3) replication in the knocked-out cells. Qualitative and quantitative analyses of lipid droplets in liver and placenta demonstrated the presence of a higher number of lipid droplets in HEV-infected rabbits compared with control rabbits. At 28 dpi, a higher concentration of FFA was found in liver tissues from HEV-infected rabbits. The FFA kinetics studied in sera revealed higher concentration of FFA with significant differences seen at 21 and 28 dpi. The lipidomic profile revealed significantly higher levels of total lipids in HEV-infected pregnant rabbits compared with noninfected pregnant rabbits. Phosphotidylethanolamines (PE) were differentially upregulated in serum (64), placenta (64), and liver (27), respectively, with 5 of them being common in all 3 different samples. Hepatitis E virus replication was significantly lower in the PISD and PCYT2 knocked out liver cells when compared with control cells. Therefore, our results indicate that PE play an important role in modulating HEV replication during pregnancy. The result from this study has important implications in understanding the mechanisms of pregnancy-associated adverse outcomes during HEV infection.

Key Words: hepatitis E virus (HEV), rabbit, lipid metabolism, phosphotidylethanolamine, pregnancy

248 - In vitro proof-of-concept evaluation of an enzyme-gated nanoparticle mRNA therapeutic platform for avian influenza virus.

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Avian influenza (AIV) poses a major global health and economic threat to the poultry industry. The disease can lead to severe illness and mortality in poultry, but current vaccines and antivirals are limited in the field. mRNA technology, successfully applied during the COVID-19 pandemic, offers a promising platform for rapid, flexible interventions. In this study we explored the therapeutic use of interferon-β (IFN-β) mRNA delivered via a novel virus-inspired nanoparticle system: liposomal metal-organic framework-nucleic acid nanocapsules (Lipo-MOF-NAN). This system enables controlled release through enzyme-gated release of payloads, extended shelf life and enhanced thermal stability, and reduced dosing requirements, positioning it as a valuable platform for both therapeutic and vaccine applications. Interferon-β, a type I interferon, is a key component of innate immunity that induces antiviral proteins to block viral replication. In vitro proof-of-concept experiments were performed using Madin–Darby canine kidney (MDCK) cells. All experiments were performed in triplicate, providing 3 biological samples per treatment group. Each biological sample was also performed in triplicate to ensure consistency and reproducibility. Statistical analysis was done using GraphPad Prism 8 software (GraphPad Software, San Diego CA). Data are presented as mean ± SD. Statistical significance was determined using one-way or two-way ANOVA, as applicable, with Tukey post hoc tests for multiple pairwise comparisons. Data were considered significant if $P < 0.05$ ($^{*}P < 0.05$; $^{**}P < 0.01$, $^{***}P < 0.0001$). Additionally, studies in a tumor-bearing mouse model confirmed successful IFN-β protein expression in vivo after delivery with Lipo-MOF-NAN. Cells transfected with IFN-β mRNA at different concentrations (1, 2, and 5 µg) demonstrated reduced replication of H3N8 influenza virus, as confirmed by qPCR. Parallel experiments using the Lipo-MOF-NAN system, with encapsulated IFN-β mRNA at 0.5 µ*M* and 5 µ*M*, also showed an effective dose-response of viral inhibition, supported by viral quantal assays and ELISA protein analysis. Overall, these findings highlight the potential of an innovative platform for treatment of avian influenza using Lipo-MOF-NAN as a stable and efficient delivery vehicle for mRNA therapeutics.

Key Words: nanoparticle, avian influenza, interferon-beta

249 - Emergence of turkey orthoreovirus as a cause of neurological disease in poults.

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Turkey orthoreoviruses (TRV) are non-enveloped, double-stranded RNA viruses with a 10-segment genome, classified within the genus *Orthoreovirus*. Turkey orthoreoviruses have been associated with diverse clinical syndromes in turkeys, including malabsorption, immunosuppression, pericarditis, myocarditis, osteoporosis, hepatitis, lameness, tenosynovitis, and viral arthritis. Here, we report the isolation and genome sequencing of TRV from the central nervous system of turkey poults submitted to the Penn State Animal Diagnostic Laboratory. Six submissions consisted of whole birds or bird heads from 2- to 8-wk-old poults representing 8 commercial flocks from the northeastern United States. All cases included a history of neurological signs such as prostration, ataxia, twisted necks, circling, and disorientation, affecting up to 10% of poults. Two submissions included 6 poults each, from which fresh brain, trachea, liver, and cecal tonsils were harvested and processed for virus isolation. Gross lesions were minimal, while histologic examination of brain sections revealed lymphocytic perivascular cuffing and gliosis, consistent with viral encephalitis. Virus isolation was achieved in cell culture and embryonated chicken eggs and confirmed by real-time RT-PCR and immunofluorescence. Infectious TRV was recovered from pooled brain tissues from 6 flocks. Whole genome sequencing was performed using sequence-independent single-primer amplification (SISPA) and the GridION nanopore platform. Alignment of reads generated complete genomes for all 6 isolates. BLAST analysis showed 89%–99% nucleotide identity across genome segments compared with GenBank sequences, with the M3 segment showing the lowest identity. Exploratory metagenomic sequencing did not detect other viral pathogens in the affected brains. Notably, in situ hybridization revealed TRV RNA in the brain, providing direct evidence of neurotropism. Taken together, the clinical presentation, histopathologic findings, recovery of infectious TRV, absence of alternative pathogens, and tissue localization of viral RNA strongly support its role as a causative agent of encephalitis in poults. These findings underscore the emerging neurotropic potential of TRV and the need for further investigation into its pathogenesis and broader implications for neurological disease in turkeys.

Key Words: avian reovirus, encephalitis, neurological disease, neurotropism, poults

250 - Toward a cell-free Marek's disease vaccine.

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Marek's disease virus (MDV) is a highly contagious herpesvirus that causes Marek's disease (MD) in chickens and poses a significant threat to poultry worldwide. Marek's disease is characterized by immunosuppression, paralysis, and tumors. Most licensed vaccines against MD are administered as cell-associated preparations that 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 was to understand the mechanisms involved in generating infectious cell-free virus (CFV) in cell culture, to develop more stable and cost-effective MD vaccines. We hypothesize that the conserved herpesvirus protein kinase (CHPK) acts as a master regulator of CFV production, influencing the subcellular localization of key viral proteins involved in MDV replication and transmission. Here, we examined the CHPK-regulated UL47 protein, which shuttles between the nucleus and cytoplasm, and is likely regulated by CHPK-mediated post-translational modifications affecting its subcellular localization. To achieve this, we first characterized the nuclear localization and nuclear export (NES) sequences within UL47. Mutagenesis was employed to generate mutations in MDV *UL47*. Transient transfection assays using fluorescently tagged UL47 identified specific regions within UL47 that are important for its subcellular localization in cells. Next, we deleted either the complete UL47 gene (vΔ47) or the putative NES (vΔ47NES) in MDV and characterized these viruses in vitro and in vivo. Using an experimental and natural infection model, we measured viral replication, oncogenesis, and transmission from chicken to chicken. Our results showed that chickens experimentally infected with wild-type (vWT), UL47-null (vΔ47), and UL47NES-null (vΔ47NES) exhibited similar replication and disease incidence. However, neither vΔUL47 nor vΔ47NES transmitted to contact chickens. This study demonstrated that UL47 and, more specifically, the UL47 NES are required for the transmission of MDV from chicken to chicken. These results suggest that the subcellular shuttling between the cytoplasm and nucleus of MDV UL47 plays a crucial role during chicken infection. Further studies are designed to investigate the role of UL47 in the generation of CFV, with the aim of developing novel MDV vaccines and therapeutics.

Financial Support: This work is supported by AFRI grant no. 2024-67015-42412 from the USDA National Institute of Food and Agriculture

Key Words: Marek's disease, chicken, transmission

251 - Molecular characterization of infectious bursal disease virus (IBDV) strains of genogroup A2B1 circulating in Delaware, Maryland, and Virginia from 2018 to 2023 uncovers an amino acid signature in the consensus sequence of the capsid hypervariable region that drives viral escape from neutralizing antibody binding.

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Infectious bursal disease virus (IBDV) causes a major immunosuppressive disease in chickens. In this study, we characterized the IBDV strains circulating in Delaware, Maryland, and Virginia (Delmarva) from 2018 to 2023. The sequence of the hypervariable region (HVR) of the VP2 capsid encoded by segment A, and a region of the VP1 polymerase gene encoded by segment B was obtained from 53 and 34 bursal samples, respectively. Phylogenetic analysis revealed that all the sequences belonged to genogroups A2 and B1, typical of US variant strains. An amino acid signature was identified in the HVR consensus sequence compared with the type-strain Delaware E (Del-E) consisting of amino acid substitutions S215N, S317R, G322E, and E323D. Strains with this signature were previously identified in 2007, in 25% of Delmarva IBDV sequences, and classified as clade 2 variants. Here, we found this signature had increased in prevalence and was present in 34/53 (64%) of the sequences from 2018 to 2023, including 13/17 (76%) of the sequences from 2023. The signature significantly reduced the virus neutralization titer of serum antibodies raised against Del-E ($P < 0.05$), suggesting the substitutions drive immune escape. Additionally, we detected amino acid substitutions in and around a key region of VP1 that correlates with virulence (residues 145–147), suggesting that different IBDV isolates could vary in pathogenic potential. Finally, 2/78 (2.5%) of the IBDV-positive bursal samples were also positive for avian reovirus (ARV), demonstrating that coinfection with multiple immunosuppressive viruses occurs in the field. These findings highlight the importance of ongoing IBDV surveillance.

Financial Support: This project was supported by Agriculture and Food Research Initiative Competitive Grant no. 2023-67015-39649 from the USDA National Institute of Food and Agriculture

Key Words: infectious bursal disease virus (IBDV), Gumboro, molecular epidemiology, surveillance

252 - Surveillance of avian metapneumovirus in live bird markets and domestic poultry farms in Pennsylvania.

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Avian metapneumovirus (aMPV) is an important pathogen of domestic poultry and wild birds, causing significant economic and ecological impacts. The virus is highly contagious and primarily associated with respiratory disease characterized by sneezing, nasal discharge, and periorbital swelling, often predisposing birds to secondary infections. Three subgroups are recognized: A and B, which are widely distributed across continents, and subgroup C, consisting of North American and Eurasian lineages, which was historically endemic in United States turkeys before eradication through targeted control programs. Since late 2024, aMPV has been detected in commercial poultry operations in the United States, affecting broilers, layers, and turkeys. Current outbreaks involve subgroups A and B, with subgroup A reported mainly in western regions. Subgroup B is circulating in the eastern United States, including Pennsylvania, where comprehensive surveillance and species-specific detection rate data remain limited. We conducted surveillance of aMPV in domestic poultry and live bird markets (LBM) across Pennsylvania and tested using 2 duplex aMPV rRT- PCR assays. Duplex I assay targets aMPV subgroups A and B, and duplex II assay targets aMPV subgroup C along with an internal positive control RNA. Between January and August 2025, 170 pooled domestic poultry samples were tested and 12 samples (7.1%) were positive for aMPV subgroup B (aMPV-B). Broiler chickens contributed the most positives (10/155; 6.5%), while guinea fowl had the highest proportion positive (1/7; 14.3%). From November 2024 to July 2025, 508 LBM pooled samples were tested, with 193 (38%) positive for aMPV-B. Chickens represented the largest number tested (n = 407), with 160 positives (39.3%), while turkeys had the highest proportion positive (7/11; 63.6%). Peak positivity was observed in the autumn season (LM3; September, October, and November months) with 47.1%. This ongoing research project indicates that although chickens provided the largest sample pool, turkeys and guinea fowl showed higher proportions of infection, suggesting these species may play a greater role in viral circulation. Findings highlight the need for expanded surveillance across avian species, particularly in LBM and during the autumn and winter months, to inform targeted prevention and control strategies.

Financial Support: This research project is funded by the Pennsylvania-PDA grant

Key Words: avian metapneumovirus, surveillance, live bird market, domestic poultry farm, reverse transcription real-time PCR, molecular biology

Featured Speaker: Session 43—AVEPM Schwabe Symposium

253 - Science reshaping the "social license to operate" on dogs with extreme conformation—The VetCompass story.

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Our human fascination with dogs led to more than 350 unique pedigree dog breeds invented over a few decades from a single species in the late 1800s. With their different understanding of welfare to today, those early inventors actively sought dogs with extreme physical mutations, such as brachycephaly, dwarfism, tailessness, and skin folds, to inbreed and fix into new breeds. Failure of current humanity to reject these extreme conformations includes failure of science to produce good evidence, normalization, disagreement between major dog welfare organizations, and confusion over the role of veterinarians on breed health. We will explore how VetCompass, a Big Data research program at the Royal Veterinary College, London, has worked to produce the necessary evidence, develop a new theory on innate health, and then instigate the organizational, legal, and social change needed to resolve a problem as complex as extreme conformation in dogs. VetCompass shares anonymized electronic clinical data from ~25% of UK veterinary practices and now includes more than 350 million clinical notes on more than 30 million animals (including 15 million dogs). These data have supported hundreds of undergraduate and postgraduate research projects to reshape the thinking of these researchers and their networks. VetCompass research has underpinned more than 160 peer-reviewed publications, with each accompanied by press releases for the general and social media, as well as an infographic. VetCompass evidence has led to a novel standard definition of extreme conformation that is internationally accepted and has supported national and international multistakeholder groups with agreed strategies on reforming extreme conformation. These include the UK Brachycephalic Working Group, the UK Legal Advisory Group on Extreme Conformation in Dogs, the International Collaborative on Extreme Conformation in Dogs, and the Royal Canin international "Choose Health" Coalition. VetCompass has led to a new theory, called "innate health," that is being embraced worldwide with the prospect of revolutionizing how the wider public think about and evaluate conformation in dogs. The UK parliamentary All Party Group Animal Welfare have released an "Innate Health Assessment" linked to current dog breeding legislation in England that gives a legal imperative to avoid breeding from dogs with extreme conformation. VetCompass is a story of science reshaping the "social license to operate" on dogs with extreme conformation.

Key Words: VetCompass, real-world, impact, welfare, legacy project, collaborative

Featured Speaker: Session 49—AVEPM Schwabe Symposium

254 - Evidence with a purpose: Stakeholder-driven research and preparedness for Japanese encephalitis and other emerging threats.

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This presentation will illustrate how stakeholder-driven research can directly shape policy and guide decision making, featuring examples from our work on Japanese encephalitis and other emerging threats such as New World screwworm. Japanese encephalitis virus (JEV), a mosquito-borne flavivirus and leading cause of viral encephalitis in Asia and the Western Pacific, is not currently established in the United States; however, the presence of competent vectors, amplifying hosts, and suitable environmental conditions signals a credible risk of introduction. Our group has developed an integrated, stakeholder-informed framework to assess, communicate, and mitigate this risk, leading to direct adoption of outputs by US and international animal-health authorities. We initially conducted qualitative and quantitative risk assessments to estimate the likelihood and pathways of JEV introduction into the continental United States. We then advanced this work through a semiquantitative model that stratified the country into 7 risk regions based on climate, domestic and feral swine density, and ecological suitability. The model evaluated multiple introduction routes, postentry transmission, and establishment probabilities, expert elicitation, and evidence from the 2021–2022 genotype IV outbreak in Australia. Findings identified the southern region from Texas to Virginia, including Mississippi, as the only area with very high overall risk, prioritizing it for enhanced surveillance and early detection efforts. Recognizing the central role of swine as amplifying hosts, we conducted a rapid systematic review of JEV infection dynamics in commercial and feral swine, which highlighted major evidence gaps, most notably, the lack of data on mosquito abundance and host feeding patterns on US swine farms. To address this, our team completed the first entomological survey of US commercial swine operations, sampling mosquitoes on 10 sow and wean-to-market farms in southern Iowa. The resulting datasets characterize vector diversity, blood-meal sources, and habitat features, providing an empirical foundation for targeted mosquito mitigation in livestock systems. By co-developing surveillance, risk assessment, and preparedness strategies with industry, producers, and regulatory partners, we ensure that scientific outputs translate into actionable decisions that strengthen animal-health policy, guide resource allocation, and improve national readiness.

Key Words: arbovirus, emerging diseases, Japanese encephalitis, preparedness, risk assessment

Session 49—AVEPM Schwabe Symposium

255 - Environmental prevalence of *Salmonella* Dublin in Midwest U.S. dairy-beef 2 operations: Associations with biosecurity and farm management.

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Salmonella enterica serovar Dublin (*S.* Dublin) poses significant risks to cattle health and public safety due to its host adaptation, antimicrobial resistance, and silent transmission. While its epidemiology is well characterized in dairy systems, limited information exists for U.S. dairy-beef operations. This study aimed to (i) design and validate a multiplex PCR (mPCR) assay for environmental detection of *S.* Dublin, (ii) estimate environmental prevalence on Midwest dairy-beef farms using mPCR and culture, and (iii) identify farm-level biosecurity and management practices associated with *S.* Dublin detection. We hypothesized that mPCR would outperform culture in detecting *S.* Dublin from environmental samples and that specific management and biosecurity factors would be associated with pathogen presence. The mPCR assay was developed using bioinformatic target selection, in silico PCR, and laboratory validation with pure cultures and spiked field samples. Subsequently, a cross-sectional study was conducted on 65 dairy-beef farms, where environmental boot swabs from calf housing areas and milk-mixing rooms were collected and tested using both mPCR and standard culture methods. Farm-level biosecurity and management data were obtained through a structured survey. Farm- and sample-level prevalences were estimated using Bayesian beta-binomial models, and associations between farm practices and *S.* Dublin detection were evaluated using multivariable negative binomial regression. The mPCR assay demonstrated high specificity and reliable detection in environmental samples. In field application, *S.* Dublin was detected on 35.4% (23/65) of farms using mPCR, compared with 6.2% (4/65) by culture. Frequent calf introductions, sourcing calves from auctions or dealers, and employee contact with outside livestock were significantly associated with increased detection. Lower biosecurity scores were positively associated with detection in univariable analysis but were not retained in the final multivariable model. Overall, the mPCR assay provides a sensitive tool for environmental surveillance of *S.* Dublin in dairy-beef systems, and results underscore the importance of strengthening biosecurity and implementing targeted surveillance to mitigate pathogen risk.

Key Words: *Salmonella* Dublin, PCR, prevalence, risk factors, dairy-beef

General Abstracts: Session 50—African Swine Fever

256 - Clinical profiling of African swine fever in domestic pigs: Toward an early diagnostic model in endemic settings.

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African swine fever (ASF) is a highly contagious and lethal disease of pigs caused by the African swine fever virus (ASFV), belonging to the family *Asfarviridae*. This study aimed to define the clinical profile of ASF in naturally infected pigs in Cameroon and to develop a diagnostic model for early recognition. Early recognition is crucial because farmers and veterinarians require practical tools that can be applied at the point of care to initiate rapid containment. An ambispective study (2013–2025) was conducted in ASF-endemic regions of Cameroon across 504 pig farms. Clinical data (signs and lesions) were collected using structured questionnaires that documented general (pyrexia, anorexia, weight loss, lethargy, dehydration) and system-specific signs (respiratory, gastrointestinal, neurological, dermatological, and hematological), following outbreak alerts of a hemorrhagic, highly lethal, and highly morbid disease. Laboratory confirmation involved the detection of ASFV nucleic acid by qPCR in blood and/or pooled tissue samples (lymph nodes and spleen), as well as the detection of anti-ASFV antibodies in serum by ELISA. A multivariate linear regression (F-statistics) model was developed from the clinical dataset. African swine fever was confirmed in 62% of farms by qPCR or ELISA, or both. Clinical data from more than 23,125 pigs were collected, and a regression analysis of 15 clinical signs yielded a significant result, $F(15, 95) = 6.573$, $P < 0.0005$, $R^2 = 0.405$. The model based on general clinical signs correctly identified 65.3% of positive farms; meanwhile, the optimized system-specific model, which combines predictive clinical signs with necropsy findings, detected more than 82% (range 65.3%–100%) of outbreaks. Integrating clinical and diagnostic indicators improved the surveillance and early detection of ASF. The proposed tool provides a practical point-of-care resource for early recognition in resource-limited settings, facilitating timely interventions and enhancing the resilience of the livestock sector in disease-endemic regions.

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Key Words: African swine fever (ASF), clinical profile, syndromic surveillance, early recognition, point-of-care tools, outbreak detection

257 - Piloting a national-scale biosecurity risk assessment and management agent–based model for building resilience against transboundary animal diseases.

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Although significant advances have been made in understanding the ecological dimensions of transboundary animal disease (TAD) transmission risk in agricultural and human systems, the integration of ecological dimensions with the social, behavioral, and economic dimensions requires fundamental transdisciplinary advances to improve our ability to assess and management TAD risk. This paper advances this transdisciplinary integration by piloting a national scale “Biosecurity Risk Assessment and Management Agent Based Model” (BRAM ABM) and applying it to forecast and control 2 types of TAD—African swine fever (ASF) and foot-and-mouth disease (FMD)—risk in the social-ecological context of securing the swine production supply chain. A BRAM ABM platform, programmed in C++ with GPU-enabled computation, leverages innovative artificial intelligence (AI) technology of deep reinforcement learned agent based models (ABM) to integrate ecological and social dimensions of TAD transmission dynamics. Dynamic data derived from online games is integrated in BRAM-ABM state, action and reward triplets of swine producers to predict the likely biosecurity adoption behaviors of supply chain network actors under alternate indemnity policies. Risk control strategies aimed at minimizing the risk from TAD outbreak are identified by testing alternate policy and behavioral response scenarios. We discovered that the low probability/high risk nature of ASF and FMD transmission processes lead to heightened importance of early detection and rapid control of a TAD. Each day of additional delay in the detection of the disease nearly triples socio-economic losses. Noncompliance with biosecurity risk control protocols by 5% of the pork producers within 100 miles of TAD introduction can lead to rapid diffusion of TAD vectors. An end-to-end BRAM ABM computational platform can not only inform the risk-reduction strategies of TAD transmission among pork supply chains of the United States, but the underlying integrated computational modeling approach can also be replicated in many other social ecological contexts to implement real-time TAD outbreak analytics and aid in the design of management strategies. This study advances the emerging field of integrated disease models that couple human behaviors and policy dynamics with traditional ecological process-based models of TAD risk.

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Key Words: artificial intelligence, biosecurity risk management, foreign animal disease, integrated modeling

258 - Enhancing biosecurity practices and outbreak preparedness for African Swine Fever in Puerto Rico.

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Following the re-emergence of African swine fever (ASF) in the Americas in 2021 after a 40-year absence, USDA-APHIS designated Puerto Rico as a Protection Zone. This underscored the need for large-scale, culturally tailored education efforts to enhance biosecurity awareness and outbreak preparedness. This study aimed to (1) implement outreach workshops on ASF and biosecurity, (2) collect and describe producer biosecurity plans, and (3) implement a multistakeholder tabletop exercise (TTX) to evaluate ASF preparedness. In 2024, 6 in-person workshops were held across Puerto Rico. Subsequently, 94 biosecurity plans were collected using the Secure Pork Supply framework by USDA Veterinary Medical Officers to document current biosecurity implementation. In 2025, a 1.5-d TTX engaged local, state, and federal stakeholders for a facilitated discussion centered on an ASF outbreak scenario. Feedback surveys from the workshops and the TTX, along with structured evaluations of participants' knowledge and perceptions during the exercise, were collected and analyzed using descriptive statistics and thematic analysis. In 2024, 92 people attended the workshops, and 38 completed postworkshop surveys. Overall, the management of biosecurity section was considered the most difficult section to complete (21%). Based on the biosecurity plans, the average herd size was 55 pigs (range: 1–665) and 78% of the sites were backyard, with 70% practicing waste-feeding. Most producers reported not taking specific measures to prevent contact with poultry (81%) or feral pigs (59%). In 2025, 26 people attended the TTX, with 15 completing surveys. Evaluators noted strengths in laboratory capabilities and technical federal expertise and identified gaps including Incident Command System coordination, depopulation planning, and producer readiness. The exercise was rated as highly useful (mean score: 4.71), with perceived ASF preparedness improving from 2.64 to 3.43 on a 5-points Likert scale. The workshops and biosecurity plans revealed gaps in biosecurity knowledge and implementation among Puerto Rican swine producers and highlighted the need for further educational opportunities. The tabletop exercise identified both institutional strengths and areas for improvement, particularly around producer readiness and interagency logistics. Together, these efforts advanced ASF preparedness and reinforce the need for continued education in the island's swine sector.

Financial Support: This project was funded by the USDA's Animal and Plant Health Inspection Service through the National Animal Disease Preparedness and Response Program, AP23VSSP0000C038

Key Words: African swine fever (ASF), biosecurity awareness, educational workshops, outbreak scenario

259 - Early detection of ASF: Comparing sampling strategies in growing pig farms.

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Early detection of African swine fever virus (ASFV) is essential for preventing large-scale outbreaks, yet the performance of within-farm sampling strategies during the early stages of infection remains poorly understood. Detection is particularly challenging when disease prevalence is low, animals are asymptomatic, or clinical signs are obscured by co-circulating pathogens. In this study, we evaluated 4 pen-level sampling strategies to detect ASFV infection within the first 14 d following the introduction of either a high or moderate virulence strain in suspected growing pig farms. Pens were selected using: (1) random sampling, (2) targeted sampling of pens with clinically affected pigs, and (3) 2 informative approaches based on estimated infection probabilities, (a) a sequential method ranking pens by descending viral load probability, and (b) a cluster-and-random method sampling from high viral load and low viral load risk clusters. Each strategy was tested across multiple sample sizes, and surveillance sensitivity was defined as the probability of detecting at least one ASFV-positive pig per farm. Sampling 30 pens with one pig per pen using the random or targeted approach resulted in the highest detection sensitivity, even under clinical uncertainty caused by other circulating pathogens. Conversely, sampling only 5 pens produced the lowest probability of detection. These findings provide evidence-based guidance for designing effective ASFV surveillance protocols in US swine systems and support broader preparedness planning for transboundary animal diseases.

Key Words: African swine fever, stochastic modeling, epidemiology

260 - Portable, smartphone-linked test for rapid field detection of ASFV.

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African swine fever virus (ASFV), a large double-stranded DNA virus, leads to a severe hemorrhagic disease in pigs, with mortality rates of approximately 100%. The rapid spread of ASFV across continents, the unavailability of a commercial vaccine, and its economic impact on the world swine industry point to the urgent need for rapid and field-deployable diagnostics. Conventional methods such as PCR and ELISA generate good sensitivity and specificity in laboratory settings, but are not suitable for use in field conditions because of their dependence on specialized equipment and trained personnel. To fill this gap, we developed a portable molecular diagnostic test platform based on a loop-mediated isothermal amplification (LAMP) assay with a clip-on detection device that interfaces with the image sensor of a smart phone to allow for real-time fluorescence-based detection. The assay targets the conserved p72 gene of ASFV and was optimized using synthetic plasmid DNA, followed by validation with ASFV p72-spiked swine serum. The test demonstrated a high analytical sensitivity of 10 copies/μL in buffer and copies/μL in swine serum samples as the limit of detection. The specificity of the LAMP assay was established against nontarget swine viruses, such as PRRSV, PDCoV, PEDV, and swine influenza A viruses with no instances of cross-reactivity noted. Amplification occurred in disposable reaction cartridges heated by a battery-powered unit while fluorescence signals were captured and analyzed through a PathTracker nanosensor system, which allowed for the rapid interpretation of amplification without laboratory support. Performance from the portable system was comparable to benchtop thermocyclers, validating its use for on-site ASFV detection. The total diagnostic process can be completed within a timeframe of 30 min and is ideally suited to remote field locations and resource-limited environments. This platform offers a practical and portable tool for ASFV field surveillance, enabling rapid decision making during outbreak scenarios.

Key Words: African swine fever virus (ASFV), loop-mediated isothermal amplification (LAMP), PathTracker nanosensor system, field deployable

261 - mRNA cocktail vaccination partially protects pigs from lethal challenge by African swine fever virus.

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African swine fever (ASF) poses a serious threat to the global swine industry, with significant economic repercussions. While live-attenuated vaccines offer efficacy, their safety concerns remain a major barrier. Subunit vaccines based on recombinant proteins or viral vectors are safer but often lack sufficient protective efficacy. mRNA vaccine technology offers several advantages, including safety, modular and adaptive antigen design, robust induction of both humoral and cellular immune responses, and rapid, cell-free manufacturing. Building on our previous work in ASFV antigen selection, engineering, and immune response profiling, here we evaluate the immunogenicity and protective efficacy of 2 mRNA cocktail vaccines in mice and pigs. Both formulations elicit strong antigen-specific immune responses in both species. Upon challenge with a high dose (1,000 HAD_{50}) of a virulent ASFV strain, vaccinated pigs exhibit partial protection against mortality. In one cocktail group, surviving pigs (40%) fully recover from clinical symptoms within 5 d of symptom onset and show low viremia and minimal viral shedding by the study's end. These survivors mount robust cytotoxic T cell responses, characterized by elevated serum TNF-α and IFN-γ following challenge, suggesting a potential role in protection. Our study represents a clear demonstration of protective efficacy against ASFV in pigs using an mRNA cocktail vaccine, providing a foundation for further development of a safe and effective subunit vaccine for ASF control.

Financial Support: This project was partially supported by Agriculture and Food Research Initiative Competitive Grant no. 2024-67012-42721 from the USDA National Institute of Food and Agriculture.

Key Words: ASFV, mRNA vaccine, protective immunity, challenge study, antibody response, cytokine

General Abstracts: Session 51—Salmonella 3

262 - Distribution and diversity of *Salmonella* serovars from beef cattle before and after processing in relation to human-derived serovars.

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Sa*lmonella enterica* remains a leading cause of foodborne illness globally, with cattle serving as an important reservoir. Understanding serovar distribution across the beef production continuum is critical for surveillance and control strategies. *Salmonella* isolate data were obtained from the National Antimicrobial Resistance Monitoring System (NARMS). We analyzed *Salmonella* isolates from cecal, HACCP, retail meat, and human clinical sources using multivariate approaches, including principal component analysis (PCA), multidimensional scaling (MDS), analysis of similarities (ANOSIM), and permutational analysis of multivariate dispersions (PERMDISP). Ordination analyses revealed clear ecological structuring of serovars by source. Human isolates were dominated by *Typhimurium*, *Newport*, and *Enteritidis* and showed the greatest diversity, consistent with multiple exposure pathways. Retail isolates formed a compact cluster overlapping with human isolates and were associated with *Montevideo* and *Infantis*, underscoring the role of contaminated meat as a transmission pathway. Cecal and HACCP isolates were the most homogeneous, clustering tightly with serovars such as *Cerro*, *Anatum*, *Kentucky*, *Mbandaka*, *Muenchen*, *Meleagridis*, and *Muenster*, reflecting stable pre-harvest reservoirs. ANOSIM confirmed significant ecological separation among sources ($R = 0.512$, $P < 0.001$), while PERMDISP revealed significantly greater variability among human isolates compared with retail, HACCP, and cecal sources. *Salmonella* serovar distributions are shaped by source, with human and retail isolates aligning closely, cecal and HACCP isolates reflecting homogeneous cattle-associated pools, and human cases exhibiting the broadest serovar diversity. These findings highlight retail meat as a critical transmission link, the value and limitations of regulatory surveillance, and the need for integrated One Health monitoring to capture the full ecology of *Salmonella* across the food chain.

Financial Support: National Cattlemen’s Beef Association

Key Words: *Salmonella enterica*, cattle, retail meat, human salmonellosis, PCA, MDS

263 - Comparison of bacterial fitness and macrophage invasion and replication among *Salmonella* serovars recovered from feedlot cattle.

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Salmonellosis is a leading foodborne illness, and cattle lymph nodes are recognized as one of the reservoirs that can introduce *Salmonella* into ground beef. *Salmonella* that cross the intestinal barrier of cattle may colonize peripheral lymph nodes by using macrophages as transport vehicles. The objective of this study was to evaluate serovar-specific differences in bacterial fitness and macrophage survival, which may contribute to lymph node colonization. A total of 127 *Salmonella* isolates representing 8 serovars (Anatum, Cerro, Kentucky, Lubbock, Montevideo, Muenster, Newport, and Virginia) were previously recovered from cattle feces, hides, lymph nodes, and the feedlot environment as a part of a longitudinal study. The isolates were selected to ensure a balanced distribution across serovars and sample types. Bacterial fitness was measured using the Bioscreen C Automated Microbiology Growth Curve Analysis System in technical and biological triplicates, and data were analyzed with a nonlinear regression 3-parameter Gompertz model in STATA. A subset of 27 lymph node isolates (Anatum, Cerro, Kentucky, Lubbock, and Montevideo) were evaluated for invasion and replication in J774A (ATCC TIB-67) macrophages in technical and biological triplicates. Assay controls included a phoPQ wild-type strain as the positive control and the corresponding phoPQ knockout strain as the negative control, as the phoPQ system enhances *Salmonella* survival and replication within macrophages. Invasion was assessed at 2 h and replication at 24 h. Linear regression models were run in Stata using the log10 cfu/mL results obtained from the 2 time points to assess differences by serovar. Montevideo isolates exhibited significantly ($P < 0.05$) higher growth rates, while Newport isolates showed significantly ($P < 0.05$) lower rates. Kentucky isolates demonstrated reduced invasion into macrophages, whereas Anatum isolates demonstrated reduced replication. All serovars, except Kentucky, displayed increased invasion compared with the control strains. These results indicate that *Salmonella* serovars differ in bacterial fitness and macrophage survival, which may influence lymph node colonization in cattle. Ongoing work includes expanding macrophage assays to additional serovars and sample types. Future studies will assess invasion of these serovars in bovine intestinal epithelial cells.

Financial Support: National Cattlemen’s Beef Association

Key Words: macrophage, *Salmonella*, cattle, lymph node, fitness, serovar

264 - Lactulose metabolism in *Salmonella*: Transcriptomic insights and nutrient-based modulation of biofilm and colonization potential.

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Lactulose, a synthetic disaccharide widely used as a prebiotic and therapeutic agent, has traditionally been considered fermentable only by beneficial gut microbes. Recently, we reported that non-typhoidal *Salmonella* (NTS), including *Salmonella* Kentucky, can utilize lactulose as a sole carbon source for growth, suggesting a conserved, serotype-independent metabolic capability in lactulose utilization. However, the molecular pathways enabling lactulose catabolism in NTS remain uncharacterized. In this study, we used *S.* Kentucky as a model to identify candidate lactulose catabolic pathways and explore potential effects on biofilm and colonization of *Salmonella* in vitro. Seven epidemiologically distinct *S.* Kentucky strains (ST198 and ST152) were cultured with lactulose or glucose (control) under standardized conditions, and total RNA was sequenced. The sequence reads were processed using fastp, aligned with Bowtie2, and gene counts generated via featureCounts. Differential expression analysis was performed using edgeR, and functional predictions were conducted using STRING. Lactulose-induced modulation of biofilm phenotypes was evaluated using Congo Red/Coomassie Brilliant Blue agar, Calcofluor agar, and microtiter plate assays. Among 4,499 protein-coding genes, 846 were upregulated and 733 were downregulated in response to lactulose, highlighting extensive lactulose-induced transcriptional remodeling. Transcriptomic profiling revealed induction of genes consistent with a functional upper pathway for lactulose uptake and hydrolysis, involving several novel transporters and hydrolases not previously linked to this metabolism. In addition, downstream metabolism through galactose and fructose catabolism showed evidence of both canonical and noncanonical routes, underscoring the metabolic flexibility of *Salmonella*. Phenotypic assays further demonstrated that lactulose impairs biofilm formation across multiple strains, suggesting a conserved effect. Current investigations are focused on whether lactulose also modulates motility and adhesion, with implications for colonization potential. This study provides the first transcriptomic characterization of lactulose metabolism in NTS and establishes a novel link between nutrient utilization and biofilm modulation. These findings lay the foundation for developing nutrient-based strategies to control *Salmonella* persistence and colonization in diverse ecological niches.

Financial Support: This study was funded in part by the Texas Tech University School of Veterinary Medicine (TTU-SVM).

Key Words: *Salmonella,* lactulose, metabolism, transcriptomics, biofilm

265 - Interrupting microbial cross-talk: Targeting quorum sensing mechanism to control *Salmonella* virulence across the One Health lens.

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S*almonella* remains a leading cause of foodborne gastroenteritis worldwide, posing a significant threat to both human and animal health. Poultry and poultry-derived products are recognized as the primary sources of transmission, linking agricultural production systems directly to public health outcomes. The increasing prevalence of antimicrobial-resistant (AMR) *Salmonella* has rendered conventional antibiotic therapies less effective, underscoring the urgent need for innovative, antibiotic-sparing interventions aligned with the One Health paradigm. This study investigates the potential of small-molecule inhibitors that target quorum sensing (QS)—a bacterial communication system mediated by autoinducer-2 (AI-2)—as a novel strategy to attenuate *Salmonella* virulence. A high-throughput screen of approximately 2,000 small molecules was conducted to evaluate their ability to suppress AI-2 production in *Salmonella enterica* without inhibiting bacterial growth. Overnight cultures were incubated with 1 µL of each compound for 6 h at 30°C, and cell-free supernatants were analyzed using the *Vibrio harveyi* BB170 bioluminescence assay to quantify AI-2 activity. Ten compounds achieved > 95% inhibition of AI-2 signaling without affecting bacterial viability. These top candidates were further characterized for their biological effects. All 10 small molecules inhibited biofilm formation by 95–100% and significantly downregulated the expression of key genes involved in quorum sensing (*luxS*), biofilm formation (*bcsA*, *csgD*), motility (*fliC*, *motA*), and virulence (*hilA*, *invF*). Hemolysis assays revealed no red blood cell lysis at concentrations up to 1 µM, and cytotoxicity screening demonstrated that 6 of the 10 compounds exhibited no adverse effects on human colorectal carcinoma (Caco-2) cells at 20 µM. Future work will focus on evaluating the non-toxic lead compounds for their ability to inhibit bacterial adhesion and colonization in human intestinal epithelial cells, followed by in vivo validation in chickens as a relevant food animal model. Together, these findings demonstrate that AI-2 quorum sensing inhibitors effectively disarm *Salmonella* without exerting selective pressure for resistance. This approach offers a promising antibiotic-alternative strategy that aligns with the One Health framework by mitigating zoonotic transmission risks and promoting safer, more sustainable food systems.

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Key Words: One Health, quorum sensing, *Salmonella*

266 - A new integrated approach for reducing *Salmonella* contamination in pig abattoirs.

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Salmonellosis is a major foodborne threat worldwide, with pigs being one of the main sources of human infection. Pork contamination at abattoirs is strongly linked to the presence of *Salmonella* in pigs' feces at slaughter, making the prediction and mitigation of shedding crucial for food safety. In this study, a predictive model was developed to estimate the risk of *Salmonella* shedding upon arrival at the slaughterhouse. Thirty batches of 50 pigs each from different fattening units were monitored through serology, pooled fecal samples, and farm-level biosecurity data collected one month before slaughter. Colon content samples were then obtained from the same pigs at the abattoir. Using random-effects logistic regression, the model achieved good predictive accuracy (AUC = 0.76; sensitivity = 71.6%; specificity = 73.6%). Key risk factors included seroprevalence, the percentage of *Salmonella*-positive pens, and internal biosecurity scores. These results show that predictive tools can identify high-risk batches, providing a framework for targeted interventions. Building on this, the efficacy of organic acids was assessed. The administration of esterified formic acid (30% formic acid, 10 kg/1,000 L) in drinking water during the 5 d before slaughter was tested in 3 *Salmonella*-positive farms. Compared with controls, treated pigs showed a significantly lower prevalence of shedding (20% vs. 50%; $P < 0.001$), with the odds of shedding being 5.63 times higher in the control group. The intervention prevented 82.2% of potential shedders and reduced bacterial loads, demonstrating its effectiveness as a pre-harvest measure. A second strategy, applied at the abattoir by supplementing lairage drinking water with the same organic acid, also reduced shedding, with prevalence decreasing from 60.7% in controls to 44.3% in treated pigs ($P < 0.01$). After adjusting for clustering, control pigs were 2.75 times more likely to shed *Salmonella*, suggesting a reduction in shedding of up to 63.6%. Together, these results illustrate a complementary framework: predictive modeling identifies high-risk batches, while the use of organic acids—applied either on-farm or at the abattoir—provides effective mitigation. Importantly, combining both approaches may yield synergistic benefits, reducing *Salmonella* shedding at multiple points in the production chain and contributing to safer pork and improved public health.

Key Words: salmonella control, pig, abattoir, prediction model, organic acid, public health

267 - Codesigning evidence-based interventions for *Salmonella* control with Irish stakeholders.

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S*almonella* remains one of the most important foodborne zoonoses in the EU, with pigs representing the primary source of human infection in Ireland. Despite the existence of a national monitoring and control program, progress in reducing infection levels has been limited. Across the island of Ireland, 28 farrow-to-finish pig farms were selected. On each farm, samples of feces, dust, water, and feed were taken across all production stages for *Salmonella* isolation, alongside testing for *Lawsonia intracellularis* and *Brachyspira* spp. Each farm underwent a BiocheckUGent biosecurity assessment, a *Salmonella* risk evaluation, and abattoir serology data review. Twenty-seven farms have completed sampling, with the proportion of *Salmonella*-positive samples per farm ranging from 4% to 57%. The most prevalent serotypes were *S*. Rissen (20%), monophasic *S*. Typhimurium (16.7%), *S.* Derby (15.6%), *S*. Typhimurium (15%), and *S.* Panama (11.7%). Dust samples were positive for *Salmonella* on 11 farms. All farms tested positive for *Lawsonia*, while *Brachyspira* spp. were detected on 22 farms. Half of the participating farms will implement control plans developed through a codesign with stakeholders, and half will be monitored as controls. Planned measures include enhanced cleaning and disinfection protocols, organic acid supplementation, *Salmonella* vaccination, and disease management based on identified vulnerabilities. Both groups will undergo regular sampling over the next 3 years to monitor *Salmonella* prevalence to assess intervention effectiveness. Concurrent smaller studies will be conducted to evaluate the effectiveness of the cleaning and disinfection protocol by assessing pathogen loads at defined set points within the pens—before cleaning, after washing, and after disinfection. These studies will target multiple pathogens besides *Salmonella*, including PRRS virus, PCV-2, Influenza A, and *Enterobacteriaceae*, using PCR, ATP bioluminescence assays, and RODAC plates. This approach aims to generate evidence-based, stakeholder-supported strategies to reduce a persistent public health risk while improving overall pig health and sustainability.

Financial Support: This material is based upon research supported by the Department of Agriculture, Food and the Marine under Grant Award No. 2023RP940

Key Words: *Salmonella*, pig, codesign, control, zoonosis

General Abstracts: Session 52—Immunology 4

268 - Host response of conventional Lohmann select layer chicks to avian segmented filamentous bacteria post hatching.

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We previously developed a treatment using avian-specific segmented filamentous bacteria (SFB) for newly hatched chicks to promote gut maturation. This treatment addresses the lack of contact between newly hatched chicks and their progenitors in industrial settings. The SFB spores were derived from the ileum of Dekalb White Leghorn chickens (D-SFB) and demonstrated an ability to enhance immune maturation in specific pathogen-free (SPF) Leghorn layers and commercial Ross 308 broilers. Testing this treatment in conventional and various commercial chicken lines is essential for its broader applicability. Lohmann Select Layer (LSL) chickens are predominantly used in US commercial laying farms. Therefore, this study aimed to test the D-SFB treatment efficacy in LSL birds obtained from commercial poultry facilities. Day-old LSL chicks were orally treated with D-SFB, and a non-treated control group (CON) was included, with both being housed in separate rooms. Samples of ileal and cecal tissues and contents were collected after euthanasia. The presence and levels of SFB were tracked weekly for a month using qPCR and microscopy. The expression of homeostatic cytokines, tight junction proteins, and antimicrobial peptides in the distal ileum was assessed using reverse transcription qPCR (RT-qPCR). Additionally, resistance to bacteria was tested using in vitro bactericidal assays for ileal tissues. Differences among treated groups were assessed at each time point with multiple *t*-tests applying Holm-Sidák's correction for multiple comparisons. $P < 0.05$ was considered as significant. The D-SFB treatment significantly increased the colonization of SFB in the ileum of LSL chickens and conferred antimicrobial properties against avian pathogenic *Escherichia coli* and *Salmonella* strains, as demonstrated in vitro. Expression levels of homeostatic cytokines IL-10 and IFNγ, as well as the Th17-associated cytokine IL-17F and Treg-associated cytokine CTLA-4, increased. Genes associated with mucin production (*muc2*) also showed elevated expression. An increase in goblet cells in D-SFB-treated birds was confirmed through immunohistochemistry analysis. Overall, the D-SFB treatment successfully promoted gut maturation and resistance to pathogenic bacteria in conventional LSL chicks after hatching. This treatment is beneficial for application in poultry farms and eliminates the need for multiple SFB-based products derived from different genetic lines.

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Key Words: segmented filamentous bacteria, gut maturation, chicken, posthatching, poultry industry

269 - Immunogenic protein profiling of *Mycoplasma capricolum* ssp. *capripneumoniae* using proteomic techniques.

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Contagious caprine pleuropneumonia (CCPP) is a highly infectious and devastating respiratory disease of goats and some wild ruminant species recognized by the World Organization for Animal Health (WOAH). Its recognition as a significant threat emphasizes the need for sustained research, surveillance, and control efforts to mitigate its impact on the worldwide goat population. The CCPP is typically caused by *Mycoplasma capricolum* subspecies *capripneumoniae* (Mccp), which belongs to the Mycoplasma mycoides cluster. The Mccp whole-genome sequences enable novel protein discovery and functional characterization through proteomics, which is the large-scale study of proteins and their functions. This experiment aimed to identify the potential immunogenic proteins of the Mccp Pakistan strain. A comprehensive proteome analysis of Mccp was performed using a combination of techniques, including protein extraction by sonication, SDS-PAGE, and 2-dimensional electrophoresis (2-DE) for protein separation and visualization, immunoblotting, and MALDI-TOF MS for protein identification. The proteome analysis revealed 3 immunogenic protein bands with molecular weights ranging from 30 to 100 kilodaltons (kDa) were found by 1-dimensional SDS-PAGE. Protein spots were detected by western blot analysis and MS in Mccp whole cell proteins (WCP) by 2-DE, in which 21 immunogenic proteins with molecular weights ranging from 22 to 74 kDa were identified using anti-goat anti-IgG horseradish peroxidase conjugate antibody. Most of the immunogenic proteins of Mccp and protein sequences of other closely related Mycoplasma species, including *Mycoplasma leachii*, *Mycoplasma mycoides* ssp. *mycoides* small colony type (MmmSC), and *Mycoplasma capricolum* ssp. *capricolum* (Mcc), indicate a high degree of sequence similarity and potential antigenic conservation among these species, using NCBI protein BLAST. This suggests that the predominant immune-stimulating WCP in Mccp may be used to develop potent vaccines and efficient diagnostic tests.

Financial Support: This research work was supported by PAK-US Science and Technology Cooperation Program Phase-7, 2017.

Key Words: *Mycoplasma capricolum* ssp. *capripneumoniae*, whole cell protein, 2-dimensional electrophoresis, immunoblotting, immunoproteomics

270 - Single-cell transcriptomic profiling of immune responses in resistant and susceptible sheep to *Haemonchus contortus* infection.

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Resolution of infection with hematophagous gastrointestinal nematode *Haemonchus contortus* in small ruminants is characterized by reduced worm burden and rapid immune activation, leading to parasite expulsion. As such, increased survivability is predicated on the early signaling of the immune response. 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, resulting in increased worm burdens and overall mortality rates. While rapid Th2 responses are implicated in parasite resistance, the frequency and diversity of immune cell populations contributing to a resistance phenotype in sheep remain uncharacterized. To investigate immune cell heterogeneity in relation to parasite resistance, peripheral blood mononuclear cells (PBMC) were isolated from parasite-naïve and *H. contortus*-primed SUF and STC for single-cell RNA sequencing. Library preparation was generated using the Evercode split-pool combinatorial barcoding, and data were processed using the Parse Biosciences Pipeline (v1.3.1, Parse Biosciences) and analyzed using Seraut v5 (Satija Lab). Cell clustering, annotation, and differential gene expression analysis were performed to identify transcriptomes associated with phenotypic differences between infection status and breed. Differential gene expression analysis within clusters identified distinct cell types in the PBMC dataset. In total, 16 cell types were identified, including T cells, B cells, monocytes, dendritic cells, and natural killer cells. Additionally, rare cell types such as low-density eosinophils and neutrophils were identified, revealing the novel characteristics of the ovine immune cell type signature. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses were performed within cell type to identify biological pathways within a disease phenotype. Profiling immune cell types and mapping the ovine cell landscape associated with resistance will improve our understanding of host-parasite dynamics. Collectively, comprehensive characterization of immune cell populations will inform improved parasite control strategies in susceptible livestock.

Key Words: helminth, transcriptome, host-parasite interactions

271 - Maternal colostral cells orchestrate intestinal immune and barrier gene networks in neonatal lambs.

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Early-life immune protection in ruminants relies mainly on maternal immune components provided through colostrum. Traditionally, its benefits were attributed to passive IgG transfer, but other bioactive components also support neonatal health and development. Chief among these are colostral cells, whose importance has been demonstrated in human and rodent models. This study investigates how maternal colostral cells influence neonatal lamb mucosal health by examining structural and functional differences of Peyer's patches using an ewe twin-lamb model coupled with single-nucleus RNA sequencing. Twin lambs (n = 2) from a single ewe were fed either cell-rich colostrum (CRC) or cell-free colostrum (CFC). Cell pellets were prepared from the ewe's colostrum and from ileal and jejunal Peyer's patches collected from each lamb at d 7 (n = 4 samples). Nuclei were processed into libraries using the 10x Genomics GEM-X v4 kit and sequenced on NovaSeq X Plus, targeting 20,000 nuclei per sample and 50,000 reads per nucleus. Raw reads were aligned to the ovine transcriptome via STARsolo, and gene count matrices were processed in R using Seurat. Gene expression enrichment analysis was performed using Enrichr, and intercellular communication was inferred using the CellChat package. The CRC-lamb tissues exhibited ileal epithelial expansion with increased T and B cells in the jejunum relative to CFC-lamb. Additionally, B cells, T cells, and enterocytes in CRC lamb tissues showed robust transcriptional activation in genes enriched for adaptive immune pathways, including BCR signaling, Th17 differentiation, and PD-1/PD-L1 signaling, particularly in the jejunum. Tracing analysis identified colostral T cells with a memory-like transcriptional profile trafficked exclusively to the CRC lamb jejunum. Compared with CFC-lambs, CRC-lamb tissues also displayed a significantly expanded intercellular communication, with highly expressed genes enriched for GALECTIN and adhesion pathways, indicating enhanced tolerance and homeostasis. Overall, maternal colostral cells have the potential to impact neonatal mucosa by orchestrating gene transcription, which may support neonatal immunity and epithelial integrity, both essential for early life health. The study provides empirical evidence advocating changes in colostrum management methods that preserve cell viability and improve neonatal health. Future studies in larger cohorts and multiple time points are required to validate these effects.

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Key Words: colostrum, Peyer's patches, neonatal mucosal immunity, maternal microchimerism, single-nucleus RNA sequencing

272 - Unraveling the cellular complexity of ovine colostrum using single-nuclei RNA sequencing.

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Colostrum supplies newborn mammals with various immunologically active components, including immunoglobulins and maternal cells. While the significance of colostral cells (CC) in neonatal health and immune development has been demonstrated in ex vivo studies, their relevance to field management remains largely overlooked because widely employed colostrum handling methods (e.g., freezing, heat treatment) adulterate CC. Thus, research efforts are needed to guide adjustments in colostrum management practices that preserve CC integrity. As the first step toward this goal, this study provides a comprehensive analysis of CC of the ovine colostrum (OC; n = 2) via single-nucleus RNA-sequencing (snRNA-seq). Single nuclei were extracted from CC pellets prepared through centrifugation immediately after parturition. snRNA-seq was done with 10X Genomics 3′ GEM-X kit, targeting 20,000 nuclei per sample and 50,000 reads per nucleus. An optimized STARsolo pipeline was used for mapping raw reads to the ovine genome. Gene counts, cell type annotation, cell cycle scoring, and differentially expressed gene (DEG) analysis were done using Seurat. Gene expression pathway enrichment analysis was done with clusterProfiler, and intercellular communication was determined using CellChat. The OC harbors heterogeneous cell populations, including multiple epithelial subtypes, macrophages, dendritic cells, T cells, progenitor cells, and a neuron-like population. Compared with the rest of the cell types, DEG of secretory epithelial cells were enriched in nutrient transport pathways, such as galactose metabolism. The DEG in specialized epithelial cells showed enrichment in tight junction, adherens, and cell adhesion pathways. The DEG in immune cells displayed activation of MAPK signaling, lysosomal function, and antigen presentation. Intercellular communication analysis revealed active PECAM1, SPP1, TNF, EGF, and FN1 signaling. Most CC were in a nonproliferative state, except T cells, which exhibited distinct mitotic activity, confirmed by the high expression of cell proliferation markers. Active transcriptional signaling and functional specialization among the CC populations highlighted the potential role of CC in maintaining intestinal epithelial integrity and supporting immune responses in neonates. Future studies with larger sample size, and multiple lactation stages are needed to validate the study outcomes and capture inter-individual variation.

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Key Words: ovine colostrum, maternal cell, snRNA-seq, neonatal heath and development

273 - Effects of Bd metabolite prophylaxis treatment on amphibian host resistance and tolerance to the deadly chytrid fungus *Batrachochytrium dendrobatidis*.

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Chytridiomycosis, caused by the aquatic fungal pathogen *Batrachochytrium dendrobatidis* (Bd), is a leading driver of global amphibian biodiversity loss. The fungus embeds in amphibian skin, disrupting osmoregulation and causing electrolyte imbalance and death. This devastation has prompted research into novel conservation tools, including wildlife vaccination, to protect individuals and sustain populations. Prophylactic exposure to non-infectious, water-soluble Bd metabolites has been shown to reduce subsequent infection intensity in naïve amphibians. However, prior vaccine trials were limited, focusing only on resistance effects in a few species (*Osteopilus septentrionalis* and *Pseudacris regilla)* from warm, humid regions. Here, we test how different doses and durations of Bd metabolite prophylaxis influence resistance and tolerance in naïve Indiana gray treefrogs (*Hyla versicolor/chrysoscelis*). Using a 5 × 5 response-surface design, we crossed metabolite concentration and exposure duration. Individuals (n = 40 per combination) were exposed daily to one of 5 concentrations or a water control for one to 16 d, then challenged with live Bd and monitored weekly for body condition, symptoms, and infection. Mixed-effects linear and Cox proportional hazards models evaluated vaccine effects on resistance (infection intensity) and tolerance (growth, mortality, and clinical signs). Exposure to Bd metabolites reduced Bd load, with treated individuals at 21 d postexposure showing significantly lower infection intensity than controls ($P < 0.001$). Effects were not confined to a threshold dose or duration. Notably, longer exposure durations, but not higher doses, produced progressively greater Bd-load reduction ($P < 0.001$; $P = 0.327$, respectively). Across infection intensities, treated frogs maintained higher growth rates, improved body condition, and reduced skin sloughing and mortality compared with controls ($P < 0.001$, $P = 0.001$, $P = 0.003$, $P = 0.005$). By identifying optimal application methods and evaluating the vaccine's efficacy, this study demonstrates the potential of Bd metabolite prophylaxis as a conservation tool and informs protocols for future field implementation. Amid disease-driven amphibian declines, such a treatment could meaningfully advance amphibian conservation in Indiana and beyond.

Key Words: vaccinology, wildlife, fungal disease, amphibian, chytrid

General Abstracts: Session 53—Vaccinology 3

274 - Lipid nanoparticle-encapsulated DNA vaccine induces robust and durable immunity against avian influenza in chickens.

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High pathogenic avian influenza virus (HPAIV) remains a major threat to poultry production, food security, and public health worldwide. In the United States, control measures rely primarily on enhanced biosecurity, surveillance, and depopulation of infected flocks. However, these approaches become unsustainable during widespread outbreaks, resulting in severe economic losses and shortages of poultry products. Vaccination represents a promising alternative approach to prevent AIV infections, offering a safe and practical means to complement existing control measures. The main objective of this study was to develop and evaluate the safety, immunogenicity, and protective efficacy of lipid nanoparticle- (LNP) encapsulated DNA vaccines as a platform for avian influenza control. To achieve this, we used low pathogenic avian influenza virus (LPAIV) as a surrogate model and tested the vaccine's efficacy in chickens. Hemagglutinin (HA) genes from H2N2, H6N8, and H5N3 were cloned separately into a eukaryotic expression plasmid and encapsulated in LNP to generate monovalent or bivalent vaccine formulations. Groups of 8 21-d–old chickens were immunized intramuscularly with a single dose of either 5 µg or 15 µg of LNP-DNA vaccine, and serum hemagglutination inhibition (HI) titers were measured weekly. Equivalent doses of naked DNA served as controls. The protective efficacy of the H5 LNP-DNA vaccine was evaluated by homologous H5N3 virus challenge at 28 d postvaccination, and viral load in tracheal swabs was quantified and analyzed using the Kruskal–Wallis test with Dunn's multiple comparisons. A single immunization with monovalent LNP-DNA vaccines induced high HI antibody titers (>1:256), whereas naked DNA failed to elicit measurable antibody responses. The bivalent vaccine induced similar levels of HI titers against both H2 and H6 subtypes without any immune interference. Additionally, H5 LNP-DNA induced durable antibody responses with HI titers exceeding 1:64 for more than 3 mo. It also conferred complete protection against challenge, with no detectable viral replication at any time point, whereas non-vaccinated chickens showed significantly higher viral titers post challenge. In conclusion, LNP encapsulation markedly enhanced the immunogenicity, durability, and multivalent potential of DNA vaccines in poultry, offering a safe, scalable, and DIVA-compatible strategy for avian influenza control.

Financial Support: This research was funded by the Strategic Funding Program of the Agricultural Research Division at the University of Nebraska–Lincoln Institute of Agriculture and Natural Resources.

Key Words: high pathogenic avian influenza virus, low pathogenic avian influenza virus, low pathogenic avian influenza virus, DNA vaccine

275 - Developing multiepitope fusion antigen vaccines of *Clostridium perfringens* against chicken necrotic enteritis.

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Necrotic enteritis (NE), primarily induced by *Clostridium perfringens*, causes ~$6 billion in losses in the poultry industry, but with few effective interventions. The *C. perfringens* sporulation protein vaccine through subcutaneous administration protected birds against clinical NE, but this delivery approach wouldn't be feasible for the poultry industry. The objective of this study was to develop multiepitope fusion antigen (MEFA) vaccines from the sporulation proteins to prevent NE using oral delivery. Proteomics analysis revealed that *C. perfringens* sporulation protein vaccines had 33 distinct proteins ($P < 0.05$) compared with the vegetative bacterium. The 19 out of the 33 proteins were assessed as more antigenic using immunoinformatic tool VaxiJen. The B-cell epitope immunogenicity of the 19 proteins was evaluated using ElliPro and IEDB BepiPred. Two 10-mer B cell epitope peptides per protein of the 19 proteins were synthesized. ELISA assay results were used to select 15 out of the 19 protein peptide epitopes, which were in situ substituted into backbone protein of C-terminal *C. perfringens* enterotoxin (C-CPE) to construct 36 CPE-MEFA vaccine candidates. These vaccine candidates were in situ evaluated for epitope probability, stability, hydropathicity, aliphatic index, half-life, and 3D structure, resulting in 12 CPE-MEFA vaccine candidates. Molecular dynamics simulations using GROMACS program narrowed the CPE-MEFA vaccines to 6 candidates. The 6 CPE-MEFA vaccines were synthesized in plasmid pET52b and subcloned into plasmid pET28a. After the vaccine candidates were expressed in BL21 cells, Coomassie blue staining showed strong bands around 30 kDa in the CPE-MEFA BL21 cell lysates compared with that of the control BL21 cells without CPE-MEFA. Consistently, western blot using His-tag antibody detected the presence of the CPE-MEFA proteins. Importantly, clear CPE-MEFA proteins at 30 kDa were detected using sera from chickens immunized with *C. perfringens* sporulation protein vaccines. In conclusion, CPE-MEFA vaccines have been constructed, expressed, and evaluated. The immunogenic western blot results suggest that the CPE-MEFA vaccines have the potential to prevent chicken NE.

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Key Words: necrotic enteritis, multiepitope fusion antigen (MEFA), vaccine, B-cell epitope, immunoinformatic

276 - Evaluation of innovative emulsion and polymer adjuvants for inactivated H5N8 HPAI virus vaccine.

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This study investigates strategies for vaccinating against Highly Pathogenic Avian Influenza (HPAI), a major threat to the poultry industry. We evaluated the safety, immunogenicity, and efficacy of an inactivated reverse genetics-based H5N8 vaccine utilizing various adjuvant technologies. The H5N8 vaccine was tested using Montanide ISA 78 VG, Montanide ISA 71R VG (water-in-oil emulsion adjuvants), and Montanide GEL P PR (a polymer-based aqueous adjuvant), comparing them to a commercial vaccine (oily formulation). Four-week-old influenza antibody-negative chickens were inoculated subcutaneously with a 0.5 mL dose of the H5N8 vaccine, formulated with either ISA 78, ISA 71 R, GEL P, the commercial vaccine, or the antigen alone. Safety monitoring involved tracking clinical signs and weight for 35 d. Immunogenicity was assessed by measuring hemagglutination inhibition (HI) antibody titers on d 0, 7, 14, 21, and 28 post-vaccination. Efficacy was determined by challenging the vaccinated chickens with an H5N1 field virus isolate 35 d post-vaccination, followed by a 10-d observation for survival and viral shedding. All adjuvanted formulations were found to be safe, with no local or systemic reactions observed. Chickens vaccinated with ISA 78, ISA 71R, and GEL P developed significantly higher HI antibody titers compared with the control group ($P < 0.0001$). The ISA 78 group showed superior immunogenicity on d 21 and 28 compared with GEL P ($P = 0.0002$; $P < 0.0001$), ISA 71 R ($P = 0.0075$; $P < 0.0001$), and the commercial vaccine ($P < 0.0001$). Post-challenge, all vaccinated groups achieved 100% survival and demonstrated reduced viral shedding compared with the control group. In conclusion, the H5N8 vaccines formulated with Montanide ISA 78 VG, ISA 71 R VG, and GEL P PR elicited substantial humoral immune responses. Montanide ISA 78 VG, in particular, induced the highest antibody titers, highlighting its potential as a potent adjuvant candidate for HPAI vaccines. These results confirm that both oil- and polymer-based adjuvants are efficacious for HPAI vaccine formulation.

Key Words: poultry, influenza, vaccine, adjuvant

277 - Chitosan mannose nanoparticle–based subunit vaccine against *Campylobacter jejuni* in broilers.

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C*ampylobacter jejuni* is the leading cause of human foodborne illness, with the CDC estimating 1.5 million infections annually in the United States. Poultry and poultry products serve as the major sources of human infections. Therefore, control of *Campylobacter* in poultry is critical to reducing the health and economic burden on both the public and the poultry industry. However, conventional vaccines do not induce strong mucosal immunity, thus are not effective in preventing preharvest *C. jejuni* colonization. In this study, we have developed chitosan-mannose (CM) nanoparticle (NP)–based subunit vaccine containing immunogenic outer membrane proteins (OMP) and flagellar (FLA) protein of *C. jejuni* that can be delivered orally through drinking water. The CM NP, being stable, polymeric, mucoadhesive, and biodegradable, can serve as a promising delivery platform to induce a stronger mucosal immune response. The CM NP were prepared via ionic gelation using tripolyphosphate (TPP) as a crosslinker to encapsulate antigenic proteins, which were obtained by growing *C. jejuni* microaerobically on Mueller-Hinton agar and extracting OMP and FLA proteins using standard protein extraction methods. Bradford assay revealed protein concentrations of 2.4 mg/mL for OMP and 1.5 mg/mL for FLA extracted from 100 g of cell pellet. The presence of OMP and FLA was further confirmed by SDS-PAGE. The NP formulation was optimized using BSA as a surrogate protein, where conditions of 1 mg/mL CM and 0.5 mg/mL TPP were identified as optimal with an encapsulation efficiency of 85%. Using this optimized condition, the antigenic protein-loaded NP was prepared and analyzed using a Malvern Zetasizer and a Keyence microscope. The CM NP loaded with *C. jejuni* antigens were 276 nm in size, optimal for mucosal uptake, with a high net positive charge (zeta potential +31.3 mV), indicating colloidal stability without forming aggregates. Morphological analysis confirmed homogeneous and intact NP formation. These characteristics support the potential of CM NP as a promising approach for the delivery of *C. jejuni* immunogenic antigens. Ongoing and future work will include in vitro studies to assess the stability and release kinetics of the NP and in vivo studies in broiler chickens to evaluate their immunogenicity and protective efficacy.

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Key Words: *Campylobacter jejuni*, chitosan-mannose, nanoparticle, subunit vaccine, outer membrane protein, flagella

278 - Enhanced immune response to porcine epidemic diarrhea virus (PEDV) harboring swine IgG Fc molecule on its surface.

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We have developed a novel vaccine platform, termed viral surface engineering (VSE). This technology enables viruses to harbor Fc molecules derived from immunoglobulins on their surface. The interaction between Fc and Fc receptors (FcR) promotes strong antigen presentation, as FcR are expressed on immune cells such as B cells, T cells, macrophages, dendritic cells, and natural killer cells. For VSE technology, we established a method to engineer susceptible cell lines so that viruses acquire Fc molecules on their surface during replication. These Fc molecules bind to FcR on immune cells, thereby enhancing antigen presentation and stimulating immune signaling. To demonstrate the potential of this technology, we applied it to the development of a porcine epidemic diarrhea virus (PEDV) vaccine. The efficacy of conventional PEDV vaccines remains controversial in many Asian countries despite large-scale vaccination campaigns. We engineered cell lines to produce PEDV harboring Fc molecules on its surface. After confirming Fc expression, we evaluated vaccine efficacy in pigs. Comparative studies between the conventional PEDV vaccine and the Fc-modified PEDV (PEDV-Fc) vaccine revealed that PEDV-Fc induced similar or slightly higher IgG responses but significantly higher IgA responses. More importantly, the PEDV-Fc vaccine elicited much stronger cytokine responses, including IL-6, IL-12, TNF-α, and IFN-γ, compared with the conventional vaccine. Most critically, PEDV-Fc demonstrated markedly higher neutralizing activity against PEDV. Collectively, these findings suggest that incorporation of Fc molecules onto viral surfaces enhances antibody production, cytokine responses, and especially neutralizing activity. This effect is likely mediated by Fc–FcR interactions that promote stronger immune stimulation through enhanced antigen presentation. Taken together, our data demonstrate that VSE technology represents a promising new generation of vaccine platforms. We plan to extend this approach to other swine viral diseases, including porcine reproductive and respiratory syndrome virus (PRRSV) and porcine circovirus.

Key Words: viral surface engineering, vaccine, porcine epidemic diarrhea virus, Fc, immune response

279 - Comparative evaluation of CPAF-based *Chlamydia* vaccine candidates in the pig model.

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Ch*lamydia trachomatis* (*Ct*) is the leading bacterial cause of sexually transmitted infections in humans worldwide. Especially repeated and untreated chlamydial infections can lead to severe complications such as pelvic inflammatory disease, infertility, and ectopic pregnancy in women. Given the limited effectiveness of current *Ct* treatment and screening programs, alongside the lack of lasting immunity from natural infection, developing an effective vaccine is crucial. Pigs serve as a valuable model for chlamydia research due to their physiological similarity to humans and natural susceptibility to *Chlamydia suis* (*Cs*), a close relative of *Ct*. While *Cs* often causes asymptomatic infections, it has been associated with clinical signs such as conjunctivitis and respiratory and reproductive disorders. Therefore, the pig acts both as a model species for vaccine development against *Ct* and as a target species for controlling *Cs*. Thus, we utilized the pig model to evaluate 3 *Ct* vaccine candidates – (1) a chlamydial-protease-like activity factor (CPAF) covalently linked to the novel STING pathway agonist CL1151, (2) CPAF combined with STING agonist ADU-S100 absorbed onto Nano-11 corn particles (NanoST), and (3) CPAF formulated with oil-in-water emulsion Montanide IMS 1313. Pigs received 2 intramuscular (IM) doses or 2 intradermal (ID) doses. Systemic T cell responses were assessed via FluoroSpots (IFNγ, TNFα, IL-17A) and multi-parameter flow cytometry. The CPAF-specific antibody levels were measured by ELISA. Among the vaccine candidates tested, the CPAF-CL1151 conjugate vaccine elicited the strongest systemic T cell response: it induced a robust IFNγ production with or without TNFα coproduction (mean 229 SFU/500K PBMC), compared with CPAF/NanoST (mean 31 SFU/500K PBMC) and CPAF/IMS1313 (mean 5 SFU/500K PBMC). This response was equally driven by all T cell subsets (CD4, CD8, and γδ T cells). The route of administration (IM vs. ID) had no significant effect on either the magnitude or the quality of the cell-mediated immune response. All candidates induced serum anti-CPAF IgG, with the strongest responses from the CPAF-CL1151 vaccine candidate. In conclusion, the conjugation of the STING pathway agonist CL1151 to the *Chlamydia* protein CPAF resulted in a highly immunogenic vaccine candidate. Future studies will focus on evaluating additional conjugate vaccines in the pig model and conducting dose–response assessments of the most promising formulations.

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Key Words: *Chlamydia trachomatis*, vaccination, pig model, stimulator of interferon genes agonist, conjugate vaccine

General Abstracts: Session 54—Virology 4

280 - Japanese encephalitis virus infection in pig fetuses causes transcriptional immunopathology sequelae in piglets.

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Japanese encephalitis virus (JEV) poses significant threats to the swine industry in Asia and Australia, causing abortions in pregnant pigs, boar infertility, and disease in piglets. The JEV is also a leading cause of viral encephalitis in humans, with pigs serving as the major amplifying host. The geographic range of JEV is expanding, raising concerns about its introduction into North America, where large populations of pigs coexist with ubiquitous Culex mosquito vectors. However, JEV-pig interactions are not well-studied. Thus, we tested whether JEV infection in pregnant pigs and fetuses causes persistent immunopathology in surviving newborn piglets. Six pregnant pigs were injected with JEV at 50 d of gestation, resulting in transplacental and fetal infections. The piglets were delivered at 115–116 d of gestation, and samples collected after birth for JEV quantification and RNA-seq analysis. Among 6 JEV-injected sows, 3 (mild infection subgroup) delivered 48 visually healthy piglets with low JEV loads in the placenta and tonsils. The other 3 sows (severe infection subgroup) delivered 8 visually healthy piglets (22.2%), 1 weak piglet (2.8%), 6 dead piglets without abnormalities (16.7%), and 21 dead piglets (58.3%) with severe pathology (decomposition, edema, mummification), out of a total of 36 piglets. All piglets, both live and dead, in the severe subgroup had very high JEV loads in the placenta and tonsils. The visually healthy but JEV-affected piglets from the mild subgroup exhibited altered expression of many genes and GO pathways in PBMC. Genes associated with cell death, cytokine signaling, and antiviral responses were affected, indicating broad suppression of antiviral pathways, including those related to IFN responses. Notably, several key innate immune genes—TRIM21, TRIM25, RIG-I, OAS2, and OAS1—were downregulated. This was further supported by the lowest *P*-value observed for the downregulated GO biological process “antiviral innate immune response.” Japanese encephalitis virus infection in pig fetuses causes transcriptional immunopathology in surviving deceptively healthy piglets. It is crucial to determine whether fetal infection caused by JEV increases the risk of affected immunocompromised piglets becoming more susceptible to secondary JEV exposure, making them more efficient in enhancing virus transmission within swine herds and to humans.

Financial Support: Department of Veterinary Biosciences, College of Veterinary Medicine, The Ohio State University, Columbus, USA

281 - Genetic diversity of equine rotavirus A viruses collected within the United States of America between 2021 and 2025.

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Despite an inactivated monovalent G3 vaccine being available for equines, equine rotavirus A (ERVA) is still the predominant infectious pathogen that causes diarrhea in foals throughout the USA. The ERVA is a dsRNA virus that contains 11 genome segments, which encode 12 proteins. The ERVA can be characterized into genotypes based on their G (glycoprotein) determined by VP7 protein (segment 9) and P (protease-sensitive protein) determined by VP4 (segment 4), respectively. The most common ERVA genotypes are G3P[12] (“G3”) and G14P[12] (“G14”). In this study, we aimed to determine the genetic diversity of ERVA strains and the predominant ERVA genotypes throughout the USA between 2021 and 2025. A total of 152 ERVA-positive samples that were submitted to diagnostic centers for rotavirus testing were analyzed with RT-qPCR using G3, G14, and rotavirus A- (NSP3) specific primers and probes. Viruses were isolated using MA104 N*V cells, a selection of the viruses that were successfully isolated were submitted for full-genome sequencing, and genetic diversity characterization was undertaken. Of the 152 total samples, 91 samples tested positive for solely G3, 21 samples for G14, and 40 samples tested positive for both G3 and G14. Twenty-three G3-positive and 9 G14-positive samples were completely sequenced using Illumina MiSeq platform. Based on whole genome SNP analysis, 12 distinct G3 groups and 7 distinct G14 groups were identified. For G3 and G14, respectively, 9 and 5 different variants of segment 4 were identified and 4 and 3 variants of segment 9. The genomes of the various viruses showed extensive reassortment within and between genotypes based on NCBI nucleotide BLAST searches. In addition, for multiple samples that were sequenced, a diverse population of heterologous and homologous virus strains were found in the same sample. This indicates that co-infection of homologous and heterologous genotypes (G3 and G14) is common in horses. This can possibly lead to the development of new strains. Together, these findings highlight a common theme in utilization of reassortment and mutations by ERVA to generate novel viruses. Taken all together, these findings suggest that genetic mutations and antigenic changes may be involved in altered vaccine efficacy.

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Key Words: equine rotavirus, genetic diversity, next generation sequencing, vaccine

282 - Highly pathogenic avian influenza A H5N1 virus infection in an immunocompromised domestic cat.

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Highly pathogenic avian influenza (HPAI) H5N1 viruses of clade 2.3.4.4b have recently caused widespread outbreaks in mammals, including domestic cats that live closely with humans and other animals. In-depth molecular and pathological characterization of naturally infected cats is urgently needed for developing better strategies to prevent interspecies transmission and further spreading of these viruses. In this case report, we characterized a unique case of HPAI H5N1 virus infection in an immunocompromised domestic cat. The pet animal was a diabetic cat with a history of feline infectious peritonitis. In early 2025, the cat developed acute fever and rapidly worsening respiratory distress and liver dysfunction despite antibiotic treatment. Due to severe clinical deterioration, the cat was euthanized. Postmortem examination revealed severe bronchointerstitial pneumonia, hepatic and lymphoid necrosis, bone marrow degeneration, and mild lymphohistiocytic meningitis. H5N1 viral RNA and antigens were detected in lung, brain, urine, and lymphoid tissues using RT-qPCR (viral RNA quantification), immunohistochemistry with anti-NP monoclonal antibody (antigen detection), and RNAscope in situ hybridization with an H5 probe (spatial localization of viral RNA). Whole-genome sequencing was performed using the Illumina MiSeq platform, and phylogenetic analysis of HA and NA genes (MAFFT alignment, IQ-TREE maximum likelihood inference, ModelFinder-selected models, 1,000 bootstrap replicates) confirmed that the virus belongs to clade 2.3.4.4b (B3.13 subgroup), closely related to HPAI H5N1 strains that are currently circulating in domestic cats and cattle. The source of infection for this particular cat might be linked to a fomite/environmental transmission route. The lethal HPAI H5N1 virus infection in an immunocompromised cat highlights the need for developing an improved prevention plan for pet animals. Clinicians should consider the possibility of H5N1 virus infection in cats with similar acute respiratory or neurologic signs, particularly in animals with chronic illness.

Financial Support: Internal Research Fund Support From University of Illinois Urbana-Champaign

Key Words: highly pathogenic avian influenza (HPAI), H5N1, feline infectious peritonitis, diabetes mellitus, whole-genome sequencing

283 - Infection dynamics and transmission of H5N1 clade 2.3.4.4b genotypes in cats (*Felis catus*).

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Since March 2024, highly pathogenic avian influenza virus (HPAI) H5N1 clade 2.3.4.4b genotype B3.13 has caused multiple cross-species spillovers in mammals. The domestic cat (*Felis catus*) is one of the most severely affected mammals, with often fatal outcomes. Here we investigated the infection dynamics, systemic spread, and transmission of HPAI H5N1 virus in domestic cats. Nine cats were oronasally inoculated with $2 \times 10^{4.5}$ PFU/cat, and 3 were mock inoculated. Nasal (NS), oropharyngeal (OPS), and rectal (RS) swabs, whole blood and sera, and 26 tissues were collected at 3, 5, and 7 d post infection (pi). To assess transmission, 16 cats were divided into 2 groups (B3.13, n = 8; D1.1, n = 8), with each group divided into infected (n = 4) and contact (n = 4) subgroups. Infected cats were inoculated oronasally with $2 \times 10^{4.5}$ PFU of genotype B3.13 or D1.1 per cat. At 24 h pi, the infected cats were transferred and cohoused (1:1) with contact cats for 14 d. Swabs (NS, OPS, RS), serum, and environmental samples (cage floor, feeder, and water) were collected and analyzed by qPCR and virus titrations. Infected cats developed fever (up to 41.1°C) and anorexia starting on d 1 pi with a gradual weight loss (up to 20%) by d 7 pi. High levels of viremia (viral RNA and infectious virus) were detected. Robust virus shedding was detected in nasal (3.7–4.9 $\log_{10}$ RNA copies/mL; 5.2–6.7 $\log_{10}$ EID_{50} mL on d 5 pi), oral secretions (2.8–5.1 $\log_{10}$ RNA copies/mL on d 3 pi; 4.5–6.5 $\log_{10}$ EID_{50}/mL on d 5 pi), and feces (2.8–5 $\log_{10}$ RNA copies/mL; 0.0–5.75 $\log_{10}$ EID_{50}/mL on d 7 pi). Highest virus titers were detected in the respiratory (nasal turbinates, trachea, lungs) and nervous systems (olfactory bulb, cerebrum, cerebellum, and pons). In situ hybridization and immunohistochemistry confirmed viral replication RNA in the brain at later times pi, initially in perivascular area and endothelial cells and subsequently in neuronal tissue, consistent with meningoencephalitis. In the transmission study, 3/4 cats in the B3.13 contact group were infected and shed virus, whereas no cat in the D1.1, suggesting genotype-specific transmissibility. Our results demonstrate that neuroinvasion in cats occurs following high-level viremia, with the virus reaching the central nervous system and invading the brain through the blood-brain barrier. Our results also demonstrate marked genotype-specific differences in transmissibility of the virus between cats.

Key Words: highly pathogenic avian influenza, H5N1, cat, pathogenesis, transmission

284 - Novel H5 recombinant protein vaccine provides effective protection against H5N1 influenza virus in cats.

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The emergence and broad circulation of highly pathogenic avian influenza (HPAI) H5N1 virus in wild birds and its spillover into dairy cows and felines pose a significant OneHealth risk. Here we developed a subunit recombinant hemagglutinin H5-based vaccine and evaluated its safety, immunogenicity, and protective efficacy against HPAI H5N1 virus in domestic cats. The full-length HA protein of HPAI H5N1 clade 2.3.4.4b genotype A2 virus was expressed using baculovirus expression system. To evaluate immunogenicity, 16 cats were divided into 2 groups: sham (n = 8), H5-vaccine (n = 8). Cats received subcutaneous injections (1 mL sham or 1 mL H5 vaccine), followed by a booster on d 21. Blood samples were collected at d 0, 14, 21, and 35 post immunization (pi) to evaluate the neutralizing antibodies (NA) levels by virus neutralization (VN) and hemagglutination inhibition (HAI) assay. We assessed H5-specific mucosal IgG and IgA antibody levels in nasal and oral secretions using a bead-based serological assay using the Luminex platform. To evaluate protective efficacy, on d 35 pi, all cats were challenged oronasally with HPAI H5N1 TX2/24 ($2 \times 10^{4.5}$ PFU/cat). To evaluate viral RNA through qPCR and infectious virus shedding, blood and swabs (nasal, oral, rectal) were collected every other day until d 14 post challenge (pc). Significance was determined by 2-way ANOVA ($P < 0.05$). The H5 vaccine elicited a robust level of NA (6–8 $\log_2$ on d 21 pi; $P < 0.0001$) against H5N1 virus. The IgG levels in nasal and oral secretions were significantly higher ($P < 0.0001$) in H5-vaccinated cats on d 35 pi when compared with sham-immunized cats. The IgA levels did not increase in H5 vaccine nor in sham-immunized cats. From H5-vaccine group, 7/8 cats did not develop any clinical sign of HPAI H5N1. One vaccinated cat (1/8) with lowest level of NA (5 $\log_2$ on d 35 pi) developed transient fever on d 3 pc and then stayed healthy d 4 onwards. The viral RNA loads in nasal, oral, and fecal secretions were significantly lower in H5-vaccine cats than in sham-immunized cats on d 1, 5, and 7 pc ($P < 0.0001$), with the peak viral RNA load (5.58 $\log_{10}$ copy number/mL^{-1}) being detected on d 5 pc. These results demonstrate that parenteral immunization with the H5-vaccine candidate provided clinical protection against H5N1 in cats and that vaccinating cats against H5N1 virus could reduce the risk of human exposure.

Financial Support: The work was funded by Medgene

Key Words: influenza, H5N1, cat, vaccine

285 - Genetic characterization of influenza D viruses from South Korean calves between 2022 and 2023.

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This study aimed to elucidate the molecular evolution and spatial dynamics of influenza D virus circulating in South Korea, with particular emphasis on regional genetic characteristics and the hemagglutinin esterase fusion (HEF) glycoprotein. The HEF glycoprotein is structurally distinct from influenza A and B counterparts. This unique glycoprotein functions as a principal determinant of IDV infectivity and replication efficiency through mediating receptor binding, membrane fusion, and immune recognition. Therefore, continuous monitoring of IDV and HEF's amino acid variations is crucial for elucidating host adaptation, interspecies transmission, and viral evolutionary dynamics at the regional level. A total of 540 cattle nasal swabs from 7 provinces were tested by PB1 gene-based RT-qPCR, with Ct values below 33 regarded as positive. Six IDV-positive samples were identified (3 from Andong, 2 from Seosan, 1 from Paju). Phylogenetic trees generated in MEGA 7 (bootstrap replicates) showed all South Korean IDVs clustering within the D/Yamagata/2019 lineage. Each gene sequence was aligned using CLUSTAL X and analyzed under gene-specific substitution models (e.g., Tamura–Nei). The BEAST analysis estimated the HEF substitution rate at 1.18×10^{-3} s/s/y (95% HPD: 9.68×10^{-4} – 1.41×10^{-3}) with tMRCA values of 1,990.8 (all IDV, 95% HPD: 1,984.4–1,996.9) and 2,018.0 (South Korea IDV strains, 95% HPD: 2,016.8–2,019.1). Bayesian spread analysis revealed frequent viral migration among Andong, Seosan, and Paju (HEF: BF = 68.53, PP = 0.70; M: BF = 84.73, PP = 0.81; M: BF = 80.94, PP = 0.80). Also, transnational movement from Japan to China (BF = 27.52, PP = 0.576) and China to Korea (BF = 21.90, PP = 0.519) indicates active local spread and diversification. Selection pressure analysis using FEL, MEME, aBSREL, and BUSTED identified episodic diversifying selection confined to HEF. The aBSREL model detected selection in one South Korean strain ($P < 0.1$), MEME identified 8 positively selected sites ($P < 0.05$), and FEL detected 3 residues ($P < 0.1$). Notably, a multidetected unique substitution site (T348G) was located near the esterase active site (aa 341–342). No pervasive selection was supported by BUSTED results. Overall, South Korean IDV belongs to the D/Yamagata/2019 lineage derived from Japan and China, with HEF displaying different substitutions. These findings highlight the need for continued genomic surveillance to assess the interspecies transmission risk of IDV.

Financial Support: This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korean government (MSIT) (RS-2024-00432287). Also this research was supported by the Animal and Plant Quarantine Agency and the Wildlife Scholarship Program of Seoul National University.

Key Words: cattle, influenza D virus, time to most recent common ancestor, spread, South Korea, positive selection model

General Abstracts: African Swine Fever—Posters

P2 - Protective antigens of African swine fever virus epidemic strain Georgia-07.

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African swine fever (ASF) is an acute, highly contagious, and often fatal viral disease of swine that poses a major threat to the global pig industry. Although substantial progress has been made in developing live attenuated ASFV strains as vaccine candidates, their use in ASF-free countries such as the United States is not feasible due to concerns over residual pathogenicity, potential reversion to virulence, and the risk of long-term viral persistence in vaccinated animals. Here, we evaluated the safety and immunogenicity of Orf virus (ORFV)-vectored ASFV single- and multi-antigen vaccine formulations in domestic pigs. In the initial study, 3 groups of 3 pigs each were primed with recombinant ORFV (rORFV) expressing the p54 antigen (rOrfΔ121/ASFV-Geo07/p54) via the intramuscular/intranasal (IM+IN), intramuscular/oral (IM+Pos), or intramuscular/intradermal (IM+ID) administration, and boosted on d 21 using the same route. Serum samples collected on d 14, 21, and 35 post-prime were assessed by the immunoperoxidase assay and the data analyzed using one-way ANOVA. Robust ASFV-specific immune responses were observed across all routes, with the IM+IN route eliciting significantly higher responses. Next, using the IM route only, pigs were primed with individual rOrfΔ121/ASFV-Geo07 antigens (p30, p54, CD2v, lectin) or multivalent pooled formulation, and boosted on d 21 and 42. Serum samples collected on d 14, 21, 35, 42, and 60 demonstrated high ASFV-specific antibody titers in both single- and multi-antigen groups. No adverse clinical signs were observed in pigs receiving the pooled formulation. Similarly, there was no diminished antigen-specific responses with the multi-antigen regimen. Notably, although antibody titers were significantly lower at 21 d post-prime, there was no significant difference between groups by d 42 and 60, suggesting that a single boost at d 21 was sufficient to maintain robust immune responses. These results demonstrate that rOrfΔ121/ASFV-Geo07-based vaccines induce strong ASFV-specific immune responses in domestic pigs, whether delivered as a single or in multivalent form and across multiple inoculation routes. This works supports further development of ORFV-vectored ASFV subunit vaccines as a safe and adaptable platform for ASF prevention in ASF-free regions.

Key Words: protective antigen, African swine fever virus (ASFV), subunit vaccine, Orf virus (ORFV), poxvirus

P3 - Partnership: Systematic screening of ASFV proteome for identification of immunogenic antigens.

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African swine fever virus (ASFV) affects many swine-producing countries, causing significant economic losses. Pigs vaccinated with a live-attenuated virus (LAV), but not with a killed virus (KV) vaccine strain, develop solid homologous protective immunity. Passive transfer of hyperimmune sera protects pigs from lethal challenge with a virulent ASFV strain, indicating that antibodies play an important role in protection. This study aimed to comparatively analyze antibody profiles between pigs vaccinated with a LAV and those vaccinated with a KV to identify immunogenic viral antigens. Thirty ASFV-seronegative pigs were divided into 3 groups. Group 1 received a single intramuscular dose of an experimental LAV vaccine. Group 2 received 2 doses of a KV vaccine at a 3-week interval. Group 3 served as a nonvaccinated (NV) control. On d 42 postvaccination, all pigs were challenged intramuscularly with a highly virulent ASFV strain and monitored for 21 d postchallenge. Whole blood samples collected at various time points postchallenge were analyzed by qPCR to quantify viral loads in serum. Serum samples collected on d 41 postvaccination and d 7 postchallenge were analyzed using a commercial ELISA kit and a luciferase-based immunoprecipitation system (LIPS) to detect antibody reactivity against a panel of 29 viral structural proteins. All pigs vaccinated with the LAV survived the 21-d observation period. In contrast, 8 out of 10 pigs in the KV group and 7 out of 10 in the NV group died within 14 d post-challenge. On d 7, blood viral loads in the KV and NV groups reached 10^6 HAD50/mL, significantly higher than in the LAV group (10^2 HAD50/mL, $P < 0.05$). When analyzed against 29 viral structural proteins, sera from the LAV group reacted with 16 proteins: p14.5, p32, p54, p17, pp62, p15, p34, pE165R, pD339L, pI73R, C-type lectin, and pCP123L. In contrast, sera from the KV group reacted with only 3 proteins: p12, p32, and p54. Moreover, a negative correlation was observed between the intensity of antibody reactivity and blood viral loads for 5 ASFV antigens: p12, p14, p15, p32, and pD205, with $R < -0.72$ and $P < 0.05$. These findings highlight distinct antibody profiles induced by LAV and KV vaccines. Viral proteins exclusively recognized by sera from the LAV group represent promising candidates for subunit vaccine development.

Financial Support: The animal experiment and antibody profile analysis were funded by the Agriculture and Food Research Initiative competitive grant number 2022-67015-37264 of the USDA National Institute for Food and Agriculture. The development of the live attenuated ASFV vaccine was funded by Vi-etnam Ministry of Science and Technology, grant number ĐT.02/DAKH-02/19-HĐ-ĐTĐL.CN-CNN.

Key Words: African swine fever virus, live attenuated vaccine, inactivated vaccine, antibody profile, humoral immunity, luciferase-based immunoprecipitation system

P4 - Thermal inactivation of African swine fever virus in swine slurry.

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African swine fever (ASF) poses a significant threat to the pork industry across the globe. The causative agent, ASF virus (ASFV), is a large, double-stranded DNA virus, with remarkable resilience when dispersed in organic matter. African swine fever virus is shed by infected swine in all secretions and excretions, which therefore serve as a critical reservoir of the virus, increasing the risk of indirect transmission on and between farms. Manure slurry generated in modern swine farms is a complex mixture of feces, urine, wastewater, disinfectants, and feed, providing a rich, organic matrix that protects the virus and impedes the efficacy of decontamination efforts. Moreover, slurry is produced in substantial volumes and stored for prolonged periods, creating a complex biosafety challenge in the event of an ASF outbreak. In general, the World Organization of Animal Health (WOAH) recommends thermal treatment of ASFV at 60°C for 20 min or 56°C for 70 min to inactivate infectious virus. However, studying ASFV inactivation in representative slurry samples is challenging. Slurry contains a highly diverse microbiota, is often toxic to cell culture, and absorbs virus, hindering virus isolation. Thus, studies demonstrating the efficacy of such treatments when virus is in swine slurry are limited. The present study was designed to determine the effect of temperature on the stability of ASFV within swine slurry. Swine slurry spiked with ASFV Georgia 2007/1 (10% vol/vol) was treated at 25°C, 37°C, 45°C, 50°C, 56°C, 60°C, 65°C, and 70°C for 0, 10, 20, or 30 min, and 1, 2, 4, 8, 16, or 24 h. Slurry was then mixed with an equal volume of fetal bovine serum, centrifuged, filtered, serially diluted and inoculated onto immortalized porcine kidney macrophage (IPKM) cells grown in 96-well plates. The cells were observed for 7 d for cytopathic effects to assess inactivation of the virus. Reduction of infectious virions was correlated with the heat and the length of time applied to slurry samples, with preliminary results demonstrating at least 90% reduction of infectious virus at the WOAH recommended temperatures (56°C and 60°C) in 10 min, suggesting heat treatment to be an effective strategy to inactivate ASFV in slurry. However, high temperatures are difficult to uniformly apply to slurry pits. Further research is needed to explore how heat treatments may be implemented in real world scenarios.

Financial Support: This project was funded by the Saskatchewan Ministry of Agriculture- Agriculture Development Fund (ADF) through the research project titled “Evaluating manure management and treatment strategies during potential African Swine Fever outbreak in Canada”(ADF) File number 20230209.

Key Words: African swine fever (ASF), DNA virus, manure slurry, swine health, thermal treatment, virus inactivation

General Abstracts: Antimicrobial Use—Posters

P5 - Developing a data management system to support accurate, reproducible, and actionable analysis of antimicrobial use and phenotypic and genotypic resistance data from swine farms.

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The main objective of this project is to develop an integrated back-end system to generate automated, standardized reports on antimicrobial resistance (AMR) trends on swine farms. Reports will contextualize on-farm AMR trends by integrating multiple data streams related to herd health status, population characteristics, environmental factors, and antimicrobial use. The goal of the reports is to provide a digestible yet appropriately contextualized summary of farm-level AMR trends over time. A review of available approaches for querying, storing and manipulating data objects was conducted. An inventory of available farm-level data sources (and their variables) was conducted in collaboration with the participating company. A use-case diagram was developed to capture a high-level contextual view of all relevant actors (i.e., end users and external data streams) associated with the company's multiple data assets. An entity relationship diagram was created to visualize the logical design and cardinality, check information requirements, identify primary and foreign keys, and enforce data integrity. The software R v4.5.1 was used to import, tidy, transform, and then join input data streams using primary and surrogate keys, based on logical design requirements from the entity relationship diagram. Informative and contextually appropriate data summaries and visualizations were identified in consultation with target end-users. Desired summary objects were generated in a Quarto v1.7.34 report template. Based on discussions with the collaborating company, the decision was made to design a logical relational database for storing data. Four data streams were identified encompassing variables such as phenotypic and genotypic AMR information, sample characteristics, farm-level health data from diagnostic and routine testing, antimicrobial purchase and use information, and herd characteristics. An automated routine for continually updating these data streams was implemented. Development of code to transform the data frames to generate report summaries and visualizations is ongoing. This project is a collaborative effort with a large swine production management company that supports independent family farms. The work being done to develop a rigorous and comprehensive data workflow based on best practices will be a useful model for other moderate-to-large sized companies that may also wish to track their farm-level AMR trends.

Financial Support: USDA APHIS Cooperative Agreement AP25VSSP0000C048

Key Words: antimicrobial use, antimicrobial resistance, relational model

P7 - Monoglyceride blend ameliorates intestinal barrier in weaned piglets through the modulation of intercellular adhesion in gut epithelium.

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The continuous application of growth promoters in swine farming was a weighted cause in resistant microbial strains dissemination. Multidrug resistant strains can infect both animals and human and are growing risk factor in the whole world. Thus, the search for antibiotic alternatives is the actual task to maintain husbandry profitability. Various monoglycerides were confirmed to be inexpensive antimicrobial substances. The aim of study was to assess the effect of monoglycerides blend (MB) supplementation on intestinal barrier integrity in weaned piglets. The control group (n = 10) was fed with standard diet plus antibiotics (colistin 4 mg/kg of BW and amoxicillin 20 mg/kg of BW). The MB-exposed group (n = 10) was fed same diet without antibiotics plus MB (0.05%) with drinking water from the 21st to 45th day of life. Statistical analysis was performed using one-way ANOVA test after assessing for normality of distribution. The barrier function was assessed via detection the relative content of epithelial adhesion markers including occludin, claudin-1, and E-cadherin in gut tissue with western blot. The innate immunity was assessed through the detection of IFN-α, IFN-γ, and TNF-α with qPCR. The administration of MB induced statistically significant upregulation in E-cadherin, claudin-1, and IFN-α content ($P \leq 0.01$) in both duodenum and jejunum of MB-exposed piglets compared with controls. Besides, statistical occludin upregulation ($P \leq 0.05$) was observed in the jejunum. Conversely, the duodenal expression level of IFN-γ was significantly decreased ($P \leq 0.01$) in MB-exposed piglet group compared with controls. In addition, the expression of both IFN-γ and TNF-α was downregulated ($P \leq 0.05$) in the jejunum of MB-exposed piglets. The measuring of total protein content in both serum and intestinal tissue extracts of piglets did not show significant differences between control and MB groups. The results on ADG have shown similar data in control and MB-exposed piglets. Thus, MB supplementation to weaned piglets can upregulate adhesion proteins expression and inhibit proinflammatory cytokine production. Together, observed effects can improve epithelial junctions and demonstrate MB's potential to modulate intestinal barrier integrity as well as regulate innate immunity in weaned piglets. In addition, comprehensive molecular markers studies of the piglet intestinal barrier may be a convenient tool to assess the efficacy of various antibiotic-free strategies.

Key Words: piglet, growth promoters, gut barrier, antibiotics ban, gut immunity

P8 - 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.

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This study aims to evaluate whether dietary fiber or commingling, or both, can be used to mitigate the development and persistence of AMR genes (ARG) within the fecal microbiome after metaphylactic antibiotic use in production pigs. A total of 84 litters from 2 rooms within a single sow farm were included in this 2 × 2 factorial design randomized controlled trial. From each litter, up to 10 newborn piglets were randomly selected and enrolled into the study (n = 833 piglets). At enrollment, piglets were tagged, weighed, and given ceftiofur. Immediately after processing (i.e., 2–5 d post-birth [dpb]), the first factor treatment was initiated. Enrolled litters were either kept within a single farrowing pen with their sow and littermates (“CONV”) or allowed to commingle and move freely across multiple pens and suckle from multiple sows (“MSCC” for multisuckle, common creep). On 17 to 18 dpb, all piglets received enrofloxacin immediately before the introduction of preweaning creep feed, which was the second study factor in the 2 × 2 design. Creep feed was either standard/low-fiber feed or supplemented with potato starch/high fiber, depending on the pre-allocated treatment group. Piglets were weaned and moved to the nursery and then the finisher facility. Rectal swabs were collected from individual piglets from birth through weaning and the nursery period, and 1 d before marketing at the finisher facility. Total DNA was extracted and pooled by sow/litter and sampling time point. A total of 1,197 DNA samples were submitted for metagenomic library preparation and sequencing. The resulting sequencing data were processed using the AMRplusplus bioinformatic pipeline to identify ARG in each sample. Resistome profiles were then compared by treatment group and time point. The resistome–microbiome profile exhibited temporal changes, with high resistome count early in life and a subsequent decline as pigs aged, with the lowest counts observed before market across all treatment groups. A significant treatment-by-time interaction effect was observed on both resistome count and overall resistome profile, which indicated differential effects of interventions on resistome dynamics (PERMANOVA R^2 = 3%–5%, $P < 0.01$). We expect that our results will provide insight into whether commingling and creep diet can be used as potential interventions to minimize AMR spread and persistence within microbial communities after metaphylactic antimicrobial treatment.

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.

Key Words: antimicrobial resistance, swine, metaphylactic, commingling, diet, metagenomics

P9 - An engineered probiotic platform with enhanced therapeutic release toward pathogen targeting.

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Streptococcus suis is a swine pathogen that also can cause zoonotic infections in humans, posing risks to both public health and the pig industry. Although antibiotics are commonly used to treat *S. suis* infections, the rising prevalence of multidrug resistance highlights the need for alternative approaches. Our long-term goal is to develop a probiotic platform to eradicate *S. suis*. The objective of this study is to engineer the probiotic *Limosilactobacillus reuteri* to optimize the release of leptin, used as a model protein for therapeutic delivery, which represents a critical first step toward the development of an anti-*S. suis* system. Previously, we demonstrated therapeutic efficacy in preclinical models following the administration of engineered *L. reuteri* that releases a therapeutic protein following natural cell lysis during gastrointestinal transit. Building on this, we hypothesized that controlled prophage activation enhances lysis and thus therapeutic release. To test our hypothesis, we placed the gene encoding a prophage-derived antirepressor protein under the control of an inducible promoter. *Limosilactobacillus reuteri* was further engineered to accumulate a luminescence reporter protein inside the cell. Antirepressor function was evaluated by monitoring bacterial growth, phage production, and extracellular luminescence; the latter is an indicator for the release of recombinant protein. We found that recombinant protein release can be maximized following the optimization of the timing of antirepressor induction. Prophage activation and subsequent cell lysis was 4-fold more efficient following antirepressor-induced lysis compared with mitomycin C–induced lysis (n = 3, $P < 0.05$, 2-tailed unpaired *t*-test). Finally, priming the cells for antirepressor-induced lysis, followed by oral administration, resulted in a 40-fold increase in therapeutic release in the mouse gut (n = 6, $P < 0.05$, 2-tailed unpaired *t*-test). In sum, our work demonstrates a novel approach to control and boost therapeutic release, which makes it a promising platform to deliver antimicrobials targeting *S. suis* and other pathogens.

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Key Words: probiotics, antimicrobial delivery, microbial therapeutics, swine, *Streptococcus suis*

P11 - Good bugs vs. superbugs: Next-generation probiotics to fight *Staphylococcal* infections.

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Staphylococcus aureus is an opportunistic pathogen that can cause severe infections in both humans and animals. Its rapid acquisition of antibiotic resistance (AMR) poses a threat to treatment efficacy across clinical, agricultural, and environmental settings, highlighting the need for new therapeutics. Among these, novel probiotics, also known as next-generation probiotics (NGP), represent a promising category of beneficial bacteria designed for use in both food and dietary supplementation, as well as specific therapeutic applications. Unlike traditional probiotics, these strains are specifically selected and optimized for their ability to target specific pathogens, regulate host immunity, and inhibit microbial virulence. In this study, 45 probiotic strains were screened for their ability to inhibit *S. aureus* using agar well diffusion assays. Several strains showed clear inhibitory activity, with inhibition zones ranging from 7.0 ± 0.7 mm to 23.5 ± 0.0 mm (mean ± SD). The most effective strains were P15 (23.5 ± 0.0 mm), P24 (22.2 ± 0.4 mm), P11 (18.2 ± 1.1 mm), and P31 (18.2 ± 0.4 mm), while others showed moderate or no inhibition. These findings highlight the promise of NGP as viable alternatives to conventional antibiotics for controlling *S. aureus*. Future work will focus on evaluating the efficacy of the selected best probiotic strains in vitro and therapeutic applications within a One Health framework to control infections in animals and humans.

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Key Words: *Staphylococcus aureus*, next-generation probiotics, AMR, animal, human

P12 - Actionable steps toward advancing antimicrobial stewardship on the dairy farm: Using a training program for improving early and accurate disease detection in dairy cattle by farm workers.

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Antimicrobial stewardship (AMS) is an evidence-based, holistic approach that promotes the judicious use of antimicrobial drugs to preserve their effectiveness and availability for humans and animals. Dairy farm workers are commonly responsible for disease identification and routine treatment decisions within the context of an existing veterinarian-client-patient relationship (VCPR). This fact highlights the importance of farm workers' knowledge and skills related to disease identification and implementation of veterinarian-guided treatment strategies because it directly affects the successful judicious use of antimicrobials in livestock production systems. Knowledge gaps in animal health and behavior have been previously identified as a key reason to provide training to farm workers. Our project goal was to develop an evidence-based training program that supports antimicrobial stewardship on the farm and is easily accessible and tailored for farm workers. We aimed to enhance farm workers' understanding of cattle sickness behavior, improve the early identification of clinical disease signs, and increase treatment success. This research project commenced in 2018 and involved several stages, including the initial development of training materials, active stakeholder input through the project, evaluation of the training program at various farms, and the final process of professionally editing the materials to ensure they were made available at no cost through an online platform. As part of the initial development and evaluation of training materials, a longitudinal evaluation of the training curriculum was developed and was tested on 12 commercial dairy farms (6 in Ohio and 6 in California), with a total of 25 farm workers receiving the training. Findings from our project revealed significant changes in knowledge and attitude toward antimicrobial stewardship by farm workers. The presentation for this project will outline the methods used to achieve our final outreach deliverable and present the guide developed for its use on the farm. It will also highlight efforts being made to support the adoption and implementation of these efforts on the farm.

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Key Words: farm worker training, antimicrobial stewardship, dairy cattle, accurate disease diagnosis

P13 - Effects of sound and air on antibiotic resistance genes during anaerobic digestion of poultry litter.

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Removal of poultry litter (PL) from broiler farms is challenging and anaerobic digestion (AD) provides a great opportunity by converting waste into energy. Our team previously showed that microaeration and sound treatments of AD increase biogas production. Here, we investigated to see whether sound, micro-aeration, and their combination had any effects on the prevalence of antimicrobial resistance genes (ARG) during AD of PL. Four AD treatments with a control were maintained at ambient temperature. A total of 12 digestate samples collected at 6, 23, and 42 wk were shotgun sequenced using Illumina NovaSeq and DIAMOND sequence aligner. Negative binomial regression in STATA was used to analyze read count data aggregated at the treatment level. Overall, 3,190 gene accessions belonging to 337 unique genes conferring resistance mainly to antimicrobials (97%) were detected. Treatment did not significantly affect the mean number of ARG; although the total number of ARG decreased in the control, micro-aeration or sound treatment, or both, increased the number of ARG. Total ARG read counts increased on wk 23 in all treatments; averaged by treatment group, only sound-treated AD significantly (P = 0.035) reduced the mean total ARG abundance compared with the control AD, with no aeration effect. The number and abundance of ARG detected from the digestate and PL feed were lower than the AD samples. More than 80% of the resistome was composed of 4 antimicrobial classes: macrolides-lincosamides-streptogramins, tetracyclines, aminoglycosides, and glycopeptides. In conclusion, AD does not effectively remove ARG from animal manure, and aeration and sound did not improve its effect. The AD system may act as a hotspot for ARG, necessitating post-AD treatments such as composting.

Financial Support: USDA-ARS

Key Words: anaerobic digestion, antimicrobial resistance, poultry litter, resistome, shotgun metagenomic sequencing

General Abstracts: Aquaculture—Posters

P14 - Use of the invertebrate model *Galleria mellonella* to assess the role of the sialidase degradation pathway in the virulence of *Mycoplasma alligatoris*.

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Alligator farming is arguably the most unique of all aquaculture enterprises and one of the greatest success stories for conservation. The alligator farming industry's "conservation through utilization" resulted in removal of alligators from the endangered species list. The economic impact of alligator farming to aquaculture is more significant than many appreciate. Although catfish and oyster production account for the majority of ~60% of value, the alligator industry is comparable or greater than other commodity groups. When coupled with its significant impact to the economy of Louisiana, the state with the second highest poverty rate in the United States, as well as the positive impact on environmental habitat and species conservation, alligator farming is a key component of aquaculture. For any intensively raised animals, infectious diseases can pose a major threat to animal health and production. *Mycoplasma alligatoris* (*Mall*) caused a severe acute disease and a die-off event in Florida alligators in 1995. The pathogenicity was confirmed by experimental infections. Most recently, a second *Mall* mortality event occurred in the same population, and the state of Louisiana now requires imported hatchlings be tested and deemed free of *Mall* infection. Thus, *Mall* is considered an emergent threat to alligator aquaculture. Experimental infections in live alligators can be challenging. The invertebrate model system *Galleria mellonella* (*Gmell*) is widely used for studying pathogenesis of multiple bacterial and fungal infectious agents. We recently reported that infection of *Gmell* with *Mall* mirrored the results of experimental infections in alligators. In other mycoplasmal species, sialidase is a known virulence factor. Whole genome sequencing revealed that the sialidase pathway is present in *Mall.* Because previous in vitro studies supported the role of sialidases in virulence of *Mall*, we tested all available mutants with disruptions in key sialic degradation pathway genes. The wild type *Mall* and transposon-generated mutants of the sialidase degradation pathway (*nanI*, *nanA*, *nagC*, and *nanE*) were used to infect *Gmell.* All mutants in the pathway had increased survival relative to the wild-type *Mall.* The greatest difference in survival was obtained with *nagC* and *nanE*. The *Gmell* model system is a promising tool to address pathogenic potential as well as identify key virulence factors, especially when infections in the natural host may be difficult.

Key Words: alligators, *Mycoplasma alligatoris*, invertebrate model

P15 - Food-grade biomaterials-based microneedles for fish vaccination in aquaculture.

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The aquaculture industry produces approximately 17% of consumed animal-derived protein but faces significant challenges in disease prevention and excessive use of antimicrobials. Vaccination, particularly for juvenile fish, can mitigate these risks, but manual and automated needle-based vaccination methods are difficult to scale and are labor- and energy-intensive. Here, a water-resistant, food-grade microneedle platform delivers vaccines to fish. Silk fibroin–based microneedles were engineered to remain waterproof from minutes to hours, penetrate fish skin in wet conditions, remain attached during swimming, and release vaccines to induce immunization. Different microneedle morphologies—shell, solid-tip, and hollow—can be used to enhance mechanical integrity, waterproofing, and cargo release kinetics. The microneedles also enable the preservation of DNA vaccines in relevant storage conditions outside the cold chain at room temperature for 1 wk. In a pilot study, microneedle-based vaccination against enteric redmouth disease demonstrated protection in rainbow trout fry (24% relative percent survival), supporting the development of a new tool for juvenile fish vaccination that increases aquaculture efficiencies and reduces environmental impact.

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.

Key Words: microneedle, vaccination, aquaculture, biopolymer

General Abstracts: Bacteriology—Posters

P18 - Phenotypic and genotypic characterization of *Staphylococcus saprophyticus* from dairy farms in the United States.

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Staphylococcus saprophyticus is one of the understudied non-*aureus* staphylococci and mammaliicocci (NASM) species isolated from dairy farms. The objective of this study was to phenotypically and genotypically characterize *S. saprophyticus* from dairy farms in the United States. *Staphylococcus saprophyticus* isolates were retrieved from the University of Missouri Udder Health Laboratory isolate collection, and species identification was reconfirmed with MALDI-TOF MS. All isolates were grown on Columbia blood agar, and phenotypic characteristics such as colony morphology, biochemical properties, and antibacterial activity against mastitis-causing pathogens were identified. Antimicrobial susceptibility testing was performed by broth microdilution assay using the Sensititer Gram Positive GPALL3F Plate. Strain typing of the isolates was determined using random amplification of polymorphic DNA (RAPD) PCR. Data were analyzed using descriptive statistics. Overall, a total of 61 isolates, originating from Missouri (n = 43), Kentucky (n = 17), and Iowa (n = 1), were characterized. Original samples were collected between 2014 and 2022 and included various dairy cow body sites and environmental samples. Phenotypically, after 24 h of growth, *S. saprophyticus* colonies were 0.5–1 mm in size, dark gray/cream/golden yellow in color, had mucoid consistency, and were smooth on the surface. All the isolates were catalase positive and coagulase negative. Approximately 3.3% (2/61) of the isolates showed antibacterial properties, inhibiting the growth of *S. aureus* isolates. Antimicrobial resistance was identified to oxacillin (69%, 42/61), tetracycline (56%, 34/61), penicillin (39%, 24/61), ampicillin (30%, 18/61), ceftriaxone (30%, 18/61), erythromycin (16%, 10/61), and tigecycline (10%, 6/61), with a 13% (8/61) multidrug-resistant value. Based on RAPD typing, 93% (57/61) of strains were unique. This study is the first to explicitly describe the unique phenotypic characteristics of *S. saprophyticus*. A high level of genetic diversity was identified among *S. saprophyticus* isolates from dairy farms. In the future, high-throughput sequencing technology will be used to further characterize *S. saprophyticus* isolates to improve understanding of traits associated with pathogenesis and antibacterial activity. Our study has the potential to benefit the dairy industry and public health because this organism is associated with diseases in humans and dairy cattle.

Financial Support: This study was funded by a University of Missouri Research Council Grant.

Key Words: characterization, dairy farms, NASM, subclinical mastitis, *S. saprophyticus*

P21 - Evidence of *Leptospira* infection in the reproductive tracts of female cull cattle.

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Leptospirosis is an underrecognized disease that affects multiple animal populations, including cattle. Although *Leptospira* infection is primarily recognized for causing acute life-threatening infection and abortion storms in cattle, it is also implicated in early embryonic death, resulting in reproductive inefficiency, a common reason for culling and economic loss. This preliminary study aims to determine the proportion of reproductive tracts of local female cull cattle with *Leptospira* present and determine the correlation between renal and genital colonization. For our research study, the uterus and bladder of nonpregnant female cattle were sampled at the time of harvest from a local abattoir. Samples were then tested for the presence of *Leptospira* by *lipL32* real time PCR and *Leptospira* culture. Swabs from the uterus were also inoculated onto aerobic and anaerobic culture media to assess the presence of other bacterial pathogens. After 24 h of incubation, the *Leptospira* cultures were filtered or treated with a selective antimicrobial cocktail (STAFF) to decrease the bacterial contamination. Cultures were routinely examined via dark field microscopy for organisms with morphology and motility consistent with *Leptospira*, followed by real time PCR confirmation. We successfully collected and tested the urine, uterus, and cervical mucous from 25 cull cattle. Out of the 25 female cull cattle, 5 (20% CI [4.32, 36.6]) cows tested positive via real time PCR with *lipL32* gene from different sample types (2/25 [8%] urine, 1/25 [4%] cervical mucous, 2/25 [8%] uterus). Successful amplification was achieved with conventional PCR of a positive urine sample using *adk* and *lipL41* gene targets. Sanger sequencing found 99.76% (*adk*) and 98.10% (*lipL41*) identity match to *Leptospira borgpetersenii* sequences found on the NCBI database. Aerobic and anaerobic culture from 17/21 uterine swabs resulted in multiple bacterial organisms. We confirmed the presence of *Leptospira* in the genital tract and urine of local female cull cattle. We continue to collect and test samples and monitor cultures for successful isolation of *Leptospira*.

Financial Support: UTCVM Center of Excellence in Livestock Disease and Human Health

Key Words: *Leptospira*, leptospirosis, cattle, surveillance, reproductive tract, PCR

P22 - Evaluation of the antimicrobial activity of clove bud oil and cinnamaldehyde against major liver abscess-causing bacterial pathogens in cattle.

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Liver abscesses (LA), a bacterial infection, are a major health and economic concern for the beef and dairy industries. The condition is typically initiated by *Fusobacterium necrophorum* invasion of the liver and is often complicated by co-infections with *Trueperella pyogenes* and or *Salmonella enterica*. High-grain diets predispose cattle to ruminal acidosis, increasing the risk of LA. Currently, tylosin phosphate is the most common feed additive used for prevention; however, the use has raised concerns regarding antimicrobial resistance (AMR) in cattle production systems. To explore natural alternatives, this study evaluated the in vitro antimicrobial activities of clove bud oil (CBO) and cinnamaldehyde (CIN) against key LA-causing pathogens, including *F. necrophorum* ssp. *necrophorum*, *F. necrophorum* ssp. *funduliforme*, *T. pyogenes*, and *S. enterica* serovar Lubbock, using 3 complementary assays: agar well diffusion, broth microdilution, and in vitro rumen fermentation. Across all assays, CIN demonstrated greater antimicrobial efficacy than CBO. In the agar well diffusion assay, CIN produced significantly larger, dose-dependent inhibition zones (all $P < 2.2 \times 10^{-16}$), with the highest susceptibility observed in *Fusobacterium* spp. and *T. pyogenes*. Broth microdilution confirmed CIN's potency, showing significantly lower minimum inhibitory concentrations (MIC; $P = 3.45 \times 10^{14}$) than CBO, requiring up to 4-fold lower concentrations for complete growth inhibition. In the in vitro rumen fermentation assay, CIN (200–400 µL) rapidly and sustainably suppressed *Fusobacterium* populations within 12 h ($P < 3.45 \times 10^{-14}$), whereas CBO produced only partial inhibition. The efficacy of both compounds was reduced in the presence of lysine, indicating substrate-dependent effects. Collectively, these results demonstrate that cinnamaldehyde is a highly effective in vitro inhibitor of major LA-associated pathogens, exhibiting dose-dependent activity and superior performance compared with clove bud oil, highlighting its strong potential as a natural alternative feed additive for controlling liver abscesses in cattle.

Key Words: liver abscess, essential oil, *Fusobacterium necrophorum*

P23 - Effect of segmented filamentous bacteria oral treatment on intestinal integrity in chickens.

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In commercial poultry farms, chicks are hatched away from their progenitors, and are thus mainly exposed to environmental microbes that may lack host-specific microbes that aid gut maturation at early life. In previous research, we demonstrated through genetic testing that early introduction of a key driver of the gut immune system, like segmented filamentous bacteria (SFB), increases the maturation of the gut immune system in chickens by upregulating genes related to immune homeostasis, gut barriers, and antimicrobial peptides. This follow-up study involved phenotypic testing to assess the effect of SFB-based treatment on gastrointestinal integrity and antimicrobial properties in chickens. One-day-old commercial Lohmann Select Layer (LSL) chicks (n = 6 per group and per time point) were orally treated with PBS (CON) or Dekalb white leghorn–derived SFB (D-SFB/~ SFB, determined by qPCR). At 4, 7, 15, and 29 d post inoculation (dpi), ileum tissues were collected following euthanasia for histological analysis to assess the effects of the treatment on the intestinal barrier. Immunohistochemistry was used to assess the prevalence of goblet cells in the ileum, facilitating their quantification. Resistance to pathogenic *Salmonella* and *Escherichia coli* was tested using in vitro bactericidal assays on ileal tissues scrapings. The treatment affected the physiological state of the intestinal barrier in the ileum, the primary colonization site for SFB, in D-SFB birds. The ancillary diagnostic tests revealed several findings associated with an improved intestinal structure in the SFB treated group, with no findings in the control group across all time points (ATP). Among the observed improvements were filamentous bacteria adhering to the ileum, heterophilic, and lymphocytic infiltrates within the lamina propria and in the villous. Additionally, there was lymphocytic hyperplasia throughout the ileum. Immunohistochemistry analysis indicated a significant ($P < 0.05$) increase in goblet cells. Bactericidal assays demonstrated a significant ($P < 0.05$) increase in antimicrobial capabilities against avian pathogenic bacteria, as shown in vitro, at ATP. The D-SFB treatment positively altered the physiology of the lower intestinal tract and increased direct antimicrobial properties. By establishing increased intestinal integrity and downstream microbial protection, the treatment has potential to increase health and productivity, ultimately benefiting the poultry industry.

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Key Words: segmented filamentous bacteria, Lohmann Select Layers, immunohistochemistry, histology

General Abstracts: Bovine Respiratory Disease—Posters

P24 - Developing a diagnostic tool for bovine respiratory syncytial virus infection through near-infrared spectroscopy.

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Bovine respiratory disease (BRD) is a multidimensional complex that causes significant economic loss in the cattle industry. Bovine respiratory syncytial virus (BRSV), one of the viruses contributing to BRD, primarily targets the lower respiratory tract and causes a variety of clinical signs, as well as subclinical infection. Nonspecific clinical signs include fever, nasal and ocular discharge, and elevated respiratory rate. Current diagnostic techniques require off-site laboratory testing, creating delays that allow BRSV to spread through herds, highlighting the need for rapid diagnostic methods. Near-infrared spectroscopy (NIRS) can be used in the field to identify diagnostic signatures in biological materials. The objective of the study was to evaluate NIRS as a diagnostic tool for BRSV detection across varying infection severities. Twenty-two Holstein bull calves were reared in research pens at Mississippi State University from 1 wk old until 12 wk of age when the study began. At 12 wk old, the calves were randomly divided into 4 groups and challenged via aerosol with , 10^4, or 10^5 $TCID_{50}$ units of BRSV, creating low-, medium-, and high-dose groups, respectively. The negative control group was nebulized with cell culture media. Over 14 d, clinical signs were assessed daily using 2 different scoring systems, and lungs were assessed via transthoracic ultrasound. In addition to the daily assessments, biofluids, including serum, plasma, nasal secretions, saliva, and breath condensate, were collected, as well as nasal swabs to detect BRSV by RT-PCR. Near-infrared spectra of biofluids were acquired using an ASD FieldSpec 4 NIR spectrometer (350–2,500 nm) and analyzed within the 1,300–1,600 nm region (first overtone of water). Data were preprocessed using different techniques and classified using soft modeling of class analogies (SIMCA) to distinguish BRSV-positive (Ct ≤ 35) from negative (Ct ≥ 35) samples. Preliminary results showed variation in accuracy, sensitivity, and specificity across biofluids, with nasal secretions performing best (66% accuracy, 44% sensitivity, 73% specificity). Incorporating advanced machine learning and AI could further enhance performance. This approach demonstrates potential for a rapid, noninvasive alternative to conventional diagnostic methods, allowing faster intervention and reduced disease transmission.

Key Words: Bovine respiratory syncytial virus (BRSV), spectroscopy

P25 - From thymic injury to TCR diversity: Defining BVDV's lasting impact on calf immunity.

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Bovine respiratory disease complex (BRDC) is the leading cause of illness and death in young cattle, and bovine viral diarrhea virus (BVDV) is a major predisposing factor within this syndrome because it undermines immunity and primes calves for secondary respiratory pathogens. Our preliminary study (under review) enrolled calves into 2 groups (n = 5 per group). On d 0, group 1 was mock-inoculated, and group 2 received an intranasal dose of BVDV-2 strain RS886 with a titer of 10^6 median tissue culture infectious dose ($TCID_{50}$/mL). On d 21, both groups were intranasally inoculated with influenza D virus (IDV) at a titer of 10^6 $TCID_{50}$/mL. Necropsies were performed on d 42 (21 d post-IDV). Findings indicate that BVDV primarily targets lymphoid tissues, including the thymus, causing atrophy and impaired T-cell development. Thymic RNA-seq at d 13 post-BVDV revealed a strong interferon-driven signature, including CXCL9, CXCL10, complement components, and other interferon-stimulated genes. The induction of CXCL9/CXCL10 in the thymus is consistent with robust interferon activity that can direct CXCR3-positive T-cell trafficking and influence maturation. The thymus induction of CXCL9 and CXCL10 reflects robust interferon activity that directs CXCR3-positive T cells and can influence trafficking and maturation. Therefore, transient increases may aid antiviral defense, while persistent elevation may promote tissue injury and dysregulation. By d 42, most calves showed structural recovery, while a subset remained dysregulated. This USDA-funded project will define how BVDV reshapes the α/β T cell receptor (TCR) repertoire across CD4 and CD8 naive and effector memory subsets using peripheral blood mononuclear cells collected in our previous study at d 0, 21, and 42 after BVDV infection. Targeted TCR sequencing will quantify V and J usage CDR3 clonotypes and diversity, including Shannon diversity. We hypothesize that BVDV narrows α/β TCR diversity most in the naive compartment, which limits effector and memory formation after heterologous challenge and informs vaccine strategies that preserve repertoire breadth to strengthen calf health and BRDC control.

Financial Support: This project was supported by the Agriculture and Food Research Initiative Competitive Grant no. 2024-67016-42406 from the USDA National Institute of Food and Agriculture.

Key Words: bovine viral diarrhea virus (BVDV), thymus, T cell receptor (TCR) repertoire, calves, immunology, bovine respiratory disease complex (BRDC)

P26 - Diversity in antimicrobial resistance patterns between two sampling time points in feedlot calves and yearlings for important bovine respiratory pathogens.

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The Canadian feedlot antimicrobial use (AMU) and resistance (AMR) surveillance program (CFAASP) began in 2019 to monitor AMU and AMR in fed cattle. The program tracks trends in recovery and AMR for 3 major bovine respiratory disease (BRD) pathogens: *Mannheimia haemolytica*, *Pasteurella multocida*, and *Histophilus somni*. Deep guarded nasopharyngeal (NP) samples were collected from 16 animals in each of the enrolled feedlots at entry processing and then again at rehandling for a targeted total of 832 samples annually. The same pen of cattle was sampled at arrival and rehandling, but not necessarily the same individual cattle were sampled at both time periods. Samples were couriered to Prairie Diagnostic Services, Saskatoon, SK, Canada, for culture and susceptibility testing using microbroth dilution, and the BOPO7F plate. The minimum inhibitory concentration (MIC) for each antimicrobial and isolate was interpreted using established by CLSI. Statistical analyses accounted for potential clustering effects of resistance using generalized estimating equations (GEE). Antimicrobial resistance increased between arrival and rehandling sampling, especially in *P. multocida.* The percentage of pansusceptible isolates for *P. multocida*, *M. haemolytica*, and *H. somni* decreased from 80% at arrival to 41% at rehandling, from 93% at arrival to 56% at rehandling, and from 64% at arrival to 52% at rehandling, respectively. Multiclass-resistant isolates also increased markedly at rehandling for all organisms, but particularly for *P. multocida*, where multiclass resistance increased from 10% at arrival to 43% at rehandling. When stratified by calf age, at rehandling sampling, the percentage of pansusceptible *M. haemolytica* isolates from calves was approximately half that of isolates from yearlings (42% vs. 78%, respectively). For *P. multocida*, the percentage of pansusceptible isolates from calves at rehandling (16%) was less than one-quarter that of isolates from yearlings (72%), and the percentage of multiclass resistant *P. multocida* isolates from calves at rehandling (63%) was approximately 4 times higher than for isolates from yearlings (16%). These data demonstrate that the shift toward decreasing susceptibility of respiratory isolates over the feeding period is driven predominantly by resistance acquisition in BRD organisms isolated from calves.

Financial Support: Beef Cattle Research Council ANH.16.24

Key Words: antimicrobial resistance, bovine respiratory disease, feedlot cattle, calf, yearling

P27 - Shotgun metagenomics characterizes consequences of disease and management practices in backgrounded beef cattle.

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Bovine respiratory disease (BRD) is a costly polymicrobial, multifactorial disease induced as an interaction between various microbial agents as a consequence of altered host immune activity following stress and pathogen exposure. Differing abundance profiles were evaluated by nasal pharyngeal swab (NPS) to elucidate the consequences of vaccination and shipping practice on cattle respiratory microbiota, with the intention of explaining ultimate health outcomes in terms of those consequences. Left and right NPS were collected from 73 bull calves enrolled in a split-plot randomized controlled trial. Thirty-three calves were randomly chosen to receive a commonly employed modified live virus 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 3 d before backgrounding. This action was conducted as part of a larger 6 time point study aiming to marry findings from the cattle transcriptome to the nasal microbiome and health outcomes. Swabs were collected for shotgun metagenomics at time points T1 (~107 d), T4 (230 d), and T5 (~234 d), corresponding to first vaccination, weaning and shipping, and arrival at the backgrounding facility, respectively. The NP swab cuttings were homogenized via bead-beating, and DNA was extracted via an AllPrep DNA/RNA/miRNA Universal kit and sequenced (150 bp; ~90 million reads/sample). Differential abundance and data exploration were performed in R with vegan and ecodist. Shipping method was the primary driver of variance among the nasal metagenomic profiles of cattle sampled, with tight clustering of Bray–Curtis distance among the directly shipped cattle, explaining more than 40% of the variance in principal coordinates analysis. Subclustering was observed between vaccinated and nonvaccinated cattle, and those that did and did not require treatment for BRD, when controlling for shipping method. Vaccination and shipping both drive differential abundance profiles in the cattle nasal microbiome, and these microbiomes exhibit unique characteristics that may be predictive of BRD risk in stocker cattle. Further evaluation may highlight the interplay between the cattle microbiome and immune system, and how the relationship between the 2 influences disease outcome.

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Key Words: metagenomics, bovine respiratory disease (BRD), vaccination, marketing, beef cattle

P28 - A comparison of AVITI and Ultima Genomics platforms for metagenomic profiling of bovine nasal and fecal samples.

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Ultima Genomics (UG) offer short-read sequencing at lower price points via a different sequencing approach compared with Illumina and AVITI platforms. Given the lower cost, researchers may wish to switch sequencing platforms, especially for large metagenomic projects. However, it is unknown whether UG generates metagenomic profiles that are comparable to traditional sequencing platforms. Therefore, the objective of this project was to compare metagenomic profiles generated from UG and AVITI using paired bovine specimens. A total of 12 nasal and 12 fecal sample types from 2 host sites were sequenced on both the AVITI and UG platforms (n = 48) in a head-to-head comparison. To reflect real-world tradeoffs, the sequencing depth of the AVITI and UG datasets were matched based on the reads/Gb cost of sequencing, rather than an equivalent read depth/sample. Data from each platform were subjected to an identical bioinformatic workflow to perform quality control (QC), host depletion, de novo assembly using MEGAHIT, taxonomic profiling with Kraken2/Bracken, and functional profiling using HUMAnN 3.9. All values are reported as medians. As designed, the UG libraries had higher raw read depth per sample in millions (M) than AVITI due to UG's lower per-read cost at 8 M/Gb and 5.05 M/Gb for AVITI pairs. The phred quality score was higher on AVITI (43.5) versus Ultima (33). The proportion of reads aligning to host was similar between platforms within site (fecal: 2.42% vs. 3.84%; nasal: 97.87% for both; AVITI vs. UG). After trimming and host removal, the number of reads for metagenomic analysis remained different between platforms (AVITI: 95.28 M vs. 2.07 M; UG: 180.98 M vs. 3.93 M fecal vs. nasal). Species richness was higher in AVITI libraries for fecal samples (10,399 vs. 7,281) but higher in UG libraries for nasal samples (635 vs. 436). Pathway richness was higher in UG samples for both sample types (fecal 342 vs. 313; nasal 93.5 vs. 59, UG vs. AVITI). The N50 was similar between platforms in fecal samples (AVITI: 2,517; UG: 2,418) but higher in AVITI libraries for nasal samples (1,463 vs. 1,002). Within each sample type, platform effects were present but generally with small effect size. At comparable overall cost, our comparison did not identify a clear advantage for either platform, and results suggested that performance may be differential by sample type and bioinformatic workflow. Therefore, selection of platform is likely to be study specific.

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Key Words: AVITI, Ultima, bovine microbiome, metagenomic sequencing, relative abundance

P29 - Lung ultrasound image scoring in bovine neonatology.

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Pulmonary ultrasonography (LUS) has become a promising tool for assessing respiratory health in neonatal calves because it is portable, safe, and provides real-time imaging. However, no standardized scoring systems are currently applicable to calves less than 30 d of age, which limits diagnostic reproducibility among evaluators. This study aimed to evaluate the interobserver agreement of a 5-point lung ultrasound scoring system adapted from human neonatology. A total of 50 thoracic images and cine loops (25 still images and 25 cine loops), were selected from a media bank of 1,575 files and evenly distributed across the 5 scores (10 media per category, from 0 to 4), ensuring full representation of the scoring spectrum. The material originated from calves on commercial dairy farms in Brazil, managed according to each farm's standard health protocols without modifications. The files were assessed by 14 veterinarians with experience in ultrasonography, selected for their familiarity with the subject, and representing different countries including Brazil, Canada, France, the United States, and Italy. Following a brief video tutorial, each evaluator independently classified the material into 5 categories: score 0 – aerated lung with presence of A-lines and a regular pleural line; score 1 – vertical artifacts occupying less than one-third of the pleural line; score 2 – artifacts occupying between one-third and two-thirds of the pleural line; score 3 – artifacts occupying more than two-thirds of the pleural line; score 4 – presence of pulmonary consolidation. Agreement was evaluated via calculation of percent agreement (PA), Gwet's AC2, Krippendorff's α (a), and Fleiss' kappa (weighted and unweighted). Results showed substantial to almost perfect agreement across all coefficients (PA = 0.957; AC2 = 0.845; a = 0.839; weighted kappa = 0.849), confirming the reproducibility of the proposed system. Variability among evaluators was lower among those reporting higher diagnostic confidence, highlighting the influence of subjective perception. Considerable variation in image quality assessment was also observed, underscoring the importance of standardized acquisition protocols. Overall, the findings confirm that the proposed score allows consistent classification of lung patterns in neonatal calves, even under field conditions and after brief training. This is the first study to formally evaluate a thoracic ultrasound scoring system for calves in the neonatal period, providing a practical, objective, and replicable tool for early diagnosis of respiratory diseases, professional training, and implementation in calf health programs.

Key Words: point-of-care ultrasound, neonatal care, respiratory disease, lung ultrasonography, neonatal health monitoring, newborn calves

P30 - Development of prototype single-cycle vaccines for bovine respiratory syncytial virus.

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Bovine respiratory syncytial virus (BRSV) is a major agent in the bovine enzootic pneumonia (BEP) and in the respiratory disease complex (BRDC), resulting in significant economic losses to the beef and dairy industry. Although BRSV vaccine use is widespread, BRSV continues to result in a substantial burden, and there is a need for improved technologies. Multiple vaccines are currently marketed for BRSV, including parenteral and mucosal delivery, but variable efficacy and safety concerns have been reported. Parenteral vaccine delivery faces interference by maternal-derived antibodies (MDA), which hampers vaccine uptake and diminishes responses to vaccination. Moreover, current live attenuated vaccines, including mucosal delivered, are replication competent, retaining the risk of reversal to and residual virulence. We aimed to develop prototype single-cycle BRSV vaccines, allowing for bona fide antiviral responses, while limiting risk associated with replication competent viruses. Our single-cycle constructs followed 2 strategies: the lack of an essential gene (Mnull) or the lack of a protein function (preF). Single-cycle viruses can be amplified in designated complementing (production) cell lines constitutively or inducibly expressing the missing gene or protein function. Following the cloning of the full-length antigenome BRSV sequence, we engineered the complementary DNA (cDNA), and the wildtype sequence was modified to include the coding sequence for green fluorescent protein (GFP). For Mnull BRSV, the M gene open reading frame was engineered to exclude M expression and to include a Tet transactivator element that drives M expression in the producer line. The initial BRSV cDNA was modified in parallel to generate the BRSV-preF cDNA. For this, the bovine F gene was subcloned and its sequence modified using site-directed mutagenesis to achieve BRSV pre-F conformation stabilization (DS-Cav-1-based preF-stabilizing mutations). Our future research will focus on the in vitro rescue and characterization of our constructs, followed by in vivo testing in the BRSV calf model.

Key Words: Bovine respiratory syncytial virus (BRSV), bovine respiratory disease (BRD), immunology, cattle, vaccinology, recombinant

P31 - Virucidal, antiviral, and immunomodulatory effects of flavonoids tiliroside, quercetin, and kaempferol against bovine viral diarrhea virus.

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Bovine viral diarrhea virus (BVDV) remains a major cause of economic losses in cattle due to respiratory disease and reproductive disorders. Given the limited treatment options in livestock, plant-derived flavonoids emerge as promising antiviral candidates. This study evaluated the virucidal, antiviral, and immunomodulatory effects of quercetin, kaempferol, and tiliroside against BVDV in vitro. Cytotoxicity was assessed in MDBK cells by MTT assay, and nontoxic concentrations were used for subsequent experiments. Virucidal and antiviral assays were conducted, followed by viral titration using the Reed–Muench method. Immunomodulatory effects were assessed through RT-qPCR analysis of IFNα, IFNβ, and ISG15 expression in infected and noninfected cells, using GAPDH as reference. Data were analyzed by one-way ANOVA with Tukey's post hoc test for viral titers and 2-way ANOVA for gene expression, considering $P < 0.05$ significant. Tiliroside was noncytotoxic up to 62.5 µ*M*, while quercetin and kaempferol were tolerated at 31.25 µ*M*. In the virucidal assays, tiliroside and quercetin reduced viral titers by 13.2- and 20.5-fold, respectively. In antiviral assays, kaempferol and quercetin decreased titers by 76- and 43-fold. Tiliroside markedly induced IFNβ and ISG15 expression in infected cells at 6–24 h, while quercetin and kaempferol triggered strong but transient peaks at 12 h for IFNβ and ISG15, and moderate upregulation of IFNα. These results demonstrate that flavonoids not only inhibit BVDV replication but also enhance type I interferon and ISG15 pathways, reinforcing host antiviral defense. In conclusion, quercetin, kaempferol, and tiliroside exhibit complementary antiviral, virucidal, and immunomodulatory properties, supporting their potential as natural antiviral agents in veterinary medicine.

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Key Words: flavonoid, veterinary, bovine viral diarrhea virus (BVDV)

P32 - Addition of probiotic to a feedlot diet alters the upper respiratory tract microbiome in feedlot steers.

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Bovine respiratory disease (BRD) remains a serious health and economic problem for cattle producers and is often caused by a combination of bacterial and viral infections. To imitate BRD pathogens without introducing any into the herd, animals were administered lipopolysaccharide (LPS). By using LPS, we hope to reproduce the inflammatory response mounted by cattle when they become infected with a gram-negative bacterial pathogen. In addition, probiotics are a promising intervention for modulating the microbiome and the immune system, promoting health benefits in cattle. By adding a commercial probiotic to the feedlot diet, we wanted to determine if it impacted the microbiome. At weaning into the feedlot environment, nasal swabs were collected from 360 steers (12 pens of 30 steers each). Shortly after weaning, steers were fed a traditional postweaning diet with a commercial probiotic (50 mg/head daily, BOVAMINE Defend® Novonesis; 6 pens of 30 calves each) or without a commercial probiotic (6 pens of 30 calves each). Approximately 4 weeks after weaning, steers were administered LPS (0.5 µg/kg; in 1–3 mL of saline) or a saline control subcutaneous. Nasal swabs were collected at weaning and at the day of administration of LPS or saline (Day0) and d 2 after administration of LPS or saline (Day2). DNA was exacted from the nostril swabs and 16S ribosomal RNA gene hypervariable regions 1–3 were subsequently sequenced. Overall, diversity of the microbiome decreased at Day0 and Day2 compared with when the steers were weaned at the feedlot 4 weeks earlier, and the bacterial profiles changed from weaning to Day0 and Day2. Addition of the probiotic also altered the microbiome of the upper respiratory tract at Day0 and Day2, including a change in abundance of *Mycoplasma*. In conclusion, these findings contribute to the understanding of the dynamic nature of bacterial diversity and the potential effects of fed probiotics within the bovine respiratory tract and provide insight for future studies of probiotics on animal health, disease prevention, and management.

P33 - Haptoglobin concentration after lipopolysaccharide treatment in cattle fed diets with or without probiotics.

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We have previously shown that haptoglobin (HPT) is an early indicator of respiratory disease in cattle. Furthermore, we have shown that intravenous or subcutaneous treatment of cattle with lipopolysaccharide (LPS) results in elevated concentrations of HPT in peripheral blood. In our current study, we hypothesize that including probiotics in the feed of newly weaned beef steers will affect the immune response to LPS as measured by HPT concentration in peripheral blood. Three hundred sixty spring-born steers were randomly placed into one of 2 feeding treatments 14 d after arriving at the feedlot. Treatments were a traditional postweaning diet (control) and traditional postweaning diet plus a commercial probiotic fed at 50 mg/head/day (probiotic; BOVAMINE Defend Plus). After 2 or 4 weeks on the treatment diets, half of each treatment group was treated with LPS (0.5 µg/kg) to mimic a bacterial infection, or with a saline sham injection, and peripheral blood was collected for haptoglobin (HPT) testing (D0). Two days later, peripheral blood was again obtained from animals to test HPT (D2) concentrations in reaction to LPS and sham treatments. Haptoglobin concentration was measured by ELISA. When all cattle were analyzed together, we found that Control Fed +LPS had an average HPT concentration increase of 24,137 ± 6,914 ng/mL at D2 as compared with Control Fed sham-treated animals with a 3,500 ± 7,021 ng/mL increase in HPT. Probiotic Fed +LPS had a 29,989 ± 6,965 ng/mL increase in HPT concentration at D2 as compared with Probiotic Fed sham-treated with a 5,723 ± 7,019 ng/mL increase in HPT at D2. Differences due to probiotic treatment and its interaction with LPS were not significant ($P > 0.10$). Probiotics may affect bovine immunity through pathways not captured in this study. Experiments are underway to measure changes in immune gene expression in peripheral blood leukocytes.

General Abstracts: Brucellosis—Posters

P36 - Latent class model analysis of diagnostic tests in the absence of a gold standard for the diagnosis of brucellosis in livestock in Costa Rica.

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Brucellosis is a major zoonotic disease affecting livestock worldwide. Accurate determination of disease prevalence and diagnostic test performance of brucellosis often pose a challenge due to absence of a single test to definitively detect the infection. This study was aimed at evaluating diagnostic accuracy of Rose Bengal Test (RBT) and the indirect enzyme-linked immunosorbent assay (iELISA), for detecting *Brucella* infection in livestock herds in Costa Rica. Retrospective data related to herd-level counts of animals testing positive or negative by both tests were obtained from SIVE (Integrated Epidemiological Surveillance System) application, developed by SENASA (Costa Rica's National Veterinary Services). The RBT was used for rapid screening, while iELISA served as the confirmatory test. Herd-level prevalence of *Brucella* infection as well as sensitivity and specificity of each test were estimated using a latent class analysis (LCA). The model accounted for potential minor disagreement between tests and used bootstrap resampling to assess parameter stability. Results showed that both tests performed well, with iELISA showing slightly higher accuracy (sensitivity = 91%; specificity = 95.6%) compared with RBT (sensitivity = 88%; specificity = 93.4%). The estimated true prevalence of brucellosis was 26.4% (95% CI: 18.2%–35.5%). This suggests the existence of a considerable degree of latent infection among the studied herds. The findings of this study highlight the importance of combining multiple diagnostic tests and using statistical models such as LCA to improve herd-level disease surveillance in the absence of a gold standard.

Key Words: brucellosis, Costa Rica, indirect enzyme-linked immunosorbent assay (iELISA), latent class analysis (LCA), Rose Bengal Test (RBT)

P37 - Development of laboratory diagnostic assays for *Brucella ceti* in stranded Hawaiian cetaceans.

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Brucella sp. bacteria are the causative agents for brucellosis, a zoonotic disease commonly found in humans, agricultural animals, feral pigs, and cetaceans (whales and dolphins). It can cause acute and systemic infections in humans and *Brucella ceti* affects the immune and reproductive systems of cetaceans. In Hawai'i, *B. ceti* is known to infect a variety of cetacean species. This project screened a DNA sample bank containing approximately 500 tissue samples from 14 species of cetaceans that stranded in Hawai'i between 1997 and 2011. Polymerase chain reaction (PCR), gel electrophoresis, and Sanger sequencing were used to detect and verify the presence of *B. ceti* using *Brucella* specific primers for the *IS711* region and *Omp2a* gene. Sequence was obtained for *B. ceti* in 3 cetaceans: *Steno bredanensis*, *Stenella longirostris*, and *Megaptera novaeangliae*. Cetaceans that strand on the beach in Hawai'i decay quickly, which causes DNA degradation in tissue samples; this also occurs from repeated freezing and thawing of DNA samples. The purpose of the second phase of this project was to design primers that target smaller fragments of the *Omp2a* gene of *B. ceti* in degraded DNA to increase the reliability of the laboratory diagnostic assay. The sequences above were used to create a consensus sequence of the *B. ceti Omp2a* gene, then used as the template to design 6 different *B. ceti* cetacean specific primers targeting a short amplicon size of 80–120 base pairs (bp). These primers were tested using standard PCR, gel electrophoresis, and Sanger sequencing against the known positive samples above to determine their ability to amplify short degraded segments of *B. ceti* DNA extracted from cetacean tissues. Of the 6 primer sets tested, 1 obtained positive sequence results. This new laboratory assay can detect 94 bp *B. ceti* DNA fragments in cetacean tissues to accurately identify the presence of brucellosis in these animals. With the laboratory portion of the PCR assay verified, the next step is to field test this PCR assay using a MiniPCR field machine. The field PCR assay would allow researchers and veterinarians conducting cetacean necropsies to rapidly diagnose an animal with zoonotic pathogens, including brucellosis. Identifying which zoonotic pathogens are present and pose a risk to human health would allow researchers and veterinarians to better protect themselves by making more informed decisions regarding necropsy protocols and personal protective equipment.

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Key Words: brucellosis, cetacean, zoonotic, Pacific Ocean, Hawai'i, diagnostic

P38 - Evaluation of brucellosis surveillance and intersectoral collaboration in Georgia: A One Health approach.

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Brucellosis, one of the most prevalent zoonotic diseases worldwide, remains endemic in Georgia, affecting both livestock and humans. Despite ongoing control measures and active communication between the veterinary and public health sectors, delayed notification and challenges in infection source tracing hinder timely outbreak control. This study aimed to evaluate Georgia's brucellosis surveillance system within a One Health framework, focusing on intersectoral collaboration, postnotification response, and infection source identification. A descriptive study analyzed human brucellosis notifications from the National Center for Disease Control (NCDC) and the National Food Agency (NFA) between 2019 and 2022. Data included regional case distribution, postnotification veterinary investigations, laboratory confirmation in animals, and slaughter outcomes. Information from electronic surveillance systems, email notifications, and regional veterinary interviews was compiled and analyzed. Between 2019 and 2022, NFA received 557 notifications, including 152 confirmed and 392 probable cases. Kakheti accounted for 38% of all notifications, followed by Kvemo Kartli (22%) and Imereti (16%), indicating these as high-risk regions. Most cases occurred in adults aged 20–59 years, with older individuals showing a higher proportion of confirmed diagnoses. The infection source remained unidentified in 80.4% of cases; where confirmed, 67% were linked to infected cattle, 22% to small ruminants, and 11% to animals on other farms. Among infected animals, 82.2% were slaughtered according to veterinary regulations, while 3.1% were sold before laboratory confirmation. Although there is active communication between the veterinary and public health sectors, timely receipt of human disease case notifications by the veterinary sector is essential to ensure rapid response. Strengthening real-time information exchange, linking animal identification systems to electronic surveillance, and improving farmer awareness and compensation mechanisms are critically important for enhancing brucellosis control measures in Georgia and advancing One Health collaboration.

Key Words: brucellosis, One Health, zoonotic disease, intersectoral collaboration

General Abstracts: Companion Animal Health—Posters

P39 - Antiviral and virucidal potential of flavonoids against canine herpesvirus, adenovirus, and distemper virus.

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Canine viral pathogens such as canine distemper virus (CDV), canine adenovirus (CAV), and canine herpesvirus (CHV) cause significant morbidity and mortality in domestic dogs and represent persistent challenges for veterinary medicine. Despite available vaccines for CDV and CAV, outbreaks remain frequent in areas with low coverage, and no vaccine or antiviral drug has been approved in the US for CHV, highlighting the urgent need for alternative therapies. This study evaluated the virucidal and antiviral effects of 5 flavonoids: tiliroside, quercetin, isoquercetin, nictoflorin, and kaempferol against CDV, CAV, and CHV through in vitro and in silico approaches. Cytotoxicity was assessed by MTT assay in MDCK and Vero cells, and nontoxic concentrations were used in virucidal and antiviral assays, with viral titers determined by the Reed–Muench method. Molecular docking was performed against predicted viral protein structures using AutoDock Vina. Results were analyzed by one-way ANOVA with Tukey's test ($P < 0.05$). Tiliroside showed the highest tolerated concentration (125 µ*M*) and significantly reduced CHV and CDV titers, whereas quercetin and kaempferol strongly inhibited CDV and CAV replication. Isoquercetin and nictoflorin also decreased CHV titers, indicating broad activity. Docking simulations revealed that tiliroside and nictoflorin bound with high affinity to CHV DNA polymerase subunits UL30 and UL42, whereas quercetin and kaempferol displayed strong (≤ −7.0 kcal/mol) interactions with CDV nucleocapsid, matrix, and hemagglutinin proteins and CAV capsid proteins, including hexon and protease. Isoquercetin showed consistent but moderate binding to replication and structural proteins. Together, these findings demonstrate that flavonoids exhibit virucidal and antiviral activity against clinically important canine viruses, targeting both structural and replication-associated proteins. Tiliroside and nictoflorin emerged as the most potent candidates for CHV inhibition, whereas quercetin and kaempferol were particularly effective against CDV and CAV. These results highlight the potential of flavonoids as natural antiviral agents for companion animals.

Key Words: canine distemper virus (CDV), canine adenovirus (CAV), canine herpesvirus (CHV), veterinary

P40 - A repeat cross-sectional/pseudo-longitudinal study of leptospiral prevalence in shelter dogs.

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Leptospirosis is a globally distributed, waterborne zoonotic disease that affects a wide range of mammals, including livestock, companion animals, wildlife, and humans. Asymptomatic reservoirs are characterized by chronic renal carriage and shedding of host-adapted leptospiral serovars, which contaminate the environment and enable transmission to humans and susceptible animals. Dogs, particularly those housed in shelters, are recognized as important sentinels for monitoring zoonotic risk. In this repeat cross-sectional study, we investigated the prevalence of leptospiral infection in clinically normal shelter dogs from the Central Appalachian region of Virginia, Tennessee, and Kentucky. Urine samples were screened for leptospiral DNA using a TaqMan-based quantitative PCR (qPCR), and sera were tested for *Leptospira*-specific antibodies by the microscopic agglutination test (MAT). Positive urine samples underwent PCR amplification and sequencing of a fragment of the rpoB gene to identify leptospiral species. A total of 286 clinically healthy dogs from 11 shelters were tested. Of the 221 urine samples analyzed, 9 (4.07%) were positive for leptospiral DNA by qPCR. Among 268 serum samples, 41 (15.3%) were seropositive by MAT. Of these seropositive dogs, 28 (68.3%) reacted to multiple serovars, and 34.5% exhibited titers = 1:800. The highest titers (1:6,400) were observed against serovars Bratislava and Pomona, while Icterohemorrhagiae was the most common reactive serovar overall. When compared with our prior survey conducted during 2017–2018, both urinary shedding and seroprevalence have decreased among shelter dog populations in this region. This decline may reflect changes in environmental exposure, vaccination practices, or dog population dynamics, although further investigation is warranted. These findings have important implications for animal and public health within Central Appalachia and beyond, as dogs adopted from shelters in this region may be relocated to other parts of the country. Ongoing surveillance of shelter populations, combined with preventive measures such as vaccination, remains critical to mitigating the zoonotic risks associated with canine leptospirosis.

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Key Words: leptospira, leptospirosis, shelter dog, Appalachia, Cumberland Gap region

P41 - Barriers to nutrition communication in small animal practice.

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When nutrition can impact health and disease processes, veterinary practitioners may recommend diet change. However, these recommendations are not always well received by pet owners. To help determine what factors were the most important barriers to this communication, and to identify potential solutions, an anonymous internet survey was circulated on the Veterinary Information Network (VIN) during 2022. Veterinary respondents identified both fixed-choice options for most common barriers and solutions to communication, as well as filling out free-text response options. The free-text response options were thematically grouped by an independent coder using Atlas.TI software. There were 561 survey respondents. The top 3 barriers identified were preconceived notions about nutrition (86% [483/561]), insufficient time during clinical appointments to discuss nutrition concerns (63% [352/561]), and owner resistance to discussing nutrition with veterinary personnel (41% [230/561]). The top 3 solutions identified by survey respondents to overcome barriers in pet nutrition communication during small-animal veterinary appointments were showing pet owners what veterinarians and pet care teams feed their pets (59% [332/561]), having honest conversations (58% [327/561]), and being specific with nutrition recommendations (49% [274/561]). Free-text responses also clarified nuances within these choices, identified several veterinary professional resources that were reported to be helpful, and highlighted how various stakeholders can contribute to improving nutrition discussions.

Key Words: canine, feline, veterinary, communication

P42 - Dogs with canine dysautonomia have altered fecal microbiome and enteric pathogen toxin genes.

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Canine dysautonomia (CD) is an idiopathic and frequently fatal disease characterized by widespread autonomic and enteric neuronal degeneration. An ingested neurotoxigenic microbe is hypothesized to be the cause. To investigate CD, we used fecal 16S and metagenomic sequencing approaches. The CD cases were within 10 d of clinical onset at the time of sampling. Full-length 16S rRNA amplicons were sequenced using the PacBio HiFi Revio platform (15 CD, 18 controls) and processed through DADA2/phyloseq pipelines for denoising, taxonomy assignment, and diversity analysis. Alpha diversity was significantly reduced in CD dogs: Shannon index (adj. $P = 0.041$) and Simpson index (adj. $P = 0.038$). Beta diversity assessed by PERMANOVA of Bray–Curtis dissimilarity demonstrated clear separation of CD and control groups ($P = 0.001$). Taxonomic profiling revealed increased relative abundances of *Enterococcus*, *E. coli–Shigella*, *Ruminococcus torques*, and *Terrisporobacter*. Pooled fecal DNA from CD and control animals was sequenced using the Illumina NovaSeq X platform. Reads were quality filtered, host DNA removed, and assemblies generated with SPAdes. BLASTx analysis against a modified DBETH toxin database identified several toxin-encoding genes in the CD group only including *C. difficile* ToxB, *Escherichia coli* heat-labile enterotoxin IIA, *C. difficile* binary ADP-ribosyl transferase CDT toxin, *C. botulinum* C2 toxin component II, and *C. sordellii* cytotoxin L. Ongoing work is evaluating the presence of these toxin genes in individual cases using quantitative PCR to determine association with CD.

Financial Support: American Kennel Club

Key Words: canine dysautonomia, 16S rRNA sequencing, metagenomics, bacterial toxin, microbiome

P43 - Total trihalomethanes in drinking water are not associated with an increase in bladder cancer test positivity in dogs.

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Preliminary research has potentially associated total trihalomethanes (TTHMs) in drinking water with bladder cancers such as transitional cell carcinoma (TCC) in dogs, although not all studies have found an association. With the advent of large-scale testing for TCC mutations in the urine of dogs using a test of mutations in the BRAF gene (associated with TCC), we have the ability to test at the population level whether BRAF mutation positivity is associated with TTHM levels in drinking water. Using BRAF test information from a large reference laboratory (IDEXX), which are typically only submitted by veterinarians when clinically indicated for suspect TCC, and including breed, sex, and neuter status information, linked to the zip-code level TTHM levels compiled by the Environmental Working Group (EWG.org) database, we fit a general linear model logistic regression on the TTHM level and individual BRAF test results (positive or negative), with covariates of age, sex, neuter status, and breed status (predisposed vs. non-predisposed binary variable) for a dataset of 64,144 dogs. Odds ratios with 95% confidence intervals were computed from the regression coefficient outputs. There was no association between TTHMs and BRAF positivity (OR 1.00, 95% CI 0.999–1.000). The covariates most associated with BRAF positivity were sex (females not predisposed, OR 0.75, 0.72–0.77), neuter status (neutered animals predisposed, OR 2.54, 2.37–2.71), and breed (with already-identified predisposed breeds more likely to be positive, OR 2.65, 2.50–2.85). In a follow-up analysis, we examined zip-code level testing rates (total number of tests divided by the estimated population of dogs based on census household information for that zip code), total positive tests, and TTHM concentration. At this population level, TTHMs were not associated with total test positivity (OR 1.00, 0.999–1.000). Only testing rates were positively associated with overall positivity (OR 1.31, 1.31–1.32). There are many limitations to this study, including that it is impossible to determine whether the dogs from the zip codes were drinking the municipal water. However, this is preliminary information to suggest that TTHMs in the drinking water are not associated with increased TCC in dogs. Future research that directly tests each dog's drinking water for TTHMs would be a better way to determine the effect of this exposure.

Key Words: canine, total trihalomethane, cancer, BRAF, epidemiology

P44 - A systematic review of factors associated with longevity in companion dogs.

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Companion dogs today are living longer lives, on average, than what is understood of their lifespan in previous decades. Individual factors contributing to this increased longevity have been studied, but the literature lacks a comprehensive evaluation assessing all of these myriad factors, combined. To better characterize what and how well-studied such influential factors on dog mortality and longevity are, and what further research still remains to be done to gain an accurate understanding of changes affecting dogs' average lifespan, we conducted a systematic review in accordance with PRISMA guidelines, and scored each paper according to GRADE criteria. Sixty-two manuscripts which included nonmodifiable factors (sex, breed, body size, brain characteristics, and drooling), modifiable life factors (neuter status, rabies vaccination status, behavior, body weight, reproductive investment, radiation, dental scaling frequency, and diet), and epigenetic factors (gene expression levels, telomere length, and methylation) that are correlated with longevity in pet dogs were included. In totality, the factors which have been most extensively studied are those which are not modifiable (i.e., size, breed, and sex). Larger breeds of dogs live shorter lives, but the underlying mechanisms for this are still only preliminarily studied. Among modifiable factors, surgical neutering is the most studied, but even this factor could benefit from a deeper understanding of its role in longevity at the individual level, in particular. For example, further research would be necessary to clarify why spaying females has such a strong positive effect on longevity, while neutering males has minimal effect, and also to determine breed-specific effects and timing of neuter procedures. Dogs who are overweight have a decreased longevity, but the effects of individual diets and ingredients have not been studied. This systematic review will inform future researchers as they seek to further illuminate factors associated with aging and longevity, and clinicians and veterinary practitioners in delivering timely and appropriate veterinary care and ensuring high quality of life standards throughout different life stages of an individual.

Key Words: small animal, dog lifespan, systematic review, aging and longevity, risk factor of aging

General Abstracts: Coronaviruses (Other Than COVID)—Posters

P46 - Porcine deltacoronavirus-related viruses in house sparrows.

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Porcine deltacoronavirus (PDCoV) is an emerging enteric pathogen in pigs and a newly recognized zoonotic coronavirus in humans. Genetic analyses suggest that PDCoV originated from avian deltacoronaviruses, with sparrow deltacoronaviruses (SpDCoVs) being the most closely related. House sparrows (*Passer domesticus*) frequently visit farms and interact directly with pigs in barns, raising the possibility of interspecies transmission. We hypothesized that PDCoV can be transmitted between pigs and house sparrows. To investigate this, 200 house sparrows near Ohio swine farms were sampled and screened for gammacoronaviruses and deltacoronaviruses using RT-PCR targeting the conserved RNA polymerase region. Deltacoronaviruses and gammacoronaviruses were detected in 18.0% (36/200) and 5.5% (11/200) of fecal samples, respectively. Genomic sequence analysis of representative samples revealed that SpDCoVs are closely related to, but not the direct ancestors of, PDCoVs. These SpDCoVs appear to be widespread in the US Midwest and may contribute to PDCoV evolution. Attempts to isolate SpDCoV from these samples in embryonated chicken eggs and 4 cell lines were unsuccessful. Because coronaviruses frequently cross species barriers to cause epidemics and/or pandemics in humans and livestock, these findings underscore the need for ongoing surveillance of deltacoronaviruses in diverse wild animals, livestock, and humans to safeguard public health.

Financial Support: The project was supported by The Ohio State University (OSU), College of Food, Agricultural and Environmental Sciences (CFAES) SEEDS grant (2020-009, Q.W., principal investigator). Salaries and research support were provided by state and federal funds appropriated to CFAES, OSU. D.L. was supported by Shandong Provincial Government Scholarship Program.

Key Words: porcine deltacoronavirus, sparrow, deltacoronavirus, coronavirus, gammacoronavirus

P47 - Investigation of antigenic characterization of highly pathogenic porcine epidemic diarrhea virus isolates.

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Porcine epidemic diarrhea virus (PEDV) has posed significant financial threats to the domestic pig industry over the last 3 decades in South Korea. Since the introduction of highly pathogenic (HP)-G2b PEDV into South Korea in 2013, HP-G2b PEDV has become most predominant and independently evolved, forming distinct nation-specific clades. The domestic isolates are phylogenetically divided into 6 disjunct clades based on the geographic distribution: nationwide (NW), Kyeongnam & Jeonnam provinces (KJ), Chungcheong & Kyeongbuk provinces (CK), Jeju Hallim region (JH), Jeju Daejeong region (JD), and geographically nonclassified (NC). In recent years, 2 CK variants, designated CK.1 and CK.2, have emerged as CK subclades. Since commercial HP-G2b vaccines in South Korea were developed with the seeds isolated in 2014, the accumulation of mutations in field strains for the past decade may have led to antigenic drift, raising immune escape mutants. To investigate the efficacy of vaccines against diverse variants circulating in South Korea, we first analyzed the spike (S) gene sequences of clade-representative strains to identify genetic variation within neutralizing epitopes. Considering these genetic data, we generated 13 recombinant viruses by replacing the S gene of a vaccine-like strain KNU-141112 with that from each clade-specific PEDV using a reverse genetics system. These viruses were then subjected to cross-neutralization assays with antisera collected from sows vaccinated with a series of commercial HP-G2b PEDV vaccines. Cross-neutralization assays revealed that the antisera against HP-G2b PEDV vaccines produced the highest neutralizing activity against the homologous icKNU-141112 strain. In contrast, their neutralizing antibody titers were consistently reduced when reacted with each recombinant virus carrying heterologous S genes. Notably, the antisera showed a lower neutralizing activity against the CK, CK.1, or CK.2 virus compared with neutralization against other clade viruses; however, the CK-like viruses had a lower genetic divergence at the S gene level compared with other clade viruses. These findings suggest the possibility that the conserved mutations sharing among the CK-like strains represent critical residues involved in vaccine-induced immune escape. Further studies will be required to investigate the functional role of these mutations, particularly in neutralizing epitope regions, and to assess their implications for vaccine design.

Key Words: porcine epidemic diarrhea virus, spike protein, antigenic variation, reverse genetics, cross-neutralization, vaccine efficacy

P49 - Bovine coronavirus: Identification of new characteristics.

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Bovine coronavirus (BCoV) is an enteric pathogen causing neonatal calf diarrhea and winter dysentery, and is also associated with respiratory disease such as bovine respiratory disease complex or shipping fever. BCoV infection occurs through the fecal–oral route or aerosol inhalation, resulting in viral shedding by nasal secretion and feces. Despite their different sites of isolation, both enteric and respiratory BCoV have been shown to replicate in the respiratory and intestinal tracts, making it difficult to accurately define their tissue tropism. Therefore, we inoculated calves with enteric BCoV to investigate its tissue tropism and sites of replication. Four colostrum-fed Holstein calves (6–13 d old) were intranasally inoculated with 6 mL of BCoV (10^{6} $TCID_{50}$/mL) isolated from diarrhea, along with 2 additional control calves. Before inoculation, all calves were tested negative for BCoV, bovine viral diarrhea virus, and group A bovine rotavirus. Nasal and rectal swabs were collected at 1, 3, 5, 7, and 9 d post-infection (dpi) and tissue samples were collected during necropsy on 9 dpi. The presence of BCoV RNA was evaluated using real-time RT-PCR. Tissue samples were also subjected to H&E staining and immunohistochemistry (IHC) to assess histological lesions and viral antigen distribution. All inoculated calves developed diarrhea starting at 1 or 3 dpi but showed no respiratory signs. Nevertheless, both nasal and fecal shedding were detected starting at 1 dpi and continued until 9 dpi. At necropsy, there was no characteristic lesions in the lungs or intestines except for the enlargement of mesenteric lymph nodes. However, histopathological lesions including enteritis, tonsillitis, mild interstitial pneumonia, and hepatitis were identified. Real-time RT-PCR and IHC showed the broad tissue tropism of BCoV by confirming the presence of BCoV antigen in liver, kidneys, and abomasum, as well as tonsils, trachea, lungs, and both small and large intestines, especially in their epithelial cells. By IHC analysis, BCoV antigen showed a greater distribution in the intestines than in the respiratory tracts. This is the first study to identify the presence of BCoV antigens in the abomasal glandular epithelium, biliary epithelium, and renal tubules. These results suggest that the enteric BCoV has the potential to cause diseases other than respiratory and enteric disorders owing to broad tissue tropism.

Financial Support: This research was funded by the Korea Institute of Planning and Evaluation for Technology in Veterinary Drugs and Medical Devices for Economic Animal Domestic Production Technology Development (Grant no. RS-2025-02216600).

Key Words: bovine coronavirus, epithelial cell, histological lesion, tissue tropism, viral antigen

General Abstracts: Diagnostic Testing—Posters

P50 - Evaluation of a novel point-of-use isothermal diagnostic tool, the HyperKit, for rapid detection and quantification of *Salmonella* in poultry samples.

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Salmonella is a major cause of foodborne illness worldwide, with poultry products being a leading source of contamination. Current testing methods for *Salmonella* are not adequate for the poultry industry needs (takes after 24–48 h) and require expensive laboratory equipment and extensive training. Consequently, there is an immediate need for rapid and reliable diagnostic strategies to detect *Salmonella* in poultry products. This study evaluated a novel point-of-use isothermal diagnostic tool, the HyperKit Total *Salmonella* (HK), for the rapid detection and quantification of *Salmonella* in poultry carcass. HK is composed of a rapid one-step sample preparation tool and a shelf-stable lyophilized isothermal master mix, with the following specifications: reaction volume: 50 µL; target: *invA*; incubation at 65°C; 495 nm fluorescent reading; results within 60 min. The detection sensitivity and accuracy of HK were assessed using artificially spiked and commercial chicken primal rinse samples (n = 64 and 21 samples, respectively). The reproducibility and repeatability of HK were assessed by 3 operators. *Salmonella* concentrations were validated by plating on selective agar (reference method). The detection specificity was validated using 46 *Salmonella* serotypes (c.a., cfu/reaction) and 37 non-*Salmonella* strains (c.a., 10^6 cfu/reaction). HK detected *Salmonella* in all artificially spiked samples (1.0 to 7.5-log cfu/mL; n = 57) with high semiquantitative accuracy ($r^2 = 0.93$; $P < 0.001$). Data demonstrated that the quantitative precision of HK was high (±0.18-log compared with the reference method) when the *Salmonella* concentration was at least 1.5-log cfu/mL. Furthermore, strong repeatability (0.16-log; 95% CI: 0.11–0.21) and reproducibility across operators were observed with HK. Similarly, trends were observed in the commercial samples; *Salmonella* was detected when the concentration was above 4 cfu/mL, while enrichment was required for lower concentrations. In the specificity study, HK detected all *Salmonella* serotypes (n = 46), while none of the non-*Salmonella* strains (n = 37) were positive. In conclusion, this study demonstrated that HK enables the convenient, rapid, and specific detection of low concentrations of *Salmonella* in chicken carcasses, highlighting the technological compatibility of HK for potentially implementing this diagnostic tool in a fast-paced production environment, such as the poultry industry.

Key Words: *Salmonella*, chicken carcass, point-of-use diagnostic, isothermal amplification, food safety

P51 - Improving diagnostics for SARS-CoV2 transmission amongst white-tailed deer.

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Since 2020, SARS-CoV2 has been identified in multiple animals across 40 states in the US. Of particular concern is over 100 spillover infections in white-tailed deer (WTD) (*Odocoileus virginianus*) followed by continued deer-to-deer transmission. Continued circulation of SARS-CoV-2 in animals increases the risk of novel variant emergence, as evidenced by the detection of the most divergent SARS-CoV2 in WTD in Ontario, Canada. The ongoing SARS-CoV-2 circulation in the WTD, together with the growing commercial success of WTD farming and the heightened risk of wildlife–livestock-human spillover, represents an emerging epidemiological vulnerability that must be monitored. Current detection and variant identification of SARS-CoV2 relies on RT-PCR followed by genomic sequencing, approaches that are costly and impractical for routine field surveillance. To address this gap, we have evaluated a cost-effective, portable approach: PCR-tiling coupled with real-time sequencing. Specifically, the PCR tiling method of SARS-CoV-2 virus with Rapid Barcoding Kit 96 V14 and Midnight RT-PCR Expansion from Oxford Nanopore Technologies was used for detection. Preliminary analysis shows detection of SARS-CoV2 USA-WA1/2020 isolate with sensitivity of 66.7% with input concentrations as low as 15,000 genome copies/µL (calculated via qPCR). Accurate lineage prediction was approximately 33% at ~20,000 genome copies/µL. Discrepancies were not due to inappropriate lineage assignment, rather due to low-quality basecalling in Nanopore data, which may be improved upon with further optimization. Performance metrics were defined as follows: True positives: exact variant matches between experimental calls and whole-genome sequencing (WGS) of the USA-WA1/2020 isolate. True negatives: agreement at nonvariant positions. False positives: variants called in experimental data but absent in WGS. False negatives: variants present in WGS but missed in experimental data. Refining and increasing accessibility of these molecular techniques will enable scalable, cost-effective, field-adaptable monitoring of SARS-CoV-2 in white-tailed deer populations and heighten response to future zoonotic disease outbreaks.

Financial Support: This project was supported by Agriculture and Food Research Initiative Competitive Grant no. 2023-70432-41395 from the USDA National Institute of Food and Agriculture

Key Words: SARS-CoV2, white-tailed deer, diagnostics, nanopore sequencing

P52 - Development of monoclonal antibodies against SARS-CoV-2: Construction of chimeric neutralizing antibodies for therapeutic applications.

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The COVID-19 pandemic caused by SARS-CoV-2 continues to be a major global health and economic burden. While human-to-human transmission is the predominant mode of transmission, SARS-CoV-2 can infect various animals, including wild and domestic feline species. This raises the concern about zoonotic transmission and reverse-zoonotic transmission, along with the virus mutations appearing as the virus goes back and forth between species. There is an urgent need for molecular and immunological tools to further develop SARS-CoV-2 diagnostics and treatment to control cross-species transmission. In this study, we generated and characterized a comprehensive panel of monoclonal antibodies (mAbs) against multiple SARS-CoV-2 proteins. Synthetic genes encoding key antigenic regions of viral proteins, including nonstructural proteins (nsp1–3, nsp7–8), nucleocapsid (N), spike (S), and ORF3a, were cloned into the pET28a vector, expressed in *Escherichia coli* as His-tagged recombinant proteins. Purified proteins were used for mouse immunization. After fusion of mouse splenocytes with myeloma cells, hybridoma screening yielded mAbs specific to each target protein, with their specificity validated by immunofluorescence assay and Western blot. Epitope mapping identified specific epitopes recognized by each mAb. Notably, several mAbs target the spike protein. Specifically, mAb #220-C1 recognized the neutralizing epitope in the S2 region, while mAbs #59–2 and #8–9 recognized the neutralizing epitopes in the receptor binding domain. This panel of neutralizing mAbs was selected for constructing mouse-feline and mouse-human chimeric antibodies by fusing murine variable regions to the feline or human constant region of the antibody. The neutralizing activity of these chimeric antibodies was confirmed using a pseudovirus system that expressed the SARS-CoV-2 spike protein. Our study provides a valuable panel of monoclonal antibodies for SARS-CoV-2 detection. Chimeric neutralizing antibodies could be used as potential therapeutic agents against SARS-CoV-2 infection in feline and human populations.

Financial Support: NIH

Key Words: SARS-CoV-2, neutralizing antibody, mouse-feline/human chimeric antibody, therapeutic agent

P53 - Species-specific genetic markers for *Listeria* identified through pangenome and core genome analyses.

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Listeria spp. are gram-positive foodborne pathogens that present ongoing public health and food safety challenges. Reliable detection requires identification of gene-specific markers that enable accurate diagnostics and species-level differentiation. This study aimed to characterize genomic diversity across the *Listeria* genus and to identify novel species-specific genetic markers for molecular diagnostic development. A total of 33 high-quality genomes representing 13 *Listeria* species were retrieved from the NCBI database and curated using CheckM. Genome annotation and pangenome analysis were performed using the Roary ILP Bacterial Annotation Pipeline (RIBAP). Candidate species-specific targets were extracted from the RIBAP presence/absence matrix, validated using NCBI MegaBLAST and Geneious Prime, and functionally annotated with the Genome Annotation and Information Analysis (GAIA) platform. Core genome analyses revealed significant diversity, with 856, 665, 362, 89, and 16 genes conserved at 60%, 70%, 80%, 90%, and 95% nucleotide identity thresholds, respectively. At the 95% threshold, highly conserved genes such as *rpmG2*, *rpmJ*, *mnmE*, *rplP*, and *rpsS* were identified, each linked to critical molecular and cellular processes. Accessory gene counts increased from 10,400 at 60% identity to 25,486 at 95%, highlighting extensive genomic variability beyond the core genome. Several species-specific gene candidates, mainly annotated as hypothetical proteins, demonstrated strong diagnostic potential with in silico sensitivity of 90% and specificity of 98%–100%, and validated with 100% sensitivity in vitro. These findings establish novel diagnostic markers that enable accurate *Listeria* species differentiation, provide insight into the evolutionary dynamics of the genus, and support improved surveillance and detection strategies for food safety applications.

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Key Words: *Listeria*, foodborne pathogen, pangenome, diagnostics, genomics, diversity

P54 - Genome-resolved metagenomics uncovers the prevalence of novel treponemes in free-ranging elk (*Cervus canadensis*) hooves with and without *Treponeme*-associated hoof disease (TAHD).

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Treponeme-associated hoof disease (TAHD) is a progressive, polybacterial infection affecting free-ranging elk (*Cervus canadensis*) in the Pacific Northwest US. Previous genome-resolved metagenomic work identified several novel treponemes associated with diseased hooves; however, their prevalence in TAHD-positive versus TAHD-negative hooves remains unknown. This study aimed to determine the prevalence of novel treponeme genomospecies using comparative metagenomics in elk with and without TAHD. Twenty-nine elk hoof biopsy samples were subjected to Illumina NovaSeq X Plus shotgun metagenomic sequencing, including 17 TAHD-positive and 12 TAHD-negative, 5 of which were from TAHD-endemic regions and 7 from nonendemic areas. Following host read removal and quality filtering, taxonomic profiles and microbial diversity were analyzed using Kraken2/Bracken, *phyloseq*, and *vegan*. The prevalence of 5 representative metagenome-assembled *Treponema* genomes (MAGs), *Treponemataceae Phy1*, *Treponemataceae Phy2*, *Treponema* sp. A13, *T. phagedenis*, and *T. pedis*, were evaluated by *inStrain* mapping at the genome (≥0.5 breadth) and contig (≥0.1 breadth) levels. *Treponemataceae Phy1* was the most consistently detected genomospecies, present in 17/17 (100%) TAHD-positive samples, 3/5 TAHD-negative samples from the endemic area, and absent in TAHD-negative samples from the nonendemic area, suggesting its role as an early colonizer or regionally maintained pathogen. *T. pedis* and *T. phagedenis* were each detected in 16 of 17 diseased samples, while *Treponemataceae Phy2* and *Treponema* sp. A13 were found in 14 and 12, respectively. The TAHD-positive samples exhibited significantly higher microbial diversity (Kruskal–Wallis, $P < 0.05$) with enrichment of *Treponema*, *Porphyromonas*, *Mycoplasmopsis*, and *Trueperella*. Seven high-quality MAGs were reconstructed from 3 TAHD-positive samples: *T. pedis*, *T. phagedenis*, *Treponemataceae Phy2*, *Mycoplasmopsis fermentans*, *Metamycoplasmataceae* sp., *Corynebacterium* sp., and *Bacteroides* sp. (all >90% completeness, <1% contamination). The recovery of non-*Treponema* MAGs corroborated community profiling results and further confirmed the polybacterial nature of TAHD. The consistent detection of *Treponemataceae Phy1* across diseased hooves underscores its potential role as a key etiological agent supporting the ongoing development of PCR assays targeting novel *Treponemataceae* for TAHD surveillance and early detection of TAHD in elk populations.

Financial Support: Funding was provided by the State of Washington through accounts dedicated to elk hoof disease research

Key Words: treponeme-associated hoof disease (TAHD), *Treponema*, metagenomics, elk, diagnostics, prevalence

General Abstracts: Disease Pathogenesis—Posters

P56 - Molecular insights into liver abscess pathogenesis through transcriptomic analysis of jejunum and liver in feedlot steers.

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Liver abscesses are among the most prevalent health and economic challenges in feedlot cattle, contributing substantially to liver condemnation and reduced carcass value. Despite their importance, the molecular mechanisms driving abscess formation remain incompletely understood. Current evidence suggests that bacterial translocation from the gastrointestinal tract to the liver occurs following disruption of mucosal barriers, initiating hepatic inflammation and abscess development. While the rumen has been the primary focus of pathogenesis models, emerging evidence indicates that distal intestinal segments may also contribute to abscess pathogenesis through altered barrier integrity and immune signaling. This study aimed to explore transcriptomic profiles of jejunum and liver tissues from feedlot steers differing in liver abscess status to gain insight into gut–liver interactions. Samples were collected from 18 steers at slaughter and classified into 3 groups: severe abscess (A+), mild abscess (A−), or no abscess (control; n = 6 per group). Total RNA was extracted, and sequencing libraries were prepared for next-generation sequencing. Differential gene expression will be analyzed using DESeq2, with significance defined as an adjusted $P \leq 0.05$ and $|\log2 \text{ fold-change}| \geq 1$. Functional enrichment using Gene Ontology and KEGG pathways will be applied to identify biological processes associated with immune regulation, epithelial barrier integrity, hepatic inflammation, and metabolic adaptation. Results are currently under analysis. We anticipate that this work will reveal key molecular signatures underlying intestinal–hepatic communication during liver abscess formation. Such insights will contribute to a better understanding of disease pathogenesis and may support future interventions to improve cattle health and production efficiency.

Key Words: liver abscess, gut-liver axis, transcriptomics, immune regulation, epithelial barrier

P57 - *Campylobacter hepaticus* transcriptomics identified the genes involved in the spotty liver disease (SLD) pathogenesis.

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Spotty liver disease (SLD) is an emerging bacterial disease of laying hens that poses a significant threat to commercial egg production. Caused by *Campylobacter hepaticus*, SLD is characterized by a sudden drop in egg production, increased mortality, and distinctive liver lesions. Despite its economic impact, the molecular mechanisms driving *C. hepaticus* virulence remain poorly defined. Understanding how this pathogen adapts to different host-relevant environments is crucial for developing targeted control strategies to control SLD. In our study, we applied RNA sequencing to investigate the transcriptional responses of *C. hepaticus* in multiple host-associated environments: exposure to bile, infection of chicken liver hepatocellular carcinoma (LMH) cells, and direct isolation from hepatic lesions of naturally infected chickens in the field. RNA sequencing was performed on 3 biological replicates per condition. Differential expression analysis was conducted using a Generalized Linear Model (GLM) with a negative binomial distribution, as implemented in CLC Genomics Workbench with stringent statistical criteria ($P < 0.01$, FDR <0.05, fold change >2). Selected gene targets were validated by quantitative real-time PCR. We observed strikingly distinct gene expression signatures across the different conditions tested, with *C. hepaticus* exhibiting differential expression of 412 genes in bile, 125 genes in LMH cells, and 26 genes in infected liver tissue. Bile exposure triggered upregulation of genes related to motility, cell envelope remodeling, glycosylation, nitrate respiration, and multidrug efflux systems, indicating a stress-adaptive, metabolically active lifestyle. LMH cell infection was characterized by downregulation of genes associated with motility, oxidative stress response, chaperones, and core metabolic processes, suggesting a low-activity, intracellular dormant lifestyle. Infected liver tissue showed selective upregulation of genes involved in nitrate respiration, sulfur metabolism, and pyridoxal phosphate homeostasis. Upregulation of the immune evasion-related gene *cmeA* and glycosyltransferase genes was also observed, along with downregulation of the major outer membrane porin and stress response chaperones. These insights provide a basis for identifying novel virulence determinants and may help in the development of targeted interventions, including vaccines and antimicrobial therapy, to control SLD in commercial poultry operations.

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Key Words: bile stress, *Campylobacter hepaticus*, intracellular persistence, layer chicken, spotty liver disease, transcriptomics

P58 - Establishment of turkey and chicken respiratory cell culture model for the isolation and propagation of avian metapneumovirus subgroup B.

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The nasal turbinate, trachea, and lungs are key sites for respiratory viral infections in poultry. Avian metapneumovirus subgroup B (aMPV B), recently identified in the United States, caused significant mortality in turkeys and chickens during a 2024 outbreak. Currently, no species-specific respiratory cell culture systems are available for isolating this virus in poultry. This study aims to develop primary respiratory cell cultures from turkeys and chickens for aMPV B isolation and propagation. Tracheal tissues were collected from 4-d-old turkey poults and 23-d-old turkey embryos. After collagenase digestion, cells were cultured in DMEM-F12 with 5% fetal calf serum (FCS) and growth factors, followed by expansion in DMEM-10. Immunohistochemistry confirmed a myofibroblast-like phenotype, with cells staining positive for vimentin and α-smooth muscle actin, and negative for cytokeratin. Twenty-two aMPV B-positive field samples (Ct 14.6–25.06) were inoculated on turkey tracheal cells, QT-35, Vero E6, and chicken embryo fibroblast cells. Virus replication occurred only in the turkey tracheal cells. To expand this model, we isolated lung cells from 5-week-old chickens, another susceptible host. Immunohistochemistry confirmed an epithelial-like phenotype, with cells staining positive for pan-cytokeratin. These cells are currently being characterized for their potential to support aMPV B growth. This research establishes a valuable species-specific model for studying aMPV B in turkeys and explores a parallel approach in chickens, addressing a critical gap in respiratory virus research in poultry.

Financial Support: South Dakota Agricultural Experiment Station

Key Words: tracheal cell, poultry, pathogenesis, avian metapneumovirus

P59 - Pan-genome-wide association study of *Avibacterium paragallinarum*.

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Avibacterium paragallinarum (AP) is the causative agent of infectious coryza, an economically important respiratory disease in poultry. The recent discovery of nonpathogenic (npAP) strains co-circulating with pathogenic (pAP) strains has necessitated a deeper understanding of the genetic factors underpinning virulence. This study aimed to identify genomic determinants of pathogenicity in AP through a pan-genome-wide association study (GWAS). Whole-genome sequences of 87 pathogenic and 14 nonpathogenic AP isolated were analyzed. The nonpathogenic isolates were obtained from naive-healthy layer chicken sites from 6 separate events across 4 states (2023–2024) while the pathogenic isolate genomes were downloaded from NCBI. Pangenome construction revealed a pangenome consisting of 5,012 gene clusters, with a large accessory genome of (~66%). Phylogenetic analysis showed distinct clustering between the pathogenic and nonpathogenic isolates, indicative of a strong population structure, while no genes were exclusive to the nonpathogenic isolates, 3 gene clusters—hmp, nsrR, and esiB—were found in pathogenic strains. To account for the confounding effects of population structure, we employed 3 distinct GWAS methods: a phylogeny-aware approach (Scoary), a linear mixed model (pyseer), and a machine learning classifier (Random Forest). The analysis highlighted the critical importance of correcting for population structure and the advantage of using multiple GWAS approaches where there is no objective reason of choice. All the initial associations identified by Scoary were nullified after phylogenetic correction; pyseer identified 2 significant gene cluster associated with pathogenicity: glnH, an ABC transporter glutamine-binding protein, and an unannotated gene. There was no consensus gene set identified by the 3 methods, suggesting that pathogenicity in AP is a complex trait. The combined results, however, provide a robust list of candidate genes for future functional validation to elucidate their potential roles in AP virulence.

Financial Support: This study was supported in part by funding from the Egg Industry Center (Grant number SG2706645) and the US Poultry, Egg Association Board Initiative Grant project # BRF-17 and USDA Capacity Grant number GR-028618-00002.

Key Words: microbial genome-wide association study (GWAS), *Avibacterium*, population structure

P60 - Modulation of intestinal barrier integrity by bacterial and viral ligands in polarized porcine epithelial cells.

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Intestinal epithelial cells play a significant role in enteric disease pathogenesis and in mediating innate immune responses in the gastrointestinal tract of food animals. Tight junctions (TJs) are essential for maintaining barrier integrity by regulating permeability and preventing translocation of harmful substances and microbes. Disruption of TJs increases intestinal permeability, or “leaky gut,” which is implicated in enteric diseases and other gastrointestinal disorders. Bacterial and viral ligands, including flagellin, poly I:C, and imiquimod, can modulate TJ function, potentially via Toll-like receptor–mediated signaling, yet their effects on porcine intestinal epithelial cells remain largely unexplored. In this study, we developed an in vitro polarized porcine intestinal epithelial model (IPEC-J2 cells) to investigate the dose and time-dependent effects of these ligands on barrier integrity. Transepithelial electrical resistance (TEER) was measured to assess barrier function, and immunofluorescence staining was used to examine localization of key TJ proteins, including Zonula Occludens (ZO-1), claudin-1, claudin-3, and occludin. Treatments with flagellin, poly I:C, and imiquimod caused significant dose- and time-dependent reductions in TEER, indicating compromised barrier integrity. These findings provide mechanistic insights into how bacterial and viral ligands affect intestinal permeability and contribute to epithelial barrier dysfunction. This model may also serve as a platform to screen interventions aimed at restoring gut barrier integrity, supporting the development of targeted strategies for gastrointestinal disorders in food animals and humans.

Key Words: tight junction, intestinal permeability, IPEC-J2, flagellin, poly I:C, imiquimod

General Abstracts: Epidemiology—Posters

P61 - Role of environmental mycobacteria on the efficacy of the BCG vaccine against bovine tuberculosis.

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Bovine tuberculosis, caused by *Mycobacterium bovis* is a zoonotic, economic, and public health importance disease. To rule out the influence of environmental mycobacteria in the diagnosis, the antigens PPD-*bovis* and PPD-*avium* are used in the comparative cervical test. This is because these bacteria may cross-react with the tuberculin test, the official test to determine herd prevalence. The same concerns arise in studies determining the efficacy of the BCG vaccine against tuberculosis in cattle. Therefore, the objective of this study was to eliminate any role of environmental mycobacteria in the efficacy of the BCG vaccine against cattle tuberculosis. Thirty-five goats, 3 to 5 mo of age, of an Alpine and Nubian crossbreed, tuberculosis- and paratuberculosis-free, were used. Goats were randomly distributed into 5 groups of 7 animals each: 4 groups vaccinated with the BCG vaccine, and 1 unvaccinated control group. Blood samples were taken every 2 to 3 weeks for a period of 7 mo; this blood was stimulated with the antigens PPD-*bovis* and PPD-*avium* to measure concentration of IFN-y with the Bovigam® kit (Prionics AG, USA). Our results show that the IFN-y response to stimulation of peripheral blood cells with the antigen PPD-*bovis* was consistently higher than that obtained with PPD-*avium* in vaccinated animals, compared with the control groups, after challenge with a field strain of *M. bovis*. Likewise, the overall average response in all vaccinated groups was significantly higher against PPD-*bovis* than against PPD-*avium*, which confirms the specificity of the immune response induced by the vaccine. These findings suggest that the involvement of environmental mycobacteria in modulating the response to the BCG-Phipps vaccine was minimal or nonexistent in this experiment, and that it did not influence its protective efficacy.

Financial Support: Secretaría de Ciencia, Humanidades, Tecnología e Innovación (Secihti)

Key Words: vaccination, BCG, goat, PPD-*bovis*, PPD-*avium*, IFN-y

P62 - A guinea pig model evaluating the potency and safety of the BCG vaccine.

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Bovine tuberculosis represents a persistent challenge for the national dairy industry, causing significant economic losses and representing a risk to public health. To control and eliminate the disease, it is important to explore new options to reduce prevalence. Vaccination with BCG is presented as a promising option; however, it is essential to evaluate safety and potency of the vaccine in a guinea pig model before using it in cattle. Twenty-five *Cavia porcellus* guinea pigs, Hartley breed, were distributed into 5 vaccinated groups and a non-vaccinated control group. For the safety test, dermal reaction was measured to determine the proportion of antigenic reactivity. In addition, a descriptive analysis of the variables weight and temperature was carried out, as well as the presence or absence of lesions suggestive of bovine tuberculosis. An ANOVA test with a *P*-value ≤0.05 was used to determine significant differences between groups. The results show no statistical differences ($P \leq 0.05$) between the groups for the variables weight and temperature, which indicates that, regardless of the treatment, the BCG vaccine is safe and does not cause weight loss. Similarly, the guinea pigs showed good appetite and no signs of disease. Antigenic reactivity was performed with PPD-*bovis* readings, and the result was interpreted based on the group that received the dose of 1×10^6 cfu, in which an antigenic reactivity of 90% was obtained. With these results, we demonstrate that the vaccine is safe and confers protection to guinea pigs and that it can be used in cattle for massive field tests.

Key Words: guinea pig, safety, potency, bovine tuberculosis, vaccination

P63 - Spatio-temporal patterns and space-time cluster analysis of foot-and-mouth-disease outbreaks in Punjab, Pakistan, from 2021 to 2022.

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Foot-and-mouth disease (FMD) is a highly contagious transboundary animal disease with widespread occurrence across Asia, Europe, and Africa, causing significant economic losses, particularly in low- and middle-income countries like Pakistan, where livestock plays a critical role in the national economy and household food security. This study aimed to investigate the spatiotemporal trends of FMD outbreaks in the Punjab province, Pakistan, and to identify the space-time disease clusters. The data of 881 FMD outbreaks reported in the Punjab province during 2021 to 2022 was analyzed to test the hypothesis of complete spatial randomness. A retrospective space-time permutation method was used to identify the space-time clusters using SaTScan. The FMD virus serotype O was the most widely distributed, accounting for more than 89% (n = 789) of the outbreaks, followed by serotype A (6%, n = 54) and Asia-1 (2.5%, n = 22), whereas in 2% (n = 16) of the outbreaks serotype A and O co-existed. In these 881 FMD outbreaks during 2021 to 2022, a total of 4,707 animals were affected. The majority of cases occurred in mixed cattle and buffalo herds (47%, n = 2,207), followed by cattle-only herds (45.4%, n = 2,138) and buffalo-only herds (6.4%, n = 297). Small ruminants were rarely affected, representing only 1% of cases (n = 45), whereas a few outbreaks involved all species (n = 32) cattle and goats (n = 6). In 2021, the highest number of outbreaks was recorded in March (n = 287), whereas in 2022, outbreak numbers were lower overall, peaking in January (n = 23). Across both years, the majority of outbreaks occurred between January and April, suggesting a potential seasonal trend. SaTScan identified 14 significant space-time clusters during 2021 to 2022, with a maximum cluster radius of 60.35 km ($P < 0.05$). Most of the clusters (n = 13) occurred in 2021, whereas only one cluster was detected in 2022. In conclusion, findings about significant spatial autocorrelation and space-time clustering of FMD outbreaks will help prioritize resource allocation and targeted surveillance and control for FMD in Punjab, Pakistan.

Key Words: foot-and-mouth disease, disease outbreak, spatio-temporal analysis, disease surveillance, space-time clusters

P67 - West Nile virus surveillance in Georgia, 2024–2025.

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Vector-borne infectious diseases, such as West Nile fever, cause a significant fraction of the global infectious disease burden; indeed, nearly half of the world's population is infected with at least 1 type of vector-borne pathogen. The considerable economic, ecological, and public health impacts of vector-borne diseases are expected to continue, given limited domestic and international capabilities for detecting, identifying, and addressing likely epidemics. A two-stage sero-survey design was used for sample size calculation for suggested risk categories. For detection of municipalities (districts) to be sampled within the regions we used confidence level = 95%; population size, number of districts = 36; expected prevalence = 10%. Of these, 23 municipalities (districts) were identified to be tested for West Nile fever diseases. Sample sizes for West Nile fever were calculated separately using online sample size calculation: confidence level = 95%; population size, number of equines = 8,000; expected prevalence = 10%; accepted error = 4%. According to this calculation, 217 animals (equine) were identified to be sampled in the 1-year project period, proportionally distributed in 23 municipalities (districts). Samples were collected from high-risk regions in Georgia. In total, 217 serum and 217 blood samples were collected from the target population (horses); a total of 138 owners from 25 villages were involved in the study. ELISA test kits were used for testing: (1) INgezim West Nile IgM Capture antibody ELISA for the detection of specific IgMs to West Nile virus (WNV) in equine serum samples, and (2) INgezim West Nile Blocking ELISA for the detection of specific antibodies to WNV in equine serum samples. Out of the tested 217 samples, 59 (27.2%) were positive, indicating the presence of specific antibodies and suggesting prior exposure to the virus. The objectives of sample collection in Georgia were early detection, risk assessment, mapping hotspots, public health response, education, and awareness. Overall, the goal of West Nile surveillance in Georgia is to protect public health by preventing and minimizing the impacts of WNV outbreaks through proactive monitoring, response, and education efforts.

Financial Support: Defense Threat Reduction Agency (DTRA)

Key Words: West Nile fever surveillance

P69 - Comparison of target-enriched resistomes from swine manure pit samples.

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The overall objective of this project is to pilot the use of manure pit sampling and enriched resistome analysis as a method for monitoring antimicrobial resistance patterns in grow-finish swine farms. Sampling without the need to enter barns minimizes biosecurity risks associated with on-farm sample collection. The methodology has the potential to provide a comprehensive profile of antimicrobial resistance genes in the manure pit matrix. Sub-objectives of the project included understanding farm-to-farm and seasonal variability of the manure pit resistome; comparing the resistome of different sampling locations within each manure pit; assessing the effects of different sample storage conditions on the resistome profile; and comparing the resistome profile between herds with different health characteristics. A convenience sample of 21 grow-finish farms in the upper Midwest of the United States was enrolled into the project. Sampling was initiated in 2024 and is ongoing. Manure pit samples from each farm are obtained using a custom sampler, from multiple prespecified locations in each manure pit. Sampling is being performed over multiple seasons, with up to 4 visits per farm. For a subset of visits, samples are being stored using 1 of 3 methods: placement on ice and then in −80°C; placement into preservation medium at room temperature; or shipment at ambient temperature for standard nutrient testing. All samples will be subjected to molecular enrichment for antimicrobial resistance genes and sequenced using the Oxford Nanopore Technologies platform. Generated data will be analyzed to characterize the resistome, and resistome metrics will be compared across farms, seasons, manure pit sampling locations, and storage conditions. Data generation is ongoing. Preliminary results indicate differences in resistome profiles across farms and seasons, although the resistome profile at the antimicrobial class level is relatively consistent across all samples. The influence of within-pit location and storage conditions on the resistome profile have not yet been evaluated. Manure pit sampling has the potential to be a minimally invasive and relatively biosecure method for obtaining information about a swine farm's resistome profile over time. Procedural details related to sample collection and storage may need to be highly standardized to support robust inferences regarding resistome differences across farms and time.

Financial Support: USDA APHIS AP25VSSP0000C004

Key Words: resistome, antimicrobial resistance, metagenomics, swine

P70 - Decay of chronic wasting disease prion detection at infectious deer carcass sites.

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Chronic wasting disease (CWD) is a fatal prion disease of cervids with significant animal health and economic consequences. CWD is transmitted both directly and through the environment, but it is unknown how long CWD prions can survive and transmit in soils in the natural environment. Our objective was to determine the risk of detecting CWD prions in soils at carcass sites of CWD-infected white-tailed deer over space and time in southwest Wisconsin, an area of high CWD prevalence. We identified 3 fresh CWD mortalities (2 in end-stage disease) and collected soil samples within a few days of death, and then longitudinally for up to 2 years. We also collected soil at 2 marked CWD-positive carcass sites from previous research; these sites were up to 4 years old. For the first 8 mo of monitoring, soils were collected at 0, 3, 6, and 9 feet from the carcass; thereafter, soils were collected only at the center of sites. CWD prions were detected in soils using real-time quaking-induced conversion (RT-QuIC). Statistical analysis using generalized linear mixed models showed that detection of CWD prions in soils was uncommon but higher at CWD-positive sites than at 8 control carcass sites (odds ratio = 4.6). Soils were increasingly likely to test positive for CWD for at least 8 mo after death, and the odds of testing positive decreased with distance from the carcass, although this latter relationship did not achieve statistical significance. At site centers, prion detection appeared to wane after about 2 years, with detections rare 4 years after death. The exact time at which prions began to wane remains uncertain, due to our small sample sizes. Nevertheless, our results indicate that, even at carcass sites from deer in end-stage CWD, detection of CWD prions in soils is relatively limited when using prion extraction methods that mitigate the false seeding events otherwise common when using RT-QuIC with soil samples. Further, although prion detection initially increased in soil as deer carcasses decomposed, after about 4 years, detections were extremely rare. More long-term research is needed to obtain a more precise estimate of the duration of prion survival in soils, and further work on the transmissibility of these prions is necessary to understand their transmission relevance.

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Key Words: disease ecology, disease management, indirect transmission, prion disease

P71 - A cryptic concern: *In situ* surveillance of *Cryptosporidium serpentis* in snakes of Killdeer Plains Wildlife Area.

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Cryptosporidium serpentis is a protozoan parasite specific to and pathogenic in squamate hosts. Knowledge of this pathogen in wild populations is limited, but studies from captive snakes indicate the parasite causes severe gastroenteritis, long-term immune impairment, and probable death. *Cryptosporidium serpentis* was discovered in quarantined captive breeding colonies of Ohio endangered plains garter snakes (*Thamnophis radix*), and it was hypothesized that the pathogen entered these colonies via breeding stock from Killdeer Plains Wildlife Area (KPWA; Wyandot and Marion Counties, Ohio). To assess prevalence of *C. serpentis* in wild snakes of KPWA, we collected cloacal swabs and opportunistically collected fecal samples from all snake species during the annual Ohio Division of Natural Resources' (ODNR) snake survey in 2024 (May and June). Following DNA extraction, we used quantitative PCR (qPCR) targeting the 18S rRNA gene region of *Cryptosporidium* spp. together with a *C. serpentis*–specific FAM-labeled probe to examine parasite loads across samples. *Cryptosporidium serpentis* was detected in 10.4% of snakes (n = 209 total samples) and in 3/7 snake species sampled, including 2 threatened species (*Opheodrys vernalis* and *Clonophis kirtlandii*). In 2025, we expanded our survey to sample snakes from April to October (377 samples and counting) to capture any apparent changes in prevalence over time, since this is an intermittently shed parasite. Although our pathogen surveillance results thus far may elicit concern, it is important to next assess whether infected wild snakes develop the associated disease state, cryptosporidiosis, as often seen in animals housed in captivity. Furthermore, our results encourage consideration of potential environmental reservoirs for the pathogen's infectious stage (oocyst) and potential mitigation strategies.

Financial Support: Ohio Northern University

Key Words: protozoan parasite, captive-breeding, conservation, cryptosporidiosis, snake, *Thamnophis radix*

P73 - Demography and bovine tuberculosis persistence in deer: An age- and sex-structured model.

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Bovine tuberculosis (bTB), caused by *Mycobacterium bovis*, remains endemic in Michigan's northeastern Lower Peninsula, where white-tailed deer (*Odocoileus virginianus*) serve as a wildlife reservoir. However, the age- and sex–specific contributions to sustained transmission are poorly quantified. We aim to (1) identify which demographic groups (age or sex) contribute most to persistence and transmission, (2) estimate the basic reproduction number within deer, and (3) evaluate targeted harvesting and testing strategies to reduce prevalence and interrupt transmission. We developed an age- and sex-structured Susceptible–Exposed–Infected (SEI) model and fitted it to voluntary testing data from hunter-harvested deer (1994–2023) in 4 high-prevalence counties (Alcona, Alpena, Montmorency, and Oscoda) using likelihood-based optimization in R (optim). Deer were classified into 2 sex groups (female, male) and 5 age classes (]0–1],]1–2],]2–3],]3–4], and >4 years). This scheme captures behavioral differences across classes, including distinctive patterns in males aged 3–4 years. The model estimates class-specific transmission, harvest, and testing rates and is used to simulate targeted management scenarios. Data cleaning and curation and model development are complete; model fitting and simulation experiments are underway. Testing data indicate differences in testing rates between the 3–4 and >4 age classes for both sexes. Next steps focus on estimating key epidemiological parameters by age–sex class and identifying demography-informed interventions. Preliminary tests with assumed data suggest that reducing transmission within specific high-contribution groups can substantially lower overall prevalence. This study will provide novel insights into age- and sex-specific contributions to bTB maintenance in deer, with direct implications for wildlife management strategies in Michigan. Our approach offers a transferable framework for other regions where wildlife hosts maintain *M. bovis*.

Financial Support: AEM acknowledges funding from the Minimizing Health Disparities Undergraduate Program, University of Arizona

Key Words: bovine tuberculosis, *Odocoileus virginianus*, structured model, age, sex, management strategy

General Abstracts: General Health and Physiology—Posters

P75 - Transcriptomic evaluation of dietary xylo-oligosaccharides derived from sorghum stover on gastrointestinal health in dairy calves.

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In preweaning dairy calves, poor immunity and gastrointestinal dysfunction are major risk factors associated with diarrhea—a leading cause of poor welfare, delayed growth, calf mortality, and significant economic losses to the dairy industry. Early-life gastrointestinal development plays a pivotal role in nutrient absorption and immune maturation in neonatal calves. Xylo-oligosaccharides (XOS), prebiotic carbohydrates produced from sorghum stover hemicellulose, have been shown to influence intestinal barrier integrity in monogastric species; however, the molecular mechanisms mediating XOS-driven responses in preruminant calves remain largely uncharacterized. This study investigates the effects of dietary XOS supplementation on host transcriptomic profiles within the gastrointestinal tracts of neonatal calves. Twenty male Jersey calves were assigned to 1 of 2 dietary treatments: control (CON, n = 10) or supplemented with 1% xylo-oligosaccharide (XOS, n = 10). Health and fecal scores were monitored daily to assess well-being. At d 7 and 21, subsets of calves (CON n = 5, XOS n = 5) were euthanized, and gastrointestinal tissues (abomasum, duodenum, jejunum, ileum, colon, and mesenteric lymph nodes) were collected for RNA sequencing. RNA was sequenced on the Illumina NovaSeq6000 platform. Differential gene expression analysis is being performed to compare XOS vs. CON groups, with differentially expressed genes being defined by an adjusted *P*-value ≤0.05 and $\log_2$ fold change >1. Functional enrichment analyses will be conducted to further characterize the biological significance of the identified differentially expressed genes. This transcriptomic approach will reveal molecular pathways responsive to XOS supplementation and provide insight into how prebiotic inputs influence gastrointestinal development at the gene-expression level.

Key Words: xylo-oligosaccharide, dairy calf, gastrointestinal health, neonatal immunity, prebiotic

P76 - The effects of volatile organic compounds in wildfire smoke on lactating dairy cows.

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Wildfires are a major source of air pollutants that are public health hazards, such as fine particulate matter ($PM_{2.5}$) and volatile organic compounds (VOC). Wildfire-$PM_{2.5}$ exposure is associated with lower milk production and altered metabolism in lactating dairy cows, but the responses of dairy cows to VOC are unknown. Across 3 wildfire seasons, daily milk production and weekly vital signs were recorded, and blood metabolites (glucose, β-hydroxybutyrate [BHB], and nonesterified fatty acids [NEFA]) were measured from lactating Holstein dairy cows (n = 53). Daily environmental conditions ($PM_{2.5}$ and temperature-humidity index [THI]) were recorded. VOC were collected in passive sorbent tubes and analyzed by gas chromatography-mass spectrometry. HYSPLIT modeling confirmed that spikes in air pollutant concentrations originated from wildfire smoke (WFS). Principal component analysis was performed on 26 environmental variables ($PM_{2.5}$, THI, and 24 VOC species) to identify environmental variable groupings (PC) explaining patterns in environmental conditions across years. Each PC received a daily score, with high scores indicating a contribution of that PC to that day's conditions. The top 4 PC (PC1–4, >80% of total variance) were used to assess relationships between cow and environmental variables. Data were analyzed in SAS using mixed models, with fixed effects of daily PC score, parity, and year, and a repeated effect of day within cow nested within year. Separate lag models were run to assess persistent and delayed cow responses. Environmental variables explaining most of the variance within each PC included toluene (PC1), trichlorofluoromethane (PC2), benzenes (PC3), and THI (PC4). Respiratory rate was positively associated with PC1 and PC4 on the day of exposure. Higher PC4 scores were associated with elevated rectal temperature on the day of exposure and for several days following ($P < 0.05$), and elevated PC2 scores from 3 to 7 d before exposure were associated with higher rectal temperature ($P < 0.05$). Glucose was decreased ($P < 0.05$), whereas NEFA and BHB were increased ($P < 0.0001$ and $P < 0.05$, respectively), with higher PC3 scores on the day of exposure, and the PC3 scores from 1–2 d prior. Higher PC3 scores were associated with lower milk yield for up to 8 d after exposure ($P < 0.05$). Specific VOC from WFS appear to impact milk production in lactating dairy cows. Further, interactions between WFS air pollutants and THI may be factors influencing cow performance.

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Key Words: air quality, inhalation, respiratory

P77 - Describing management risk factors associated with youngstock health, welfare, and performance on organic dairy farms.

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Recent modification to the origin of livestock requirements for organic dairy operations has rendered organic youngstock as the primary source of lactating organic cows. As a result, organic dairy farmers need to optimize youngstock outcomes to sustain herd size, with youngstock performance becoming a primary driver of profitability and long-term resilience. Because the health, performance, and welfare of youngstock on US organic dairy farms are largely unknown, this study aims to identify the management risk factors affecting these outcomes. Up to 50 USDA organic-certified dairy farms will be enrolled across 5 regions for quarterly visits over 1 year. At the initial visit a survey on current management practices will be administered, and at least 6 calves will be enrolled at each quarterly visit. Calf enrollments will include at least 2 neonatal calves (<7 d old) and up to 2 preweaning and 2 postweaning calves per quarter. All enrolled calves will undergo hygiene scoring, health scoring, and body condition scoring. Jugular blood samples will be collected from neonatal calves for IgG testing. Pre- and postweaning calves will have bilateral nasal swabs and fecal swabs collected, and thoracic ultrasound performed to assess subclinical pneumonia. Whole blood samples will be used for IgG testing to assess transfer of passive immunity. Nasal and fecal swabs will be used to identify respiratory agents and parasites, respectively. Thoracic ultrasound will be used to detect lung lesions and help in assessing subclinical pneumonia. Statistical analysis will be completed using directed acyclic graphs in R Studio. Analysis will encompass survey responses and collected health data to measure associations between management practices and calf health outcomes. This study will be used to improve youngstock health through optimized management and welfare. Future aspects of this study will involve identifying on-farm sources of *Staphylococcus aureus* exposure in organic youngstock, as well as the development of curriculum specific to organic dairy farming, targeted for animal science and veterinary students and educators.

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Key Words: respiratory, lung ultrasound, dairy calf

P78 - Lung immunophenotype and lung and immune function in dairy heifer calves in the weeks following wildfire-$PM_{2.5}$ exposure.

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Fine particulate matter in wildfire smoke (wildfire-$PM_{2.5}$) is associated with increased mortality risk, coughing, and lung consolidation in dairy calves. However, pulmonary and systemic immune responses to wildfire-$PM_{2.5}$ in the weeks following exposure are not known. Our objective was to investigate longer-term effects of wildfire-$PM_{2.5}$ inhalation on pulmonary and blood immune cells and pulmonary function. Holstein heifer calves were housed at Oregon State University (CON, n = 18) or the University of Idaho (WF, n = 20). WF calves were exposed to wildfire-$PM_{2.5}$ (17–33 mg/m^3) for 6 d when they were 2 wk old. Age-matched CON calves were not exposed to wildfire smoke ($PM_{2.5}$ <3.8 mg/m^3). Calves were born healthy; nutrition and management protocols were the same between groups. Health scores and respiration rates were recorded, blood was collected, and tidal volume and minute ventilation were determined by pneumotachography at 3, 9, and 12 wk of age. Transtracheal washes (TTW) were performed at 3 and 12 wk of age and used for immunophenotyping via flow cytometry. These data are presented as percent of live cells. Respiratory burst of phagocytes in whole blood was determined. Serum albumin, serum amyloid A, and haptoglobin concentrations were measured in plasma. Data were analyzed by general or generalized mixed models. WF had higher odds of coughing relative to CON at 12 wk (P = 0.02). Tidal volume was lower in WF than CON at 12 wk (P = 0.001), and minute ventilation was greater overall in WF compared with CON (3.09 vs. 2.48 ± 0.04 L/min; P < 0.001) because of higher respiration rates. TTW fluid from WF had fewer B lymphocytes at 3 and 12 wk, fewer macrophages at 12 wk, but more granulocytes at 3 wk compared with CON (macrophages: 6.77 vs. 29.45 ± 2.7%; granulocytes: 63.83 vs. 48.95 ± 3.38%; P < 0.01). Phagocyte respiratory burst was lower in WF compared with CON at 3 wk (42.96 vs. 65.62 ± 4.41% rhodamine 123-positive cells; P < 0.01). Overall, serum amyloid A concentration was higher in WF compared with CON (P < 0.01). Haptoglobin concentration was higher in WF at 9 and 12 wk, and serum albumin concentration was higher in WF compared with CON at 9 wk (P < 0.03). Our results indicate local and systemic immune responses to wildfire-$PM_{2.5}$. Minute ventilation was greater in WF, potentially increasing particle deposition in calf lungs. Understanding responses to smoke inhalation can aid in developing strategies to mitigate poor health associated with wildfires.

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.

Key Words: air quality, pulmonary function testing, acute phase protein, flow cytometry

P79 - Effect of lauric acid and microencapsulated zinc oxide on nursery pig performance and intestinal integrity.

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Feed additives such as zinc oxide (ZnO) and lauric acid (LA) are commonly included in nursery diets to enhance growth performance and intestinal health of weaned pigs. Despite extensive evaluation of ZnO and LA independently, evidence regarding their potential synergistic effects on growth and intestinal integrity in nursery diets is limited. Therefore, the objective of this study was to evaluate the effects of dietary ZnO, LA, and their combination on growth and intestinal integrity of weaned pigs. A total of 256 crossbred pigs were weaned at 21 d of age, blocked by sex and body weight and housed in pens of 8. Following a 3-d acclimation period, pens were randomly assigned to 1 of 4 diets: control (CON), 2% lauric acid (LA), 150 ppm microencapsulated ZnO (ZnO), or ZnO + LA in combination (LAZ). Body weights and feed disappearance were measured weekly during the study. After 27 d, 24 pigs (6 pigs/treatment) were euthanized, and fresh jejunum explants were mounted in modified Ussing chambers to assess intestinal barrier integrity and function. Data were analyzed using the mixed model procedure of SAS to examine the fixed effect of diet. On d 14, pigs fed ZnO were heavier ($P < 0.05$) than CONT pigs, with LA- and LAZ-fed pigs intermediate. By d 21, both ZnO and LAZ pigs were heavier than CONT pigs, and LA pigs remained intermediate ($P < 0.005$). Average daily feed intake and feed efficiency did not differ among treatments on d 27 ($P = 0.139$). Transepithelial resistance tended to be greater in ZnO-fed pigs compared with CONT ($P = 0.07$), whereas LA and LAZ were intermediate. Glucose transport ($P = 0.187$) and FITC-dextran flux ($P = 0.461$) were unchanged across diets. In summary, dietary ZnO and LA influenced growth and intestinal integrity of weaned pigs; however, their combined use did not produce additive benefits.

Financial Support: Maxx Performance

Maxx Performance Key Words: nutrition, health, medium-chain fatty acid

P80 - Efficient pig posture recognition using the Segment Anything Model (SAM) and YOLOv8n-seg for enhanced farm management.

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Posture changes in pigs during the fattening period are often precursors of disease. However, in fattening barns, it is often difficult to monitor individual pig postures, which can delay necessary interventions. Although computer vision offers an effective solution for posture recognition, existing techniques struggle to perform recognition in crowded environments. Therefore, this study aims to develop a robust pig posture recognition model by combining the Segment Anything Model (SAM) for efficient image segmentation with YOLOv8n-seg, an algorithm well-suited for crowded environments. For this purpose, we continuously recorded video data (24 h/d) of 2 groups of fattening pigs (~550 pigs each) from their introduction to a fattening barn until slaughter on a commercial farm in Japan. Cameras were installed on the ceiling, positioned to capture the posture and behavior of pigs in 2 pens per camera and used to record video data. Using this video data, we aimed to develop a model to recognize 5 distinct postures: standing, sternal lying, lateral lying, sitting, and front legs sitting. The methodology involved 2 steps: (1) creating a training data set for the 5 postures by leveraging SAM for efficient annotation, and (2) developing a method to simultaneously recognize the postures of multiple pigs using the YOLOv8n-seg algorithm. The performance of this model was evaluated using Recall (R), which indicates the proportion of actual positive instances that were correctly identified by the model. Training data for each posture were created using a combination of OpenCV, LabelImg, and SAM. The model was trained for 120 epochs. The result showed that it achieved the following R scores for each posture: standing R = 0.85, sternal lying R = 0.85, lateral lying R = 0.95, sitting R = 0.96, and front legs sitting R = 0.89, at a confidence threshold of 0.01 when the trained model was applied to infer postures from field data. In conclusion, this study successfully developed a high-response model for pig posture recognition, demonstrating the effectiveness of combining SAM for data annotation with YOLOv8n-seg for robust recognition in crowded pens. Future work will focus on developing a system to automatically identify pigs exhibiting problematic postural patterns using this model.

Key Words: fattening pig, computer vision, posture recognition

P81 - Gut microbiota-mediated remodeling of postpartum inflammation in dairy cows.

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The gut microbiota modulates host immunity and can influence inflammation. In dairy cows, this interaction is critical during the postpartum transition, when prolonged inflammation affects long-term health. This study aims to understand how an exacerbated postpartum acute-phase response is associated with an altered fecal microbiome, accounting for bias from contamination, technical batches, and biological confounders. We analyzed fecal and blood samples from 71 Holstein cows at 1 and 3 d postpartum. Inflammation was assessed via haptoglobin and fibrinogen, considering covariates as parity, days in milk (DIM), body condition score (BCS), and qPCR bacterial load. Negative (environmental, reagent, extraction blanks) and positive controls (2 microbiome standard communities) were included. Fecal DNA was extracted and sequenced (V3–V4 16S rRNA, AVITI platform), and reads were processed with DADA2. Contaminants were removed with Decontam and SourceTracker, reducing ASVs from 24,011 to 21,727. SourceTracker attributed most contamination to extraction blanks (99.61%). Batch effects by extraction date were corrected with MBECS. Aitchison β diversity analysis showed significant association between microbiota composition and elevated haptoglobin status (PERMANOVA R^2 = 0.008, *P* = 0.001), but not elevated fibrinogen (R^2 = 0.006, *P* = 0.27). DIM and parity (PCoA1: 4.74%, PCoA2: 3.82%) were correlated with compositional shifts over time and between uniparous vs. multiparous cows. Parity explained more variance in β diversity than inflammation measured by fibrinogen (R^2 = 0.015, *P* = 0.001) or haptoglobin (R^2 = 0.015, *P* = 0.001), whereas BCS was not significant. Alpha diversity was analyzed using a linear mixed-effects model with cow ID as random effect. Only parity was significant, with multiparous cows showing lower richness (Chao1 = −7.49) and diversity (Shannon = −0.12) than primiparous cows. Our results show that elevated haptoglobin predicts early compositional shifts in the fecal bacteriome of postpartum cows in 1–3 DIM. However, contamination from extraction kits, batch effects, and biological confounders can bias interpretation of microbiota–inflammation relationships. Parity and DIM emerged as key covariates, and extraction date drove batch and contaminant effects. Future work will include functional microbiome profiling to further delineate microbiome drivers of acute postpartum inflammatory response.

Financial Support: Dr. Neves's discretionary funds. No grant agency sponsored the work.

General Abstracts: Immunology—Posters

P82 - Sexual dimorphism in metabolic responses to influenza vaccination in a mouse model of diet-induced obesity.

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Our prior study showed that female mice with obesity produce significantly higher antibody responses to the influenza vaccine and are better protected compared with males with obesity. The B-cell responses in the spleen, however, were comparable. We hypothesized that sexual dimorphism in influenza vaccine efficacy during obesity is driven by differences in B-cell metabolism and function. In this study, our objective was to determine sex- and obesity-specific differences in influenza vaccine-induced metabolites in splenic B-cells. Male and female mice with and without diet-induced obesity were vaccinated with an inactivated pandemic 2009 H1N1 influenza vaccine twice at a 3-week interval. At 35 d post-vaccination, magnetically sorted $CD19^+$ B-cells from the spleen were analyzed using liquid chromatography mass spectrometry. Log-transformed data were analyzed by 2-way ANOVA, and $P \leq 0.05$ was considered a significant difference. Principal component analysis showed a clear separation of the analyzed biochemicals between males with and without obesity, but no such separation was observed between females. A total of 184 biochemicals showed statistically significant differences in B-cells. When focusing on the differences between males and females with obesity, 139 biochemicals were significantly higher and 3 were significantly lower in females compared with males. The 3 metabolites that were lower in females compared with males with obesity included hypotaurine, S-(1,2-dicarboxyethyl) glutathione, and uridine 5′-monophosphate. The biochemicals significantly higher in females with obesity compared with males included dipeptides, which are the sources of amino acids for biosynthesis; polyunsaturated fatty acids, required for B-cells membrane remodeling, signaling, and eicosanoid-mediated regulation; acylcarnitines, involved in metabolic reprogramming of B-cells during activation and differentiation; acylcholines, involved in lipid remodeling; glycerophospholipids, sphingolipids, and other bioactive lipids, involved in B-cell expansion, signaling, survival, and membrane structure; and vitamins, cofactors, and nucleotides that support antioxidant defense, coenzyme function, and nucleotide metabolism. In conclusion, the higher antibody production following influenza vaccination in females compared with males with obesity is associated with a favorable metabolic and lipid environment that supports robust B-cell activation and differentiation.

Key Words: sex difference, diet-induced obesity, influenza, vaccination, metabolomics, B-cell

P83 - Swine influenza A infection–induced antibody responses to homologous H1N2 (clade 1B2.2) and heterologous H1N1 (clade 1A.3.3.2) virus.

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Influenza A virus (IAV) is a significant respiratory pathogen of pigs. Multiple subtypes (H1N1, H1N2, and H3N2) circulate in swine, with diverse antigenic variation in the viral hemagglutinin (HA) and neuraminidase (NA). Inactivated whole influenza virus (WIV) vaccines provide reasonable protection against viruses with homologous HA but limited protection against viruses with heterologous HA and NA. Additionally, cross-protective immunity induced by natural infection is poorly understood. To further study infection-induced immunity, we assessed antibody reactivity to homologous and heterologous viruses. Five pigs were exposed to an H1N2 (clade 1B.2.2), with 5 IAV-naive pigs serving as controls. At 21 d post-infection (DPI), the pigs were necropsied to collect serum and bronchoalveolar lavage fluid (BALF), and antibody reactivity was evaluated by hemagglutinin inhibition (HI), neuraminidase inhibition (NI), and ELISA to homologous virus and a heterologous H1N1 (clade 1A.3.3.2). All exposed pigs were confirmed to have been infected via viral shedding in nasal swabs, with elevated temperature readings further confirming infection. Lesion scores at necropsy indicate recovery post-infection. In serum, there was no cross-reactivity by HI or NI, but serum whole virus–specific IgG ELISA showed cross-reactivity between the 2 viruses. Additionally, BALF whole virus–specific IgG and IgA were also cross-reactive between viruses by ELISA. In conclusion, we demonstrate that infection with IAV can induce cross-reactive whole virus–specific antibodies in serum and the mucosa, even with divergent HA and NA. Ongoing studies are evaluating cross-reactivity between individual IAV proteins and assessing cross-reactive T-cell responses. Further studies are necessary to understand how infection-induced cross-reactive antibodies may play a role in immune protection from divergent IAV infection.

Financial Support: USDA-ARS; ORISE

Key Words: swine, antibody, influenza A

P84 - A spatial transcriptomic evaluation of vaccine-associated enhanced respiratory disease (VAERD) in porcine.

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Vaccine-associated enhanced respiratory disease (VAERD) occurs when vaccination, rather than conferring protection, results in more severe respiratory illness upon pathogen exposure. Although rare, the molecular mechanisms driving VAERD vary by virus and remain poorly defined. In this study, we employed the 10x Genomics Visium HD 3′ spatial transcriptomic platform to map gene expression patterns in pigs vaccinated against and subsequently challenged with influenza A virus (IAV) to induce VAERD. We hypothesized that VAERD would be associated with severe pneumonia, bronchiolitis, edema, hemorrhage, and elevated proinflammatory cytokine expression within the lung. A total of 19 commercial mixed breed piglets were assigned to 1 of 4 groups: vaccinated with adjuvant, vaccinated without adjuvant, non-vaccinated, or non-vaccinated non-infected. Select groups were vaccinated at −28 and −14 d post infection (d.p.i) using a whole, UV-inactivated human-like δ1-cluster H1N2 A/swine/Minnesota02011/2008 (MN08) influenza A virus either with or without the oil-in-water adjuvant Emulsigen D. Pigs were intratracheally inoculated with 2×10^6 $TCID_{50}$ of the H1N1 pandemic A/CA/04/2009 influenza A virus at 0 d.p.i. and euthanized for necropsy at 5 d.p.i. Infected pigs vaccinated with adjuvant (n = 5) exhibited lower average pulmonary lesion scores compared with infected pigs vaccinated without adjuvant (n = 5; 13.2 vs 17.8), whereas vaccination overall was associated with higher lesion scores than infection alone (n = 5; 15.5 vs. 6.8). Spatial profiling revealed that VAERD-associated pulmonary lesions were enriched for *JCHAIN*, a marker of B-cells, and chemokine genes involved in lymphocyte recruitment, including *CXCL8*, *CXCL10*, *CXCL14*, *CCL2*, *CCL5*, *CCL8*, *CCL19*, *CCL21*, *CCL22*, *CCL26*, and *AMCF-II*. Lesions also displayed increased expression of T-cell–associated genes (*CD3*, *CD4*, *CD8A*, *CD8B*, *CD28*, *CD80*, *ICOS*), which were not enriched in adjacent, non-diseased lung tissue. VAERD has previously been linked to non-neutralizing antibodies directed against conserved regions of the hemagglutinin 2 stalk. Our preliminary spatial transcriptomic analysis identifies localized inflammatory and immune gene expression signatures associated with VAERD lesions, offering new insight into vaccine priming events that potentiate dysregulated immune responses to IAV, including disruptions in cell–cell communication within the lung microenvironment.

Financial Support: National Institutes of Health grant AI158477 and the U.S. Department of Agriculture grant 2021-67015.

Key Words: VAERD, influenza A, vaccine-associated enhanced respiratory disease, spatial transcriptomics

P85 - Binding of bovine WC1 γδ T-cell co-receptor/PRR SRCR domains to *Mycobacteria* spp.

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Gamma delta T-cells are a crucial component of the immune response to several 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 in 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 and other species. In cattle there are 13 unique WC1 genes (WC1-1 to WC1-13), each comprising 6–11 SRCR domains that selectively bind unprocessed antigen in a manner that resembles a pattern recognition receptor (PRR). WC1 functions as a hybrid PRR and co-receptor for the gamma delta TCR. We hypothesized that the multigenic WC1 arrays across species have co-evolved with pathogens and thus sought to characterize their diversity and ligand-binding potential. Sequences encoding SRCR domains from cattle were cloned into a mammalian expression vector. After transfection of the SRCR domain vector into suspension 293F cells, recombinant SRCR domain proteins were purified and quantified. ELISA and bacteria-binding pull-down assays were carried out with recombinant WC1 SRCR proteins and sonicated or fixed whole *Mycobacteria* spp. and *Leptospira* spp. Out of the 13-member bovine WC1 multigenic array, the WC1-12a1 SRCR domain bound with the highest affinity to sonicated and whole fixed *Mycobacteria* spp. This was in contrast to WC1.1-type *Leptospira*-binding WC1-3 SRCR domains, which did not show binding to *Mycobacteria* spp. WC1.2-type WC1-4a1, WC1-7a1, and WC1-9a1 SRCR domains showed low-level binding to sonicated *Mycobacteria* spp. in an ELISA but did not show binding to *M. bovis* surface ligands in a whole-cell bacteria pull-down assay. Of the 4 WC1-12 SRCR domains that bind to *M. bovis*, 2 SRCR domains overlap with the WC1-3 SRCR domains that bind to *Leptospira* spp. Selected WC1 SRCR domains from cattle bind differentially and with high affinity to *Leptospira* spp. and *Mycobacteria* spp., supporting the hypothesis that WC1 genes and individual SRCR domains 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.

Key Words: γδ T-cells, scavenger receptor, *Mycobacteria*

P86 - Transcriptomic profiling of equine myometrium identifies immune networks underlying placentitis-induced activation.

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Equine ascending placentitis is a leading cause of abortion, stillbirth, and perinatal mortality in horses. A key event of placentitis-induced preterm labor is loss of myometrial quiescence, which triggers the onset of labor. However, the molecular drivers of myometrial activation in horses are not well understood. To address this, we examined the myometrial transcriptome in mares with induced ascending placentitis (n = 6) at ~290 d gestation compared with gestationally age matched controls (n = 6). RNA sequencing was performed using Illumina NovaSeq6000, with raw reads processed using TrimGalore 0.4.3 before mapping to EquCab3.0 via STAR 2.4.3a. Mapped reads were quantified using Cufflinks 2.2.1 with NCBI annotation. Differentially expressed genes (DEGs) were identified with Cuffdiff 2.2.1 with a false discovery rate (FDR) <0.05. We identified 263 DEGs, including 7 genes (*APOBEC3Z1B*, *PLA2G2D*, *CXCL17*, *MMP8*, *MMP13*, *S100A8*, *S100A9*) uniquely upregulated during placentitis. Functional groups of DEGs pointed to key pathways of myometrial activation: connexins (*GJC2*), matrix metalloproteinases (*MMP1*, *MMP8*, *MMP13*, and *MMP25*), prostaglandin signaling (*PTGS2* and *PTGER3*), and calcium regulation (*TRPM2*, *RAMP2*, *CACNA1D*, and *PRKCB*) point to a clear pathway for equine placentitis-induced myometrial activation. Several chemokines (*CCL2*, *CCL3*, *CCL4*, *CCL5*, *CXCL1*, *CXCL3*, *CXCL16*, and *CXCL17*) were also enriched, correlating with pronounced leukocytic infiltration, a hallmark of myometrial activation. To identify potential upstream regulators of myometrial activation, we performed upstream regulator analysis using Ingenuity pathway analysis, which revealed 12 potential regulators: *TLR2*, *STAT1*, *IRF7*, *SPI1*, *PTGS2*, *S100A8*, *S100A9*, *ICAM1*, *NRG1*, *HIF1A*, *PRKCB*, and *TYROBP*. Immunolabeling confirmed protein-level upregulation of TLR2, PTGS2, S100A8, S100A9, and HIF1A. Overall, this study enhances our understanding of immune networks and molecular mechanisms involved in myometrial activation during equine placentitis, which could inform therapeutic development to prevent preterm labor and treat placentitis. In conclusion, these data highlight TLR2 as a central initiator of inflammatory signaling in the equine myometrium, promoting contraction-associated transcripts such as PTGS2, PTGER3, MMP1, MMP8, and GJC2. Therefore, targeting TLR2 and its associated pathways, as through TLR antagonists, could offer potential therapeutic strategies to prevent placentitis-induced preterm birth.

Financial Support: Boehringer Ingelheim Equine Research Award

Key Words: equine, placentitis, immune network, myometrium, transcriptomics, abortion

P87 - Multimeric mRNA vaccine for broad protection against rheumatic heart disease.

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Infections caused by group A *Streptococcus* (GAS), exclusively the species *Streptococcus pyogenes*, can lead to diseases ranging from pharyngitis to acute rheumatic fever, rheumatic heart disease (RHD), and invasive infections. Although GAS infections are commonly reported in developing countries, recent trends indicate a rising global incidence. Despite its significant health impacts, no vaccines are currently licensed. Developing a vaccine is challenging due to the existence of over 220 GAS serotypes, making a serotype-specific approach impractical. To overcome this challenge, we developed multimeric messenger RNA lipid nanoparticle (mRNA–LNP) vaccines encoding conserved epitopes from both M and non-M proteins to induce broad and durable immunity. To evaluate the immunogenicity and protective potential of mRNA–LNP vaccines incorporating conserved epitopes (StreptInCor, J8, and *S. pyogenes* cell envelope protease) in wild-type A/J mice. We used an mRNA construct encoding 3 conserved GAS epitopes (GAS-3Epi-MITD). A group of mice was injected twice with the GAS-3Epi-MITD vaccine or left unimmunized as controls. Serum, lymph nodes, and spleens were harvested on d 21 post-immunization. Antibody responses were evaluated using ELISA with the StreptInCor peptide as antigen, and cellular responses were assessed by lymphocyte proliferation assays. Mice immunized with GAS-3Epi-MITD produced strong antibody responses against the StreptInCor peptide, characterized by the induction of IgG1 and IgG2a isotypes. Control mice showed minimal reactivity. Lymphocytes from vaccinated animals, but not from controls, proliferated in response to StreptInCor stimulation, demonstrating antigen-specific T-cell activation. These findings provide clear proof-of-concept for the immunogenic potential of GAS-3Epi-MITD mRNA constructs and lay the foundation for further optimization toward an efficacious GAS vaccine. Our data demonstrate that multimeric mRNA–LNP vaccines encoding conserved GAS epitopes can elicit both antibody and T-cell responses in mice. This approach represents an effective GAS vaccine that could substantially reduce the incidence of RHD and other GAS-related diseases.

Key Words: group A *Streptococcus* (GAS), rheumatic heart disease (RHD), GAS-MITD lipid nanoparticle vaccine

P88 - Single-cell RNA sequencing of bovine PBMCs to characterize immune dysfunction associated with bovine leukemia virus infection.

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Bovine leukemia virus (BLV) is a deltaretrovirus that infects ~84% of US dairy herds, resulting in an estimated $2.7 billion in annual losses. The virus infects B lymphocytes, leading to immune dysfunction, reduced production, and secondary infections. Despite its impact, the mechanisms of BLV-induced immune dysregulation remain unclear. This study aims to characterize molecular pathways associated with BLV infection using single-cell RNA sequencing (scRNA-seq) of peripheral blood mononuclear cells (PBMCs) from affected cows to reveal transcriptomic alterations underlying immune dysfunction. Clinically healthy, mid-lactation Holstein cows were screened for BLV antibodies (ELISA) and proviral load (PVL) by qPCR. Animals were classified as BLV-negative (n = 4) or BLV-positive (n = 16) based on the presence of anti-BLV antibody and stratified into low (n = 4), moderate (n = 4), moderate-high (n = 4), and high (n = 4) PVL groups. PBMCs were isolated by Ficoll-Paque density-gradient centrifugation and processed for scRNA-seq using the Parse Evercode WT Mega v3 platform. Libraries were sequenced on the Ultima Genomics UG 100 instrument and data processed with the Evercode Whole Transcriptome pipeline. A total of 1,329,050 PBMCs were sequenced, with a median of 3,126 transcripts and 1,468 genes per cell, confirming high-quality data for analysis. Comparative analyses will be performed in Seurat to identify differentially expressed genes and cell populations across PVL strata, with pathway enrichment (GO and KEGG) used to determine functional changes associated with infection status. Bioinformatic processing will be used to generate cell clusters, identify PBMC subtypes, and evaluate transcriptional signatures linked to BLV infection and PVL. Analyses include principal-component analysis, clustering, pseudotime modeling, and pathway enrichment to identify immune-cell subsets differing in abundance and activity between groups, with focus on B- and T-cell activity as PVL increases. This study will produce the first single-cell transcriptional atlas of PBMCs from BLV-infected cattle, advancing understanding of how BLV reshapes immune cell populations and gene expression networks. By identifying genes and pathways associated with immune dysfunction and disease progression, this work will provide targets for further study and experimentation, and may directly inform improved diagnostics, herd-level screening, and control strategies within the dairy industry.

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Key Words: bovine leukemia virus, dairy cow, single-cell RNA sequencing, immune dysfunction, lymphocyte, gene expression

P89 - Advancing snRNA-seq applications in adipose biology through optimized nuclei isolation.

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White adipose tissue (WAT) is a key metabolic organ in dairy cows. WAT cellular components include adipocytes and non-lipid-laden cells. This cellular complexity is not well understood, which limits our understanding of WAT's metabolic functions. Single-nucleus RNA-sequencing (snRNaseq) enables transcriptomic analysis of lipid-rich tissues such as WAT, where single-live cell isolation is difficult. Published protocols for nuclei isolation from bovine WAT require large tissue amounts (≥500 mg) and flow cytometry to isolate nuclei. However, these methods are time-consuming and may reduce nuclei integrity and RNA quality. In this study, we optimized a nuclei isolation protocol for frozen bovine subcutaneous WAT that avoids flow cytometry and reduces tissue input to 300 mg. Key modifications included liquid nitrogen pulverization, Dounce homogenization, lipid removal, and optimized centrifugation. Nuclei were assessed for yield, morphology, and RNA quality. Microscopy showed that more than 85% of nuclei were intact, with clear membranes and minimal debris, indicating preserved structural integrity. Libraries were prepared using the Parse snRNaseq kit. Our protocol consistently yielded high-quality nuclei (~5,000 nuclei/mg tissue) suitable for snRNaseq. Sequencing generated an average of 78,152 reads, with median detection of 1,720 genes and 3,530 transcripts per nucleus, indicating strong coverage. Saturation reached 71.6%, suggesting sufficient depth for downstream analyses. Clustering revealed distinct cell populations in WAT, confirming the quality of the isolated nuclei. Our modified protocol provides robust nuclei isolation, resulting in high yield and excellent quality. This developed protocol should prove useful for snRNA-seq applications of bovine WAT samples.

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Key Words: snRNAseq, adipose tissue transcriptomics, nuclei isolation, adipose tissue

P90 - Evaluating the impact of *Aspergillus oryzae* postbiotic on immune development and gut health in calves during weaning.

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This study evaluated the effects of *Aspergillus oryzae* (AO) supplementation from birth to weaning (d 49) on feed intake, growth performance, fecal consistency, health status, intestinal mucosal development, and resilience against enteric disease. Sixty beef-on-dairy crossbred calves were randomly assigned to 1 of 2 treatments: CNT (silica-based carrier, n = 30) or AO supplementation (n = 30) administered through milk replacer. Individual grain and milk intakes were recorded daily, body weight weekly, and fecal samples collected from d 1 to 21 to determine fecal dry matter. Colon biopsies were collected 1 week before weaning (d 35) and 1 week after weaning (d 56) to isolate and characterize intraepithelial leukocytes (IELs) using monoclonal antibodies to target monocytes, macrophages, dendritic cells, and gamma delta (γδ) and CD4+ T-cell subsets. Blood was collected on d 14, 35, and 56 to isolate polymorphonuclear cells. Data were analyzed in R (version 4.5.0) using a nonlinear mixed-effects model with repeated measures. AO supplementation modestly increased milk intake on d 1, 2, 12, and 26 compared with CNT ($P = 0.03$) and tended to increase grain intake on d 56 ($P = 0.06$), without affecting fecal dry matter, body weight, or neutrophil function ($P = 0.78$). AO tended to increase the intestinal CD4+ T-cell subset ($P = 0.10$), whereas γδ T-cells, monocytes, macrophages, and dendritic cell populations were not affected. Independent of treatment, marked maturation of the IEL compartment was observed, with significant increase in γδ T-cell and dendritic cell frequencies between d 35 and 56 ($P < 0.01$). These findings suggest that early-life supplementation with *Aspergillus oryzae* modestly improved feed intake, whereas overall mucosal immune maturation during the weaning period was driven primarily by age rather than treatment.

Key Words: calf development, calf nutrition, health, intestinal immunity, weaning

P91 - Effects of *Saccharomyces cerevisiae boulardii* CNCM I-1079 supplementation on the immune function of dairy cows during the periparturient period.

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The periparturient period is a critical stage for dairy cows, marked by extensive metabolic and physiological adaptations that affect immune function. Nutritional interventions, including probiotics such as *Saccharomyces cerevisiae boulardii* (SCB), have been proposed to modulate rumen fermentation and gut health, but their impact on the immune function during this period remains unclear. This study aims to characterize systemic and mucosal immune function in dairy cows across the periparturient period, and to determine whether SCB supplementation affects those components. Holstein cows (n = 79) were blocked by parity and, within block, randomly assigned to 100 g of cornmeal (CON; n = 39) or 87.5 g of cornmeal with 12.5 g of SCB (SCB; n = 39) to deliver 1×10^{10} cfu/head per day from d −28 prepartum to d 70 postpartum. Blood samples were collected on d −30, −7, 7, 21, and 70 relative to calving, and colon biopsies at d −30, 7, and 70. Peripheral blood mononuclear cells (PBMCs) and intraepithelial leukocytes (IELs) were isolated and analyzed by flow cytometry to assess T and B subsets, activation markers, macrophages, and dendritic cells. Pre- and postpartum data were analyzed separately using linear mixed effect models in R, with fixed effects of treatment, day, and their interaction, and the random effect of cow within block, and d −30 as a covariable. Independent of treatment, time exerted a strong effect on immune populations. In blood, CD4 expression declined from d 7 to d 21 before recovering at d 70 ($P < 0.01$), and CD28 expression on $CD4^+$ T cells peaked at d 7 and then decreased ($P < 0.01$). The percentage of $CD8^+$ T cells increased from d 7 to d 21 and remained elevated through d 70 ($P < 0.01$), and IgG^+ B cells showed an increase CD21 expression from d 7 to d 70. In IELs, dendritic cells percentage peaked at d 7 ($P < 0.01$). Supplementation with SCB increased postpartum CD86 expression on IgG^+ and IgM^+ B cells ($P < 0.05$), suggesting a modulatory effect on humoral activation. Overall, markers of cell-mediated immunity peaked early postpartum, whereas SCB further enhanced B cell activation. These results highlight dynamic immune changes during the transition period and the capacity of SCB to influence humoral function.

Financial Support: Dairy Innovation Hub, Lallemand, Elanco, NRSC alliance, Alberta Milk and Dairy Farmers of Ontario

Key Words: transition cow, probiotic, immunity

P92 - Development and characterization of bison respiratory epithelial and non-epithelial cell cultures for studying the innate immunity and disease pathogenesis.

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Bison are a keystone animal species and an important food source. Respiratory diseases pose a significant threat to bison health and result in substantial economic losses to the bison industry. Preventive and treatment options for respiratory diseases in bison are limited, and clinical tools available for cattle are not reliable in bison. This raises the need for the development of bison-specific research tools. Our understanding of the bison's immune system is in its infancy, as bison-specific immunological reagents and cell cultures are not readily available. This study aims to develop and characterize bison respiratory epithelial and non-epithelial cell cultures to study innate immunity and pathogenesis of bison respiratory diseases. After collection, bison lung, bronchial, and tracheal tissues were dissected into small pieces and digested using collagenase. The digested cells were incubated in collagen-coated flasks in DMEM-F12 media supplemented with 5% fetal calf serum and selected growth factors. Epithelial cell enrichment was conducted using 0.05% trypsin-EDTA treatment on the original cells, and the cells obtained after the treatment were cultured in DMEM-10 medium in normal tissue culture flasks. Cell cultures showing cobblestone morphology in supplemented DMEM-F12 medium and stellate morphology in DMEM-10 were used to prepare cytospins for immunocytochemistry. Cytokeratin, vimentin, and α-smooth muscle actin (ASMA) were used as cell-specific markers. The lung, bronchial, and tracheal cells cultured in supplemented DMEM-F12 medium showed positive staining for cytokeratin, confirming their epithelial phenotype. The cells cultured in DMEM-10 medium were negative for cytokeratin but positive for ASMA and/or vimentin, indicating their non-epithelial phenotype. The bison bronchial epithelial cells were seeded on the trans well membranes for cell polarization. These cells showed the increased trans-epithelial electrical resistance (TEER), indicating their polarization capability. These bison respiratory epithelial and non-epithelial cell cultures may prove to be good culture models for studying the bison's innate immunity and pathogenesis of bison respiratory infections.

Financial Support: The work was funded by Centre of Excellence in Bison Studies and SDSU-AES Hatch Project.

Key Words: bison, epithelial cell culture, innate immunity, disease pathogenesis

P94 - Investigation of the neuroinflammatory response in horses with selected neurologic diseases.

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Equine protozoal myeloencephalitis (EPM) is a devastating neurologic disease of horses in the Americas caused by the protozoan parasite *Sarcocystis neurona*. Although seroprevalence to *S. neurona* is relatively high, EPM incidence is reported to be less than 1%. The goal of this study is to determine the cytokine/chemokine profile in sera and cerebrospinal fluid (CSF) from EPM cases and compare these with horses affected by cervical vertebral malformation (CVM), equine degenerative myeloencephalopathy (EDM), equine herpesvirus myeloencephalopathy (EHM), and normal controls. The clinical history of neurologic horses subjected to EPM immunodiagnostics (SnSAG2/4/3 ELISA) was reviewed to identify cases and controls fitting the inclusion criteria: (1) EPM, with serum/CSF titer ratio ≤25 and exclusion of other neurologic etiologies; (2) CVM and EDM controls, with serum/CSF titer ratio ≥100/negative with a specific index (SI) <1, and postmortem diagnosis confirmation; (3) EHM controls, horses experimentally infected with EHV-1; and (4) normal controls, with serum/CSF titer ratio >100/negative and absence of neurologic signs. Paired serum and CSF from 10 horses per group was sent for analysis at Cornell's Animal Health Diagnostic Center for the following analytes: sCD14, IFN-α, IFN-γ, IL-4, IL-10, IL-17, IL-1β, TNF-α, CCL2, CCL3, CCL5, and CCL11. The Kruskal–Wallis test with Dunn's correction for multiple comparisons was used to assess statistical difference of median levels among groups. Serum concentrations of IFN-α, IL-4, IL-17, and Il-1β were not detected in more than 2 horses/group, and IFN-α was not detected in CSF. Outliers were present in all groups, and there was no disease-specific cytokine or chemokine profile. The EHM group presented significantly higher CSF levels of sCD14, IFN-γ, IL-10, IL-1β, TNF-α, CCL2, CCL5, and CCL11 ($P < 0.05$). These findings are likely due to the differences in sample origin (clinical cases previously treated with anti-inflammatories versus controlled experimental infection) and disease progression (chronic clinical cases versus acute onset of neurologic signs following experimental infection). The next steps in this research include a targeted lipidomic analysis of eicosanoids and specialized pro-resolving lipid mediators in serum/CSF samples from this cohort. Collectively, the findings from this study are expected to better define the immunopathology of EPM and provide groundwork for novel ancillary diagnosis and immunomodulatory therapies.

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Key Words: equine protozoal myeloencephalitis, cytokine, *Sarcocystis neurona*

P95 - A novel oral immunotherapeutic approach: Anti-Fel d 1 hagfish antibodies for reducing human allergy to cats.

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Approximately 20% of adults in the United States are sensitized to cats, which poses daily challenges for pet owners and individuals in animal-related occupations. Symptoms range from mild rhinitis to severe allergic asthma. Cat allergens are the most common mammalian allergens. *Felis domesticus* 1 (Fel d 1), the major cat allergen, is a small glycoprotein produced in cat saliva and sebaceous glands. In susceptible individuals, initial exposure leads antigen-presenting cells to capture Fel d 1 and present it to T helper cells, driving a Th2-polarized immune response. This promotes the production of Fel d 1-specific IgE antibodies by B cells, which bind to mast cells and basophils, priming them for subsequent encounters. Upon re-exposure, Fel d 1 cross-links these surface-bound IgE molecules, triggering inflammatory mediators. Cat allergy symptoms include sneezing, watery eyes, and nasal congestion, and in more severe cases, bronchoconstriction and asthma. Because this cascade is initiated by the binding of Fel d 1 to IgE, blocking this interaction represents a promising strategy for preventing allergic reactions. The objective of this study was to evaluate whether oral administration of anti-Fel d 1 hagfish antibodies could neutralize salivary Fel d 1 and thereby prevent its binding to Fel d 1–specific IgE, ultimately reducing the severity of cat-induced allergic responses. A total of 7 cats (4 males, 3 females) received daily oral administration of an anti-Fel d 1 antibody solution (1 mg/mL per day) for 14 d. Oral swab samples were collected before and after treatment to monitor the level of Fel d 1 complexed with antibody using a competition ELISA. We found that baseline Fel d 1 levels did not differ significantly by sex or breed but was higher in cats aged 3 to 4 yr. Oral administration successfully promoted Fel d 1-antibody complex formation, ranging from 21.7% to 75.7%. Male cats (average 59.7%) and those aged 3 to 4 yr (average 70.5%) showed the highest levels of complex formation. All cats tolerated the treatment well, with no harmful symptoms observed. These findings suggest that oral administration of a Fel d 1–specific antibody is a promising method for reducing salivary allergen levels in cats. Further studies with larger sample sizes, diversified delivery methods, and direct evaluation in cat-allergic humans are warranted to confirm these results and assess long-term efficacy.

Key Words: cat allergy, hagfish antibody, Fel d 1, neutralization

P96 - The development of antigen-specific regulatory T cells in autoimmune myocarditis.

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Autoimmune myocarditis is inflammation of the myocardium from the breakdown of immune tolerance, and is associated with a poor prognosis. Approximately 30% of cases progress to inflammatory dilated cardiomyopathy (iDCM), and 66% of patients die within 15 years of diagnosis. Regulatory T cells (Tregs) are essential modulators of the immune system and play a key role in maintaining peripheral tolerance. Antigen-specific Tregs and chimeric antigen receptor (CAR)-Tregs have recently emerged as novel therapies for treating autoimmune disorders; however, antigen-specific Tregs remain understudied in inflammatory cardiovascular diseases. In this study, we hypothesize that antigen-specific Tregs play an essential modulatory role in the heart during autoimmune myocarditis. WT BALB/cJ mice were immunized with α-cardiac myosin heavy chain (α-MyHC) peptide for experimental autoimmune myocarditis (EAM) induction. Hematoxylin and eosin staining was used for disease assessment, and spectral flow cytometry was used for immune profiling in the heart, including the presence of α-MyHC–specific CD4+ T cells. In mice with EAM, there was significant infiltration in the heart, whereas control mice did not show inflammation. Flow cytometry analysis revealed an increase in CD4+ T cells, CD8+ T cells, neutrophils, macrophages, and monocytes in EAM compared with controls. Most interestingly, there was an increase in α-MyHC–specific CD4+ T cells, both effector T cells and Tregs, in the heart with EAM, whereas they were not present in controls. These findings suggest the development of α-MyHC–specific Tregs during autoimmune myocarditis. Our ongoing studies aim to determine the modulatory role of α-MyHC–specific Treg cells in myocarditis.

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P97 - Defining chicken host responses to spotty liver disease.

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Spotty liver disease (SLD) is an acute bacterial infection of layer hens, caused by *Campylobacter hepaticus*, and occurs most frequently in barn-housed and free-range production systems. The disease is characterized by a sharp decline in egg production and increased mortality. The hallmark pathological feature is the presence of 1 to 2 mm white to gray necrotic foci distributed across the liver surface. Despite its substantial and growing economic impact on commercial poultry, the molecular mechanisms underlying host responses to *C. hepaticus* infection remain poorly understood. To address this gap, we performed a comprehensive analysis using RNA sequencing of liver tissue from naturally infected chickens compared with uninfected controls. Total RNA was extracted from samples and subjected to high-throughput sequencing, yielding 9,277 differentially expressed genes, of which 3,063 were significantly upregulated and 6,214 were downregulated. Functional pathway enrichment analysis revealed major alterations in immune and metabolic processes associated with SLD pathophysiology. Infected chickens exhibited a significant activation of immune response pathways, particularly cytokine-cytokine receptor interactions involving interleukins IL-22, IL-21, and IL-6, along with enhanced cell signaling, cell adhesion, and calcium ion binding pathways. Among individual genes, C1QTNF1 and the adhesion molecule ADGRD1 genes were notably overexpressed, indicating enhanced inflammatory activity. In contrast, core hepatic metabolic functions were profoundly suppressed, as evidenced by the downregulation of oxidative phosphorylation, fatty acid metabolism, carbon metabolism, iron ion binding, and heme binding pathways. A marked increase in serum amyloid A gene expression further confirmed robust acute-phase responses and compromised liver function during infection. Together, these findings highlight strong immune activation accompanied by marked suppression of metabolic pathways during SLD. The strong upregulation of acute-phase proteins and proinflammatory cytokines demonstrates the host's vigorous attempt to combat bacterial infection, and the concurrent suppression of essential metabolic pathways reflects the pathological consequences of SLD. This study provides the first detailed transcriptomic characterization of host responses to *C. hepaticus* infection, offering critical insights into SLD pathogenesis and potential avenues for targeted intervention.

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Key Words: *Campylobacter hepaticus*, chicken liver, immune response, spotty liver disease, transcriptomics

General Abstracts: Mastitis—Posters

P98 - Effects of systemic inflammation in postpartum dairy cattle on performance and immune function during an intramammary *Streptococcus uberis* challenge.

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Elevated concentrations of proinflammatory cytokines during the peripartum period have been associated with an increased risk for postpartum diseases including mastitis in dairy cattle. Nevertheless, these associations do not demonstrate a cause-and-effect relationship. Therefore, the objective of this study was to evaluate the effects of recombinant bovine tumor necrosis factor α (rbTNF-α) on performance and immune function in postpartum dairy cattle during an intramammary challenge. Parous Holstein dry cows were blocked by parity, expected calving date, and genetic merit for milk yield (PTAM) and randomly assigned within block to receive a daily s.c. injection of either (1) rbTNF-α (n = 10; 2 µg/kg BW in 10 mL PBS) or (2) saline (CON, n = 10; 10 mL of PBS) from d 1 to 7 postpartum. All cows received an intramammary *Streptococcus uberis* challenge (2,500 cfu) in a randomly selected rear quarter at 4 d postpartum. Blood and milk samples (composite and quarter) were collected once daily from d 1 to 8. A multiplex bovine cytokine kit was used to quantify interleukin (IL)-10, macrophage inflammatory protein (MIP)-1α, MIP-1β, and tumor necrosis factor (TNF)-α in plasma and milk from the challenged quarter. Data were analyzed using linear mixed models (PROC GLIMMIX in SAS). Cows treated with rbTNF-α produced less milk (28.2 vs. 32.9 ± 1.2 kg/d; $P = 0.01$), milk protein (1.1 vs. 1.3 ± 0.05 kg/d; $P < 0.01$), milk fat (1.4 vs. 1.5 ± 0.05 kg/d; $P = 0.02$), and energy-corrected milk yields (35.7 vs. 40.7 ± 1.1 kg/d; $P < 0.01$) than CON. Similarly, cows treated with rbTNF-α tended to consume less dry matter than CON (16.5 vs. 20.0 kg/d; $P = 0.06$). Plasma ($P < 0.0001$) and milk TNF-α ($P = 0.05$) were greater in rbTNF-α as compared with CON. Plasma MIP-1α ($P < 0.01$) concentrations were greater in rbTNF-α as compared with CON. There was a treatment × time interaction ($P \leq 0.05$) for plasma IL-10 and MIP-1β. Plasma IL-10 and MIP-1β concentrations were greater in rbTNF-α on d 1 through 7 ($P < 0.001$). Treatment did not affect milk concentrations of IL-10 ($P = 0.89$), MIP-1α ($P = 0.73$), or MIP-1β ($P = 0.88$). No difference was found for *S. uberis* bacterial counts ($P = 0.51$), challenged quarter foremilk somatic cell score (SCS; $P = 0.98$), or composite SCS ($P = 0.32$) during the intramammary challenge. In conclusion, induction of systemic inflammation via rbTNF-α induced a broad systemic cytokine response; however, aside from enhancing milk TNF-α, rbTNF-α did not affect mammary gland immune responses.

Financial Support: This project was supported by Agriculture and Food Research Initiative Competitive Grant no. 2024-67016-42630 from the USDA National Institute of Food and Agriculture.

Key Words: cytokine, postpartum dairy cow, mastitis

P99 - Whole genome sequencing of *Staphylococcus chromogenes* causing persistent intramammary infections reveals limited associations between genotype and mastitis phenotype in a longitudinal observational study of cattle from 10 dairy herds.

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Staphylococcus chromogenes is frequently isolated from dairy cattle mastitis cases. Our objective was to use whole genome sequencing to characterize strains of *S. chromogenes* associated with distinct intramammary infection (IMI) phenotypes. Isolates originate from a longitudinal, observational study of 10 organic dairy farms in Vermont, where aerobic culture and somatic cell count (SCC) measurement were repeated monthly for all quarter-milk samples from at least 30 cows per farm. Persistent IMI was defined as isolation of *S. chromogenes* on 2 or more consecutive milk samples where the same random amplified polymorphic DNA (RAPD)-PCR DNA banding pattern was observed for the repeated isolates from a quarter. Two IMI phenotypes were defined based on repeated milk SCC observations within an udder quarter: (1) persistent high SCC IMI, where all samples had a SCC of ≥200,000 cells/mL; and (2) persistent low SCC IMI, where all samples had a SCC of <200,000 cells/mL. Representative isolates from 15 low SCC IMI and 15 high SCC IMI were submitted for whole genome sequencing. Isolate strain types (ST) were predicted from the annotated genomes according to a 7-locus multilocus sequencing typing scheme. Antimicrobial resistance genes (ARGs) and virulence factor (VF) genes were identified from assembled genomes by comparison to reference databases. Ten different ST were identified, including 4 novel ST. Seven ST were identified in each SCC category, with 3 unique to each. The *blaZ* gene, encoding for resistance to penicillin, was the only ARG identified (33.3% of isolates; 6/15 in the high SCC category and 4/15 in the low SCC category). Sixty-two unique VF were identified. Thirty-nine VF were present in all isolates, including genes associated with iron uptake and metabolism, production of phenol-soluble modulins, hemolysins, and an exfoliative toxin. Presence of VF associated with adherence, host immune evasion, type VII secretion system, and production of exoenzymes and exotoxins was variable among isolates. In the high SCC category, 677 VF total were identified versus 670 in the low SCC category. Genes encoding for exfoliative toxin type C (etc) and staphylocoagulase (coa) were identified, neither of which have been widely reported for *S. chromogenes* isolates of bovine origin. *blaZ* carriage and number and type of VF may be associated with *S. chromogenes* ST. More research is needed to confirm these findings.

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Key Words: molecular epidemiology, mastitis, non-*aureus* staphylococci, dairy cattle, genomics

P100 - Effects of dietary supplementation of *Bacillus subtilis* PB6 to mid-lactation cows on local inflammatory responses during an intramammary lipopolysaccharide challenge.

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Objectives of this study were to evaluate the effects of dietary *Bacillus subtilis* PB6 supplementation on inflammatory mediators in milk during an intramammary lipopolysaccharide (LPS) challenge in dairy cows. Mid-lactation Holstein cows (n = 20; 147 ± 48 DIM; 2.2 ± 0.93 parity; 42.1 ± 7.6 kg/d of milk; mean ± SD) were enrolled in a crossover design. Treatments included *Bacillus subtilis* PB6 (BSP; CLOSTAT®, Kemin Industries Inc., Des Moines, IA) or limestone (CON) at 13 g/d, which were top-dressed daily onto a basal lactating TMR for 28-d periods. An intramammary LPS challenge (10 µg) was conducted in a rear quarter on d 24 of each period. Milk samples were collected at 0, 3, 12, and 24 h relative to the intramammary LPS challenge from the challenged quarter. Milk samples were treated with rennet and centrifuged to obtain whey, and 13 cytokines/chemokines were measured using a multiplex assay (IFN-γ, IL-1α, IL-1β, IL-4, IL-6, IL-10, IL-17A, IL-36RA, IP-10, MCP-1, MIP-1α, TNF-α, and VEGF-A). Linear mixed models included the fixed effects of treatment, period, and time, and their interactions, and the random effects of cow nested within period, block, and block by period. There was a treatment by period by time interaction ($P < 0.01$) for whey MCP-1 where dietary BSP supplementation increased MCP-1 at 3 h ($P = 0.03$), 12 h ($P = 0.02$), and 24 h ($P = 0.01$) during period 1, but decreased whey MCP-1 at 3 h ($P = 0.05$) and 12 h ($P = 0.04$) during period 2 as compared with the CON. These data suggest that dietary BSP supplementation enhanced innate immune training during period 1 that may have carried over into period 2. Dietary BSP supplementation did not affect the concentrations of all other whey cytokines ($P \geq 0.20$). Independent of treatment sequence, period 1 had greater concentrations of whey IL-1α and IL-1β at 3 h following the LPS challenge as compared with period 2, suggesting the development of immunological tolerance to LPS. Finally, aside from IL-4, all other cytokines measured were affected by time ($P \leq 0.02$), where we found increases (IFN-γ, IL-1α, IL-1β, IL-6, IL-10, IL-36RA, IP-10, MCP-1, MIP-1α, and TNFα) or decreases (IL-17A and VEGF-A) in their concentrations following the LPS challenge. In conclusion, our data suggests that dietary BSP supplementation may have a limited impact on whey cytokine concentrations during an intramammary LPS challenge.

Financial Support: Funding was provided by Kemin Industries (Des Moines, IA).

Key Words: cytokine, innate immune training, immunological tolerance, direct-fed microbial

P101 - Efficacy of soluble epoxide hydrolase inhibition on modulating clinical disease severity in experimental bovine coliform mastitis.

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Severe bovine mastitis caused by *Escherichia coli* is characterized by major alterations in cytochrome P450 (CYP450)-derived lipid inflammatory mediators, known as epoxy fatty acids. Soluble epoxide hydrolase (sEH) further metabolizes and alters the epoxy fatty acid profiles during mastitis. The sEH activity increases as early as 6 h after mammary *E. coli* inoculation in Holstein cows, preceding clinical signs. Based on improved clinical outcomes in comparable disease models, we hypothesized that limiting epoxy fatty acid metabolism through sEH inhibition (sEHI) modulates experimental bovine coliform mastitis signs. Holstein dairy cows (n = 21) were enrolled in a randomized noninferiority study comparing sEHI efficacy to standard nonsteroidal anti-inflammatory treatment with flunixin meglumine (FM). The cows were enrolled into 3 treatment groups (n = 7/group), namely 3 mg/kg sEHI, 1.1 to 2.2 mg/kg FM, and control (CON) cows receiving the sEHI solvent. Cows were enrolled based on lack of clinical disease signs, including mastitis, negative bacterial milk cultures (<3 cfu/µL), SCC <200,000 cells/mL, and absence of systemic inflammation on CBC and plasma fibrinogen. Intramammary inoculation (IMI) of 400 cfu of *E. coli* P4 strain was performed into the right front quarter in all cows. Cows were monitored for systemic clinical signs, including elevated rectal temperature (RT), heart rate (HR), and respiratory rate (RR), physical signs of mammary inflammation (swelling, redness, firmness), and milk production. All treatments were administered intravenously 6 h post-IMI. Blood and milk samples were collected for CBC, inflammatory cytokines (Interleukins 1 and 6), acute phase proteins (serum amyloid A, haptoglobin, and fibrinogen), and analyses of CYP450-derived epoxy fatty acids. Individual quarter milk samples were analyzed for bacteriological status and SCC. Data were compared using repeated measures ANOVA with time, treatment, and time-by-treatment interaction as the variables of interest using SAS software (Cary, NC) with α set at 0.05. All cows developed systemic clinical signs (increases in RR, HR, RT) and mammary gland inflammation by 12 h post-IMI, but no treatment differences were detected. Milk production was lowest in all groups at 12 h, with production in CON significantly lower than sEHI treated cows. Additional data will be available in the next 2 mo, and conclusions on sEHI efficacy will be discussed.

Financial Support: This project was supported by Agriculture and Food Research Initiative Competitive Grant no. 2020-67015-38956 from the USDA National Institute of Food and Agriculture

Key Words: cytochrome P450, dairy, epoxy fatty acid, inflammation, mastitis, soluble epoxide hydrolase

P102 - Evaluation of induced *Staphylococcus chromogenes* intramammary infections in dairy heifers.

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The objectives of this study were to (1) determine if phenotypic behaviors of *Staphylococcus chromogenes* (SC) are reproducible using in vivo challenge models and (2) determine the ability of SC teat colonization or intramammary infection (IMI) to prevent *S. aureus* (SA) IMI in dairy heifers. The study was conducted at the University of Missouri Foremost Dairy Research Center (ACUC #59201). Holstein heifers were sampled before enrollment, and only intramammary infection–negative quarters were enrolled. Enrolled quarters were randomly assigned to 1 of 8 treatment groups. Treatment groups included assignments to treatment with one of 3 SC strains (SC62, SC99, and SC304) and one SA strain (SA29740). Using these isolates, the 8 treatment groups included one group challenged with each SC strain only (groups 1, 2, 3), each SC strain plus the SA secondary challenge (groups 4, 5, 6), a group only challenged with the SA strain (group 7), and a broth negative control (group 8). A double teat dip challenge technique with bacterial concentration of 1×10^5 cfu/mL was used. Enrolled quarters were dipped with the corresponding broth once a day for 14 d. Next, assigned quarters were dipped with the SA broth for 14 d. Quarters were sampled 2 to 3 wk before parturition. After calving, aseptic milk samples were collected throughout lactation using National Mastitis Council milk sampling protocols. All isolates were speciation using MALDI-TOF mass spectrometry. Data analysis was completed using SAS software to evaluate the relationship between quarter-level SCC and the incidence of SA IMI relative to SCIMI/teat skin colonization. Overall, 206 quarters were enrolled in the study. No SC IMI were identified 14 d post-challenge. Among postpartum milk samples, 38/198 (19%) quarter were SC IMI positive, whereas 9/198 (5%) were SA IMI positive. A total of 24/38 (63%) SC positive quarters were treated with a SC strain, whereas 5/9 (56%) SA-positive quarters were treated with the SA strain. In the future, strain typing will be used to differentiate the SC and SA strains to confirm if infections are associated with the challenge strains. This experimental challenge model has the capacity to provide a method to evaluate strain level variability among SC strains. We anticipate that the SC IMI will be protective against the SA challenge. These results could open the door to novel non-antimicrobial methods for SA control in heifers.

Financial Support: This project was supported by Agriculture and Food Research Initiative Competitive Grant #2022-67015-37123 from the USDA National Institute of Food and Agriculture.

Key Words: heifer, mastitis, dairy, staphylococcal species, induced infection

General Abstracts: One Health and Public Health—Posters

P103 - Persistence and infectivity of Seneca virus A and porcine epidemic diarrhea virus in houseflies (*Musca domestica*).

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Houseflies (*Musca domestica*) are ubiquitous synanthropic insects closely associated with human and animal environments. Their feeding and breeding behaviors bring them into frequent contact with animal waste, decomposing matter, and secretions, making them well-documented mechanical vectors of bacteria, protozoa, and, to a lesser extent, viruses. This study investigated the persistence and infectivity of Seneca virus A (SVA) and porcine epidemic diarrhea virus (PEDV) in house flies. For each virus, 180 houseflies 24 to 48 h post-emergence were allocated in 9 groups (n = 20; 10 females and 10 males). Flies were exposed to SVA- or PEDV-spiked medium (final input titers: SVA = $10^{8.68}$ TCID50/mL; PEDV = $10^{5.8}$ TCID50/mL) for 2 h. At 2, 6, 12, 24, 36, 48, 60, 72, and 96 h post-exposure, flies were euthanized by freezing at −20°C and processed. Infectious virus was assessed by isolation and virus titration (TCID50) using the Reed–Muench approach on ST and Vero cells, respectively, for SVA and PEDV. Viral RNA was extracted and quantified by RT-qPCR. Synthetic double-stranded DNA fragments (gBlocks) were used as a standard curve for estimating genome copy number. Infectivity assays revealed that SVA remained infectious up to 96 h ($10^{4.5}$ TCID50/mL), whereas PEDV retained infectivity for up to 6 h ($^{.3}$ TCID50/mL). Viral RNA from both SVA and PEDV was detected in flies up to 96 h after exposure. In addition, qPCR analysis revealed a progressive decline in viral RNA copies over time, indicating decreasing viral loads (PEDV: $10^{5.57}$ copies/mL at 2 h vs. $^{.15}$ copies/mL at 96 h; SVA: $10^{5.93}$ copies/mL at 2 h vs. $10^{4.99}$ copies/mL at 96 h). Under laboratory conditions, houseflies acquired and harbored significant infectious SVA and PEDV for up to 96 h and 6 h, respectively. Notably, the viral loads and persistent titers observed correspond to infectious doses capable of causing disease in both neonatal and weaned pigs. These findings highlight the potential of houseflies as mechanical vectors of livestock viruses, warranting further investigation into their role under natural conditions and the broader implications for veterinary public health.

Financial Support: USDA-APHIS-NADPRP grant AP24VSSP0000C064

Key Words: housefly, mechanical vector, Seneca virus A, porcine epidemic diarrhea virus, persistence, infectivity

P104 - Avian influenza: Multi-disciplinary approaches to educating small-scale poultry owners.

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Since 2022, over 182 million US domestic poultry have died from highly pathogenic avian influenza, with more than half of the affected flocks being backyard flocks. Additionally, 15,340 wild birds tested positive. In 2024, our project team surveyed backyard poultry owners across Indiana, including adults (n = 195), youth in grades 3 to 6 (n = 171), youth in grades 7 to 12 (n = 226), and 117 extension educators. The survey revealed common knowledge gaps in biosecurity and reliable information, especially among youth and adult owners. Over half of extension educators reported low confidence in advising on backyard bird management, with ~60% considering disease transmission to humans a minor issue. Our current project aims to create tailored educational materials to address these gaps, distribute them across Indiana's 92 counties, and evaluate their impact. An online interactive course was developed and shared with 4-H and agricultural extension educators via listserv, reaching ~1 to 4 educators per county. The course included a survey link for testing purposes and monetary incentives, along with an invitation to join a focus group. We also developed a 4-H class with hands-on activities and a card game, supported by marketing materials such as superhero stickers and a printed egg bucket with biosecurity tips and a QR code linking to a webpage with trustworthy contacts for future information. These resources were distributed statewide for use by educators and volunteers in youth poultry programs. The class included pre- and post-quizzes for youth, with results submitted via Qualtrics and optional feedback. Data analysis will include descriptive statistics of educator responses, qualitative summaries of focus groups, and assessments of knowledge gains among youth through pre- and post-quiz comparisons using *t*-tests and chi-squared tests as appropriate. The webpage linked to the bucket QR code will also be evaluated. By engaging extension educators in our co-creation process alongside experts in poultry, mass communication, and education, we have developed several innovative educational and marketing products. These have been well received by our stakeholders. We hope to use feedback to improve our products, and also to adapt and expand them for future dissemination in other states.

Financial Support: This project is funded by USDA's Animal and Plant Health Inspection Service through the National Animal Disease Preparedness and Response Program. (Grant number AP23VSSP0000C048)

Key Words: avian influenza, poultry, biosecurity, education, One Health

P105 - Foodborne zoonotic bacterial diseases from animal-origin foods: Knowledge, practices, and health-economic burden in Northern Ethiopia.

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Unsafe animal-derived products along the farm-to-fork continuum are a major source of foodborne zoonotic diseases, particularly in low-income settings. In Ethiopia, the substantial health and economic burden associated with these diseases has been largely overlooked. Comprehensive local studies are therefore needed to fill critical data gaps and support evidence-based prioritization of food safety interventions. A cross-sectional survey was conducted among 440 household heads across various districts of East Gojjam, Northern Ethiopia from May to July 2024 to assess knowledge, attitudes, and practices (KAP) as risk factors for foodborne zoonotic bacterial diseases. The disease burden was estimated using 765 reported cases of foodborne illnesses from 5 governmental hospitals between November 2024 and January 2025. Public health impact was quantified using disability-adjusted life years (DALYs), whereas the economic burden was evaluated in terms of direct losses and indirect healthcare costs. The KAP assessment revealed that 90.55% of households, regardless of demographic characteristics, had a history of contracting foodborne diseases and shared similar consumption behaviors and handling practices for foods of animal origin. Indigenous preservation techniques, the availability of cooling or pasturing facilities (P = 0.0001), and prior awareness of transmission pathways (P = 0.002) were significantly protective. Conversely, poor hygiene practices in food handling and catering, along with the consumption of raw or undercooked animal-origin foods, significantly increased disease risk (P = 0.001). The mean healthy life years lost was estimated at 207.96 DALYs per 100,000 population annually in the 5 study districts. When extrapolated to the entire East Gojjam zone (2,632,632 people across 21 districts), this amounted to 193.61 DALYs per 100,000 population per year. The direct and indirect economic burden was estimated at US$2,085,931.60 annually. This estimate excluded losses to animals and their products, temporary subsidies of treatment and diagnostic costs by the central government and USAID, longer fasting seasons, and lower wage rates in the area. In conclusion, foodborne bacterial zoonotic diseases of animal origin represent a growing public health and economic challenge in East Gojjam. Addressing this issue requires a coordinated, integrated preventive approach involving One Health actors.

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Key Words: bacterial; zoonoses; disability-adjusted life years (DALY); East Gojjam; food-borne; knowledge, attitudes, and practices (KAP)

P106 - Evaluation of the variability of per- and polyfluoroalkyl substances serum concentrations in beef cattle.

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Per- and polyfluoroalkyl substances (PFAs) are a group of synthetic chemicals commonly used as protectants against heat, water, or grease. Per- and polyfluoroalkyl substances are a public concern due to their long half-life and association with the development of several forms of cancer. Animals destined for food production who have been exposed to high concentrations of PFAs are euthanized to maintain food safety. Recently, a strong association was reported between PFA concentration in muscle and blood at the time of slaughter, suggesting that antemortem blood PFAs determinations could be used to assess the suitability of animals for the food supply. However, the extent to which PFAs blood concentrations show diurnal, weekly, and/or monthly variations remains unknown. This could limit the suitability of a single blood PFAs quantification to predict muscle concentrations. Thus, this study aims to determine the variability of PFAs concentrations in cattle serum. For this, 10 female adult beef cattle from a PFA-contaminated farm were repeatedly blood sampled (1) every 2 h for 1 d, (2) every day for 5 d, and (3) every 2 wk for 3 mo. Serum was analyzed for PFAs concentrations via LC-MS/MS. Changes in serum PFAs concentrations over time were analyzed via repeated measures mixed models, with independent models built to assess daily, weekly, and fortnightly variation. Concentration of PFAs does not significantly vary across a day ($P = 0.17$), indicating that blood tests could be conducted at any time of day. The PFAs quantification results to evaluate weekly and fortnightly variation are pending. However, based on the long half-life of PFAs, we hypothesized that the expected gradual decline over time would not reach statistical significance.

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Key Words: per- and polyfluoroalkyl substances (PFAs), antemortem, serum

P107 - Emerging insights on leptospirosis from Central Appalachia and their broader national implications.

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This study aims to define the host range and infection dynamics of *Leptospira* among companion animals, livestock, wildlife, and aquatic or semi-aquatic species in the Cumberland Gap Region (CGR) of Central Appalachia. Serological testing was performed using the microscopic agglutination test (MAT) to assess exposure, and quantitative PCR (qPCR) was employed to detect urinary shedding and renal colonization. Genotyping and molecular characterization were conducted using rpoB and lipL32 gene PCR, followed by sequencing. Results from our surveillance studies in the CGR has revealed a complex, multi-host ecology with implications beyond the region. These studies documented widespread *Leptospira* exposure, including high renal carriage in small wild mammals (~60%) and detection in surface waters (~2%), establishing an environmental bridge for transmission at the human-animal interface. Shelter-based studies showed that clinically normal dogs can shed *Leptospira* (13%) and are frequently seropositive (18%), underscoring dissemination risks through interstate adoptions. Pathogenic *Leptospira* DNA identified in 12.9% of herpetofauna and 4.6% of fish further suggests a role for aquatic and semiaquatic hosts in persistence and environmental contamination. Collectively, these findings depict an Appalachian leptospirosis system defined by multi-host reservoirs, environmental contamination, and animal movement, patterns likely mirrored in many US regions with comparable hydrology, wildlife, and shelter practices.

Financial Support: This study was funded by the LMU-CVM intramural research grant #25AV001

Key Words: *Leptospira*, leptospirosis, shelter dog, herpetofauna, Appalachia

P108 - Seasonality of antimicrobial resistance genes in community wastewater (a systematic review study).

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Changes in seasonal parameters, particularly temperature, are known to modulate antimicrobial resistance mechanisms in bacteria. However, whether these effects translate into global-scale patterns in the community remains poorly understood. Here, we aimed to review seasonal patterns of antimicrobial resistance on a global scale by systematically reviewing ARG abundance and presence in wastewater across diverse climates. We conducted a systematic review of the PubMed, Web of Science, and SCOPUS databases for articles published between 1998 and 2024 with the aim of identifying seasonal patterns of antimicrobial resistance genes detected in influent community wastewater. Using the random effects model with a restricted maximum-likelihood estimator, we synthesized standardized mean effect sizes between seasons across 44 studies. This study observed a significant increase in the abundance of sulfonamides resistance genes (SMD 0.55 [0.08; 1.02]) and a decrease in β-lactamase gene abundance (SMD −0.55 [−0.77; −0.33]) from spring to summer. No significant changes in gene abundance were detected for other seasonal transitions. Additionally, the abundance of tetracycline resistance genes remained stable across all 4 seasons. These findings provide robust evidence for global community patterns demonstrating that warmer periods are associated with predictable changes in the baseline of some resistance genes in community wastewater.

Financial Support: Water Research Foundation

Key Words: community wastewater, antimicrobial resistance, One Health, resistant gene

P109 - Antibody testing against *Coxiella burnetii* in beef cattle using 2 different antibody ELISA kits.

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Coxiella burnetii is a select agent that causes Q fever in human and abortions in ruminants. Antibody tests are more commonly used as a standard diagnostic method. We evaluated 2 commercially available Q-fever antibody testing kits with relatively a large number of serum samples collected from beef cattle operations. A collection of 1,129 serum samples were tested with a commercial *Coxiella burnetii* antibody ELISA kit (kit 1). All positive samples identified by kit 1 were tested with a different commercial ELISA kit (kit 2). Animal age and pregnancy status were recorded at sample collections. Bovine leukemia virus (BLV) was tested with a qPCR assay as a potential immunodeficiency condition indicator. Test kit 1 identified 147 antibody-positive animals. Among these 147 samples, 73 samples were also tested positive with the test kit 2. The remaining results by kit 2 included 17 suspected positive (borderline between positive and negative) and 57 negative samples. There were 137 of the 147 (93.2%) Q-fever antibody positive animals by test kit 1 were pregnant animals. Similar results were obtained by test kit 2, from which 69 of the 73 (94.5%) antibody-positive animals were pregnant. The proportion of BLV positive samples also remain consistent across 2 assays. There were 58 animals out of 147 (39.5%) Q-fever antibody positives were also positive to BLV by test kit 1; and there were 27 animals from the 73 (36.9%) Q-fever positives were also BLV positive. Despite the significant different results observed between the 2 Q-fever antibody testing kits (13.0% by kit 1; 6.5% by kit 2), Q-fever antibody was clearly detected in beef cattle operations. Our results demonstrated that *Coxiella burnetii* has been circulating not only in dairy cattle (as reported by others), but in beef cattle as well. Its effects on public health, though, are not yet clear and need more investigations. It appears that more pregnant animals were Q-fever antibody positive. However, 1,013 of the 1,129 animals we collected samples from were pregnant. Thus, there is no clear relationship between pregnancy and Q-fever status. From the 1,129 animals that we collected serum samples, whole blood samples from 567 were also collected and test by a *Coxiella burnetii* qPCR assay. None of the 567 blood samples were positive, indicating the infection stage at sample collection may be critical to qPCR testing.

Financial Support: CDC - Association of Public Health Laboratories Fellowship, Kansas Veterinary Diagnostic Laboratory

Key Words: *Coxiella burnetii*, Q fever, ELISA, beef cattle, diagnosis, public health

P111 - One Health systems-thinking approaches to understand interrelationships among factors influencing the spread of antimicrobial resistance: A scoping review of current approaches.

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Antimicrobial resistance (AMR) is a global health challenge affecting human, animal, and environmental systems. The One Health approach has been adopted to study AMR, recognizing the inherent interconnectedness of these domains. However, most published studies focus on a single domain. This gap in multidomain AMR data resources hinders a better understanding of the interrelationships among factors influencing the spread of AMR. To address this, we conducted a scoping review to answer the following question: What systems-thinking approaches have been applied to study factors influencing AMR spread across One Health domains? Following PRISMA guidelines, we systematically searched the PubMed database for research studies on AMR across all One Health domains. The compound search strategy was developed to retrieve only research articles in English on AMR from across all One Health domains. The initial 1,897 articles were screened by 3 reviewers using preselected inclusion and exclusion criteria, yielding 37 articles for the study. Preliminary analysis revealed that nearly all studies (36; 97%) referenced the One Health framework. The majority were cross-sectional designs (30; 81%), followed by longitudinal studies (7; 19%). We also found considerable variation in methodologies used to assess AMR across sectors. Most studies focused on indicator bacteria or specific AMR genes (14; 38%), whereas microbiome- and resistome-focused studies accounted for 8 (22%). Data analysis is currently ongoing; however, we expect that this review will generate insights into gaps and opportunities for applying systems-thinking approaches to address AMR across One Health domains. Ultimately, the knowledge and multi-domain data resources generated from this study will contribute to the existing AMR literature, support researchers, and facilitate collaborative research efforts to address AMR through a One Health approach.

Financial Support: This study was funded in part by the Texas Tech University School of Veterinary Medicine (TTU-SVM).

Key Words: antimicrobial resistance (AMR), One Health, systems thinking, scoping review

P112 - Evaluating hepatic metabolic dysregulation during hyperglycemia in a chicken embryo model of gestational diabetes mellitus.

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Gestational diabetes mellitus (GDM) affects ~20% of pregnancies worldwide, contributing to maternal hyperglycemia that disrupts the gut microbiota and facilitates the transplacental transfer of microbial components to the fetus. Chicken embryos, offer a promising alternative to mammalian models due to lack of maternal influence. However, this model has limitations, including the absence of a placenta and maternal metabolic influences, which hinder direct simulation of transplacental nutrient exchange and hormone. This study examines hyperglycemia-induced metabolic changes in chicken embryos as a GDM model. We hypothesize that glucose overload causes metabolic and developmental disruptions, which probiotics may mitigate. Fertile Cobb500 eggs (n = 36 per group) were inoculated with 20 µL of 0.9% normal saline (control), 138.8 mmol/L D-glucose, 10^{10} cfu/mL live *Leuconostoc pseudomesenteroides* (Live MLS3), heat-inactivated MLS3 (HI MLS3), or combinations of Live MLS3 or HI MLS3 with D-glucose, administered on alternate days from embryonic d 4 (E4). Embryos were euthanized by decapitation, and samples were collected on E18. Data were analyzed using one-way ANOVA with multiple comparisons in GraphPad Prism. Although not statistically significant ($P = 0.069$), blood glucose levels increased in the glucose-treated group (146.8 ± 6.314 mg/dL) compared with the saline control (136.8 ± 1.911 mg/dL), indicating transient hyperglycemia induced by in ovo glucose administration. Relative liver weight was elevated by 19.92% in the glucose group ($P = 0.016$), an effect normalized by Live MLS3 ($P = 0.009$) and HI MLS3 ($P = 0.003$). Pearson correlation analysis demonstrated a positive association between blood glucose levels and relative liver weight (r = 0.61). Transcriptomic profiling revealed significant glucose-mediated alterations in genes associated with metabolism (e.g., *PCK1*, *LDHA*, *FOXA1*, *AKIRIN2*, *HEXA*), inflammation (e.g., *CD163*, *TNFSF10*), stress response (e.g., *DUSP1*, *SGK1*, *RASD1*), and mitochondrial function (e.g., *HIGD1C*, *AK3*, *ATP5F1AW*). Both live and heat-inactivated probiotics attenuated these hyperglycemia-induced changes. These findings indicate that in ovo hyperglycemia elicits hepatic metabolic disruptions, impairing key pathways in chicken embryos. Given inherent differences in metabolism and development between avian and mammalian systems, future investigations should extend to post-hatch analyses and incorporate translational validation in murine models.

Key Words: gestational diabetes mellitus, chicken embryo, hyperglycemia, probiotic, leaky gut, translational model

P113 - Serological evidence of leptospiral infection in sheep from East Tennessee.

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Leptospirosis is a globally important zoonotic disease affecting multiple animal species, but its prevalence in small ruminants in the United States remains poorly understood. This study aimed to determine the seroprevalence of Leptospira infection in sheep across farms in Tennessee and Virginia. A total of 257 sheep representing different breeds from 17 farms were screened using the microscopic agglutination test (MAT). Of these, 44 animals tested positive, yielding an overall seroprevalence of 17.12% (95% CI: 12.38%–21.86%). These findings indicate that exposure to *Leptospira* is relatively common among sheep population in this region. The presence of seropositive animals across multiple farms highlights the potential role of sheep in epidemiology of leptospirosis, with implications for both animal health and zoonotic transmission. Further studies are warranted to identify circulating serovars in sheep, risk factors associated with infection, and strategies to mitigate the spread of disease.

Financial Support: This study was funded by the LMU-CVM intramural research grant #25AV001

Key Words: leptospirosis, *Leptospira*, small ruminant, sheep, Appalachia, One Health

P114 - Wild encounters: Characterizing the frequency and nature of human interactions with peridomestic species, Minnesota State Fair, 2024.

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Increased urbanization allows for frequent human-wildlife interactions and opportunities for pathogen transmission. Although peridomestic species (animals adapted to living near humans) likely have greater opportunities to transmit pathogens, they are often overlooked by public health. The main purpose of this study was to describe the frequency, location, and nature of encounters with peridomestic species of public health importance during summer 2024. A cross-sectional survey was conducted in 2024 at the Minnesota State Fair, an event that attracts nearly 2 million people annually. Respondents were asked about the nature of wildlife encounters between May and August. Direct contact was defined as hunting or physical contact. Respondents were presented with wildlife scenarios and asked how they would respond. Descriptive statistics were calculated using SAS 9.4. A total of 701 respondents reported 6,290 encounters with 3,759 animals (mean of 5.4 species and 9.0 encounters per respondent). There was no difference in total encounters by urbanicity ($\chi^2 = 1.98$, $P = 0.372$). We found that 11.9% of encounters were with species associated with pathogen transmission (bats and raccoons) and 71.4% were with squirrels (20.0%), songbirds (17.7%), rabbits (16.9%), and ducks (16.8%). Most encounters (71.3%) occurred either daily (45.5%) or weekly (25.8%) around homes (51.0%) or local parks (23.1%); 36% reported recent direct contact, and 4.1% reported rescuing a sick or injured animal (re-nesting or taking for care). In addition, 48.4% reported having a bird feeder or providing outdoor bird food, and 31.2% reported having outdoor water or food (pet food or table scraps). Those with outdoor food had 1.3 times more daily wildlife encounters than those without (RR = 1.31, 95% CI 1.18, 1.45). When asked about common wildlife scenarios near their homes, 52.1% indicated they would have contact with either a strange-acting raccoon (8.7%), very young rabbit (9.0%), or dead squirrel (44.9%). We documented frequent interactions with peridomestic species around respondent homes. This interaction increased with owners providing outdoor food for wildlife. Given the frequent public contact with wildlife, future work includes developing methods to characterize species demographics, census numbers, and evaluating zoonotic pathogen risks based on numbers and species.

Key Words: One Health, human wildlife interaction, public health, zoonoses, disease transmission

P115 - Characterizing viral receptor gene diversity in white-tailed deer (*Odocoileus virginianus*).

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White-tailed deer (WTD) are an abundant and widely distributed species in North America that interact closely with humans, livestock, and other wildlife, creating opportunities for viral transmission. Viral entry is strongly shaped by host receptor variation, a pattern seen across many mammalian systems. Examples include changes in ACE2 affecting SARS-CoV-2 evolution, virus-driven selection on receptors such as NPC1 in bats and TFRC in rodents, and the ability of several viruses to utilize multiple host receptors during infection. These trends highlight that receptor diversity in wildlife species can play a key role in viral susceptibility and spillover potential. Studies have demonstrated WTD susceptibility to viral pathogens such as SARS-CoV-2, suggesting their potential role as both sentinel and reservoir species. To focus on knowledge gaps in WTD-specific viral receptors, a prior comprehensive study of cellular receptors for mammalian viruses was cross-referenced with the WTD reference genome (Ovbor1.2), identifying 186 viral entry–related cellular receptor genes present in WTD. These 186 genes include receptors targeted by individual viruses and those utilized by multiple viral families to enter cells. Nasal swabs collected through US Department of Agriculture surveillance programs were used for genomic analyses. The DNA was extracted using the ZymoBIOMICS Quick DNA/RNA kit, and a hybrid capture panel from Twist Biosciences was used to selectively enrich for the 186 receptor genes for downstream sequencing. Sequencing was performed on an Illumina NextSeq 2000 platform, followed by demultiplexing, alignment, variant calling, and annotation using standard workflows with samtools, bcftools, vcftools, and a custom WTD SnpEff database. High-impact variants are defined by SnpEff based on predicted disruption to coding sequences, indicating potential effects on protein structure and thereby viral binding. Preliminary results from n = 24 samples (n = 8 per region) from Kansas-Missouri, Minnesota-Wisconsin, and New York-Maine identified 24 to 30 high-impact variants across 18 to 21 genes per region. These findings reveal variations in wild WTD populations that may influence viral susceptibility. Future work will expand to more regions and further explore the functional consequences of these variants.

Key Words: white-tailed deer, viral receptor, host genetics, hybrid capture, zoonotic risk

P117 - Characterization of biosecurity practices among swine producers in Puerto Rico.

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Effective biosecurity practices are essential to protect swine producers from infectious transboundary threats, particularly high-impact foreign animal diseases (FADs) such as African swine fever (ASF). Puerto Rico was designated as an ASF Protection Zone in 2021, requiring intensified biosecurity measures across the island. However, the current level of biosecurity implementation among local producers remains largely undocumented. The objective of this study was to describe biosecurity practices on swine farms across Puerto Rico using frameworks adapted from the Secure Pork Supply (SPS) initiative. A total of 95 farms participated in the study. All the farms were distributed across 33 municipalities and reported having a premise identification number and a designated biosecurity manager. Nevertheless, some critical gaps were identified. Notably, none of the farms reported having a specific site veterinarian on record, although they commonly worked with veterinary officials. In addition, 93.7% of farms lacked records of animal, vehicle, equipment, and personnel movements, and 91.6% did not have cleaning and disinfection (C&D) stations at the time of data capture. Approximately 70.3% of farms practiced waste feeding, and 51.6% had multiple species on-farm. The use of controlled entry points and perimeter fencing was limited, with fencing only present at 26.3% of sites. Approximately one-third of farms (35.8%) reported training their workers in biosecurity, typically annually; however, almost none of the farms documented the use of personal protective equipment (PPE) for entering and exiting biosecure areas. These findings highlight the need for targeted education and prevention efforts to address identified vulnerabilities and improve on-farm biosecurity. Enhancing farm-level biosecurity is essential to improving preparedness and protecting the swine industry from the introduction and spread of animal infectious diseases.

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Key Words: biosecurity awareness, disease prevention, preparedness, swine farm

General Abstracts: Parasitology—Posters

P118 - Unique glycolytic enzymes as targets for novel anti-*Cryptosporidium* drugs in bovine calves.

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Cryptosporidium parvum is a zoonotic protozoan parasite that commonly causes a serious diarrheal disease called cryptosporidiosis in neonatal calves, lambs, goat kids, and humans globally. No fully effective drugs nor vaccines exist. We identified inhibitors of *C. parvum*'s unique bacterial-type lactate dehydrogenase (CpLDH) and plant-like pyruvate kinase (CpPyK) enzymes in the glycolytic pathway. Some of those inhibitors block parasite replication and cure cryptosporidiosis in calves. We now focus on the derivatization of chemical analogs of CpLDH and CpPyK inhibitors with optimized selective superior inhibitory activity, translating into improved anticryptosporidial efficacy. We investigated safety and anticryptosporidial synergistic efficacy of CpPyK and CpLDH inhibitors in a mice and subsequently tested the efficacy of those combinations in neonatal Holstein bovine calves. Further, we determined pharmacokinetics of CpPyK and CpLDH inhibitors in calves. We then chemically synthesized a library of chemical analogs of CpPyK and CpLDH inhibitors and tested them for improved anticryptosporidial potency. In infected mice, combination CpPyK+CpLDH, including NSC303244+NSC158011 and NSC252172+NSC158011, showed efficacy against *C. parvum* and ameliorated intestinal lesions at doses 4-fold lower than doses of individual inhibitors. Notably, combination NSC303244+NSC158011 completely cleared the infection in mice. In neonatal calves, combination NSC638080+NSC158011 showed the most efficacy, with amelioration of clinical signs, intestinal pathology, and parasite oocysts loads. Pharmacokinetic analyses showed that NSC638080 is rapidly absorbed, attaining maximum systemic levels 2 h after administration, and is eliminated in 10 h, with a half-life of 1.58 h. NSC158011 attains maximum systemic levels 10 h after dosing, but is slowly eliminated with a systemic half-life of 31.5 h. We found that 4 and 2 chemically derived analogs of NSC638080 and NSC158011, respectively, had better in vitro anticryptosporidial efficacy than parent compounds. Collectively, we have unveiled compounds that block essential enzymes in the metabolic energy pathway of *C. parvum* and cure cryptosporidiosis in calves. Further, we have derived optimized superior chemical analogs of those compounds that will serve as lead compounds for developing effective anticryptosporidial drugs.

Financial Support: This study was funded, in part, by USDA-NIFA-AFRI grant number 2022-67015-36347 to W.H.W.

Key Words: *Cryptosporidium parvum*, enzyme inhibitor, novel drug, calf

P119 - Detection of β-tubulin resistance alleles in Alabama and Georgia isolates of *Ancylostoma caninum* using a novel qPCR assay.

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Ancylostoma caninum is a common intestinal nematode of dogs in the United States. It is also an important zoonotic parasite, capable of causing cutaneous larval migrans. Over the last 8 years, there has been an increase in reports of anthelmintic resistance, particularly to the benzimidazoles fenbendazole and febantel. Resistance has been associated with single-nucleotide polymorphisms (SNPs) in the parasite's β-tubulin gene, specifically at codons 167 (F167Y) and 134 (Q134H). Accurate detection of these SNPs is crucial for monitoring resistance patterns and guiding treatment decisions. Our objective in this study was 2-fold: (1) to develop and evaluate a novel quantitative PCR (qPCR)–based approach for detecting benzimidazole resistance–associated SNPs in *A. caninum,* and (2) to apply these methods to detect and quantify these mutations in isolates from naturally infected dogs in Alabama and Georgia. We hypothesized that these mutations are prevalent in hookworms in dogs in Alabama and Georgia. *Ancylostoma caninum* eggs were isolated from the feces of 94 naturally infected client-owned or shelter dog samples submitted to Auburn University's College of Veterinary Medicine parasitology diagnostic laboratory. The DNA was extracted using a commercial kit. We developed a novel SYBR green–based qPCR to specifically detect susceptible or resistant alleles at codon 134. We used a previously published primer set specific for alleles at codon 167. We compared the amplification efficacy of both qPCRs using 4 plasmids containing the *A. caninum* β tubulin isotype-1 gene alleles cloned into a TOPO-TA backbone. We generated qPCR Cq values for each allele, and relative allele frequencies were calculated for each codon. Resistance-associated SNPs were detected in both codons. We found significant variation in allele frequencies within and between samples at both codons. Mean resistant allele frequency was 25.1% at codon 167 and 3.7% at codon 134, which is less than the 40.2% and 16.6% reported in a previous study. Approximately 13.8% of samples had >75% allele F167Y, whereas no samples had Q134H at >75% allele frequency. Our findings demonstrate that resistance-associated β-tubulin alleles are present in *A. caninum* populations from Alabama and Georgia. These results will further inform monitoring and management strategies for anthelmintic resistance in canine populations.

Financial Support: American Kennel Club Canine Health Foundation

Key Words: canine, resistance, *Ancylostoma caninum*, quantitative PCR (qPCR)

P120 - Uncovering the role of a *Cryptosporidium parvum* thrombospondin protein in macrogamont development.

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The protozoan parasite *Cryptosporidium parvum* is a global cause of diarrheal illness, primarily affecting calves, immunocompromised people, and children. No drug or vaccine is available to treat or prevent cryptosporidiosis or reduce disease transmission. Ingested oocysts excyst sporozoites that invade the host's intestinal epithelial cells. Post-invasion, the parasite replicates asexually and then proceeds to a sexual cycle, forming male (microgamonts) and female (macrogamonts) gamonts. Male gametes released from the microgamont fertilize the macrogamonts, resulting in oocysts. The molecular mechanisms underlying macrogamont maturation and signaling interactions between gametes leading to fertilization are not understood. We identified a *C. parvum* thrombospondin repeat-containing protein (CpTSP2) with a Lin-12 repeat/Notch signaling domain predicted by the SMART protein domain prediction tool. Because Lin-12/Notch signaling proteins are known to mediate development and cell-cell interaction, we hypothesize that CpTSP2 may be involved in the maturation of the macrogamont and communication with the male gametes for fusion. To determine the localization of CpTSP2 during the parasite's developmental cycle, we generated a stable transgenic *C. parvum* line with a hemagglutinin tag appended to the C-terminus of the protein using CRISPR/Cas9 editing and an immunocompromised mouse infection model (n = 2 cages of 4). Using super-resolution and ultrastructure expansion microscopy, we found that CpTSP2 localizes to macrogamont, oocyst, and asexual stages. All immunofluorescence assays and expansion microscopy imaging were repeated twice, and images shown are representative images. Our imaging analysis reveals that macrogamonts of various sizes express CpTSP2 differently. Although some macrogamonts show an intracellular localization with the protein in the cytoplasm, others exhibit punctate expression of CpTSP2 near the membrane. These variations in CpTSP2 expression at different stages of macrogamont development suggest a role in gamont maturation and/or fertilization. Our plans include using a pharmacological agent to inhibit CpTSP2 function, delete the *tsp2* gene, and determine the effect on macrogamont maturation and fertilization. Understanding the mechanisms controlling macrogamont development and fertilization for oocyst production will pave the way for developing future strategies to reduce disease transmission.

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Key Words: *Cryptosporidium parvum*, microscopy, macrogamont development

P121 - Assessment of the seroprevalence of *Toxoplasma gondii* in healthy and neurologic horses from diverse geographic locations.

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Equine protozoal myeloencephalitis (EPM) is a common neurologic disease in horses in the Americas. The disease is caused primarily by the apicomplexan parasites *Sarcocystis neurona* and less commonly by *Neospora hughesi*. Recent reports have proposed that the related parasite *Toxoplasma gondii* could contribute to EPM development as either an etiologic agent or comorbidity. Although *T. gondii* exposure in horses has been reported worldwide, clinical toxoplasmosis is rare with only one case report so far. The goal of this study is to assess *T. gondii* seroprevalence in varying geographic locations and determine whether this parasite is associated with EPM. Western blot using whole *T. gondii* tachyzoite lysate was used to test 777 serum samples from healthy and neurologic equids. Samples from healthy horses originated from the United States (n = 297, all from Kentucky), Colombia (n = 71 horses and 29 mules), and Europe (n = 199). Samples from horses affected by various neurologic conditions originated from Kentucky (n = 24), Pennsylvania (n = 33), and Brazil (n = 124). The seroprevalence of *T. gondii* observed in healthy horses was 6.60% in Kentucky, 3% in Europe, and 54% in Colombia. In comparison, the seroprevalence observed in neurologic horses was 8.33% in Kentucky, 12.12.% in Pennsylvania, and 18.55% in Brazil. Our results show that the seroprevalence of *T. gondii* tends to vary greatly by geographic location. Additionally, the overall seroprevalence in horses affected by miscellaneous neurologic horses was slightly higher (16.02%) than in healthy horses (12.42%). However, additional testing is needed to confirm if *T.* gondii plays a role in the onset of neurologic conditions, especially EPM. To facilitate testing larger sample sets, an ELISA based on recombinant *T. gondii* surface antigen 1 (rTgSAG1) is being developed. Additional serum sample sets will be tested in the ELISA, such as normal horses from additional US states and Brazil, to better understand the discrepant seroprevalences. Additionally, cerebrospinal fluid of neurologic horses will be tested with the rTgSAG1 ELISA to determine if intrathecal antibody production occurs. This continued work will allow us to better address whether there is an association between *T. gondii* and EPM in horses. Future research should investigate direct detection methods of *T. gondii* in the central nervous system of neurologic horses to further identify if an association is present.

Financial Support: This work was supported by funds from the American Quarter Horse Foundation (AQHA).

Key Words: Apicomplexa, equine protozoal myeloencephalitis, serology, diagnosis

General Abstracts: Pathogenic E. coli—Posters

P122 - Evaluation of antimicrobial peptides as alternatives to antibiotics for controlling avian pathogenic *Escherichia coli* in poultry.

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Avian pathogenic *Escherichia coli* (APEC) is an extraintestinal pathogenic *E. coli* that causes colibacillosis in poultry, a multisystemic disease manifesting as omphalitis, swollen head syndrome, enteritis, polyserositis, cellulitis, and salpingitis. APEC poses a major challenge to the poultry industry, leading to major economic losses and contributing to spread of antibiotic resistance. Its zoonotic potential—highlighted by transmission to humans as a uropathogen via contaminated poultry products—underscores its significance as a public health concern. Current measures, including antibiotics and limited vaccines, remain inadequate, emphasizing the need for effective alternatives. We characterized 2 antimicrobial peptides (AMP), P1 (NPSRQERR) and P2 (PDENK), derived from *Lacticaseibacillus rhamnosus* GG (LGG), with potent bactericidal activity against multiple APEC serotypes, including antibiotic-resistant strains. In specific-pathogen-free chickens, oral administration of P1 and P2 in drinking water (50–200 mg/L) significantly reduced cecal APEC colonization even at the lowest dose (50 mg/L) (P1: 1.1 log cfu/g, $P < 0.05$; P2: 1.6 log cfu/g, $P < 0.001$), without affecting intestinal morphology or gut microbiota. We further optimized in-house synthesis of P2, which showed equivalent antimicrobial activity equivalent to the commercial preparation while preserving the microbiome. In addition, a combination therapy of the AMP with *LGG* improved pathogen clearance (up to 1.7 log reduction), supporting synergistic applications. Mechanistic studies revealed P1 and P2 disrupt outer membrane lipid asymmetry by targeting the MlaA–OmpC/F system, confirmed by bacterial cytological profiling, gene expression, immunoblotting, and in silico binding analyses. To probe structure–activity relationships, modified P2 variants were synthesized and tested against APEC O78. These studies showed that the full-length PDENK sequence is essential for potency, while acetylation preserved near-complete activity. Collectively, our findings demonstrate that P1 and P2 are safe, effective, and scalable therapeutic candidates with novel mechanisms of action against APEC. Future work will include testing efficacy infield-simulated condition, pharmacokinetics, tissue residue analyses, and mechanistic validation. This research advances antibiotic alternatives that may reduce reliance on conventional antimicrobials, enhance poultry health, and mitigate public health risks from AMR pathogens.

Financial Support: This project was supported by Agriculture and Food Research Initiative Competitive Grant no 2022-67015-44185 from the USDA National Institute of Food and Agriculture.

Key Words: avian pathogenic *Escherichia coli*, antimicrobial peptide, poultry therapeutics, antibiotic alternative, membrane lipid asymmetry, MlaA–OmpC/F system

P123 - Potential therapeutics: Small-molecule quorum-sensing inhibitors for extra-intestinal pathogenic *Escherichia coli*.

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Extraintestinal pathogenic *Escherichia coli* (ExPEC) has emerged as a public health concern, causing severe infections such as urinary tract infections, sepsis, and meningitis. The recent studies indicate that poultry are reservoirs for ExPEC strains pathogenic to humans. Avian pathogenic *E. coli* (APEC), the avian equivalent of ExPEC, causes multisystemic infections known as colibacillosis, leading to high morbidity, mortality, and economic losses in poultry production. Current control approaches rely on antibiotics and vaccines which are less effective due to increasing multidrug resistance and limited vaccine protection against diverse serotypes. Small molecules (SMs) functioning as quorum-sensing inhibitors (QSIs) offer an alternative strategy to disrupt bacterial regulatory systems controlling virulence, motility, and biofilm formation by blocking autoinducer (AI) signaling, thereby reducing bacterial pathogenicity. In this study, 6 derivatives (OA4–107, OA4–108, OA4–109, OA4–111, OA4–112, and OA4–115) were synthesized from the piperazine-based parent small molecule QSI-5 and evaluated against APEC O78 as a model organism. Two derivatives, OA4–108 and OA4–109, exhibited enhanced AI-2 inhibition in bioluminescence assays, showing significant activity at 50 µ*M* compared with 100 µ*M* for QSI-5. Further in vitro assays revealed that both derivatives significantly inhibited APEC O78 motility and biofilm formation, key virulence traits, while exhibiting minimal toxicity in HD 11 cells, THP-1 cells, Caco-2 cells, and sheep and chicken erythrocytes. Further, in intracellular survival assays using HD-11 and THP-1 macrophage cells, both QSIs eliminated intracellular APEC O78 at a concentration of 50 µ*M*. In vivo wax moth assays also confirmed the safety and efficacy of both QSIs. Both QSIs significantly reduced bacterial load and enhanced larval survival at 50 µ*M* compared with the control group. Metabolomic and lipidomic analyses revealed that treatment of APEC O78 cultures with OA4–108, OA4–109, and QSI-5 disrupted folate metabolism, stress-response pathways, and lipid homeostasis, resulting in broad alterations in cellular metabolism and lipid profiles. Furthermore, preliminary AI-2 bioluminescence assays showed a reduction in AI-2 production in uropathogenic *E. coli*. These results suggest a potential for these improved QSIs as new antibacterials against human and animal ExPEC infections.

Key Words: avian pathogenic *E. coli*, poultry, colibacillosis, quorum-sensing inhibitor, piperazine, uropathogenic *E. coli*

P124 - Genomic markers differentiating Shiga toxin-producing *Escherichia coli* using pangenome and phylogenetic analyses.

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Shiga toxin-producing *Escherichia coli* (STEC) is a foodborne bacterium that poses a significant public health concern due to its genetic diversity. It causes a range of illnesses, from mild to severe, with around 265,000 cases reported each year in the United States. This study aimed to identify conserved genomic markers for each serogroup of STEC that could enhance the detection of these pathogens in clinical and food samples. A total of 160 STEC genome assemblies representing clinical and environmental isolates were obtained from the NCBI Pathogen Detection Database and then verified for quality using CheckM. These assemblies underwent pangenome analysis, which compares genes shared by all strains and those unique to particular strains using Panaroo and the Roary ILP Bacterial Annotation Pipeline (RIBAP), 2 computational tools that cluster genes and increase genome annotation accuracy. Potential candidates were identified using the RIBAP's presence/absence profile, cross-checked with a Panaroo matrix, and then verified using the BLASTn and Geneious Prime tools. For these targets, gene ontology annotation was done. Our analysis identified several high-confidence candidates, including metabolic genes (*dgcE*, *fcl*_2, *dmsA*, *hisC*) and surface polysaccharide-associated genes (*capD*, *rfbX*, *wzzB*). The comparative pangenome analysis revealed that RIBAP's pangenome was the largest, with 22,238 gene clusters, exceeding Panaroo's pangenome (11,515), and the core gene content displayed a genomic diversity across STEC serotypes. Furthermore, some genomes belonging to the O104:H1, O145:H28, and O45:H2 serotypes did not match their anticipated canonical serotype clade in the core-phylogenetic tree. Moreover, functional annotation of the targets revealed that over 22.9% of them were classified as hypothetical, indicating uncharacterized genes that may contribute to STEC pathogenicity. Thus, our findings demonstrate that the genetic variability among STEC serotypes can be leveraged to identify markers. These markers have the potential to serve as candidates for future study into novel virulence mechanisms or vaccine targets to combat STEC infections, particularly as the pathogen evolves. Furthermore, core-genome phylogenies indicated discordant placements explained by differences in H-serogroup, despite possessing an identical O-serogroup, emphasizing the importance of full O:H serotype reporting. Ultimately, this will improve STEC detection and diagnostic approaches.

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Key Words: Shiga toxin-producing *Escherichia coli* (STEC), pangenome analysis, serogroup-specific markers, phylogenomic analysis

P125 - Genomic characterization of *Escherichia coli* from ruminants: An examination of diversity, virulence, and antimicrobial resistance patterns.

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Escherichia coli (*E. coli*) is a complex and ubiquitous commensal organism in both food-producing animals and humans, comprising diverse pathogenic strains. In ruminants, pathogenic *E. coli* can asymptomatically colonize the intestinal tract, leading to fecal shedding and contamination of the food chain, making them important reservoirs of infection. Notably, studies have attributed approximately 75% of human *E. coli* O157:H7 outbreaks to food products of bovine origin. In this study, we characterized *E. coli* isolates from 116 fecal samples collected from cattle (n = 48) and goats (n = 68) in Tuskegee, Macon County, AL. Presumptive identification of 80 isolates was performed using coliform chromoselect agar and standard biochemical tests, followed by genomic confirmation via whole-genome sequencing on the Illumina MiSeq platform. Of these, 55 isolates (26 cattle, 29 goats) were confirmed as *E. coli*. Genome assemblies analyzed on the GalaxyTrakr platform using the Shovill, *E. coli* serotyper, ABRicate, and staramr pipelines revealed diverse serotypes, including Shiga toxin-producing *E. coli* (O93:H28 [20%], O126:H19 [2%], O76:H19 [7%]), extraintestinal pathogenic *E. coli* O180:H14 (13%), enteroinvasive *E. coli* O8:H19 (5%), and enterohemorrhagic *E. coli* O80:H19 (4%). Virulence genes identified included toxins (*stx1B*, *stxA*, *hlyE*), adhesins (*fimH*, *yagZ*/*ecpA*, *afaC*-VIII), biofilm-associated genes (*csgG*, *csgF*, *csgE*), serum resistance determinants (*ompT*, *iss*, *traT*), and iron acquisition systems (*chuX*, *iroN*, *iutA*). Resistome analysis showed a predominance of the multidrug resistance gene mdf(A)_1 (82%), alongside resistance determinants for β-lactams (blaACT-6), aminoglycosides [ant(3")-Ia_1], quinolones (oqxA, oqxB), tetracyclines [tet(B)_2, tet(C)_2, tet(C)_3], and sulfonamides (dfrA1, sul2_2). Six plasmid types (Inc.FIA, Inc.FIB, Inc.I1-I, Inc.FIC, Col156, Inc.B/O/K/Z) associated with both virulence and antimicrobial resistance were also detected. The occurrence of highly pathogenic *E. coli* serotypes with multidrug resistance potential highlights a significant public health risk posed by fecal contamination of food and the environment. At the same time, plasmid carriage underscores the potential for horizontal dissemination of virulence and AMR determinants. These findings underscore the importance of ongoing genomic surveillance and rigorous farm-to-fork control measures to mitigate transmission risks.

Financial Support: NI22AHDRXXXXG040

Key Words: ruminants, whole-genome sequencing (WGS), *E. coli* serotypes, virulence, AMR, plasmid

P126 - Comparative genomic analysis of *Escherichia coli* from liver abscesses and ruminal epithelial tissues of feedlot cattle.

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Liver abscesses (LA) are polymicrobial infections in feedlot cattle, primarily caused by *Fusobacterium necrophorum* (subspecies *necrophorum* and *funduliforme*) and *Trueperella pyogenes*. However, other bacterial species are sporadically isolated with *Escherichia coli* being the more frequent. Ribosomal RNA gene amplicon sequence-based analyses of LA purulent material have shown *Fusobacteria* dominance, followed by *Proteobacteria* and *Bacteroidetes*. Our recent study showed that *E. coli* in 68 of 96 (70.1%) LA samples from feedlot cattle that originated from feedlots that did not include tylosin in the diet, most after enrichment, suggesting a potential opportunistic role. In addition, *E. coli* was isolated without enrichment from 92.7% of ruminal epithelial tissues, indicating higher colonization in gut epithelial tissues. Whole-genome sequencing of 95 *E. coli* (liver = 65; rumen = 30) revealed a diverse array of virulence and antimicrobial resistance-associated genes. Core virulence determinants, involved in adhesion, motility, iron acquisition, and secretion systems, including *che*, *csg*, *ent*, *fep*, *fim*, and *flg/flh/fli* operons, were conserved across nearly all isolates. Type III and VI secretion system effectors were widely distributed, whereas colibactin (*clbA*-*Q*) and hemolysin (*hlyA*-*D*) genes were detected in a smaller subset. Ninety-five antimicrobial resistance (AMR) genes were identified, with β-lactamase-associated genes (*blaAmpC2*, *blaPBP*, *blaampH*) and multidrug efflux determinants (*acrA*, *acrF*, *mdtA*, *mdtP*, *emrA*, *emrY*) representing RND, MFS, and MATE-type efflux systems being most prevalent. Regulatory genes (*baeSR*, *evgAS*, *marA*, *soxS*) were also identified, reflecting complex transcriptional control of efflux-mediated resistance. Functional classification grouped these AMR genes into β-lactams, aminoglycosides, macrolides, tetracyclines, sulfonamides, peptide antibiotics, fluoroquinolones, and multidrug efflux systems. Among these, β-lactam and multidrug efflux genes dominated, with fluoroquinolone resistance genes (*qnrB5*, *qnrB19*) detected only in liver isolates, suggesting stronger selective pressures or antimicrobial exposure with enhanced pathogenicity in hepatic environment.

Key Words: *Escherichia coli,* liver abscess, feedlot cattle, comparative genomics, virulence genes, antimicrobial resistant genes

P127 - Longitudinal metagenomic insights into diversity and temporal prevalence of *E. coli* virulence genes in the gut of pastured goats.

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Escherichia coli a common inhabitant of gastrointestinal tract of animals can be a commensal, pathogenic but also opportunistic pathogen in altered gut conditions. Some strains of *E. coli* cause diarrhea in young ruminants during the first weeks of life and during stressful immunocompromising periods. Other strains found possess virulence factors that help them colonize human tissues causing diarrhea and other severe systemic infections. Understanding the temporal age-based variation in *E. coli* virulence factors in ruminants will help us understand the risk including identifying critical periods of vulnerability and shedding, which is important in designing prevention and control strategies. In this study, we used shotgun metagenomic sequencing to understand the diversity and age-related differences in the abundance of *E. coli* virulence factors in 105 fecal samples from pastured healthy goats from 2 d of birth to 10 mo of age. In total 63 unique virulence factors associated *E. coli* genes ranging from colonization, fitness, toxins, effectors and antimicrobial resistance were detected. Significant differences in diversity and prevalence of individual virulence genes were detected in the different age groups. A significantly higher number of virulence factors genes were detected during the preweaning period with very few or none beyond 1 wk after weaning. Several virulence factors were also enriched during the preweaning period but the specific virulence genes differed in different age groups. The top 10 virulence genes detected in the goats included *E. coli* gene *lpfA*, *E. coli* gene *ompA*, *E. coli* gene *traA*, *E. coli* gene *ehxA*, *E. coli* gene *lacY*, *E. coli* gene *efa1*, *E. coli* gene *gad*, *E. coli* gene *tsh*, *E. coli* gene *espI*, and *E. coli* gene *f17G*. Uniquely, primary *E. coli* genes associated with human virulence; *E. coli* gene *eae*, *E. coli* gene *ehxA*, and Stx2-converting phages were all enriched at 7 wk of age. This study highlights the repertoire and age-based prevalence of important *E. coli* virulence factors in the gut of apparently healthy pastured goats. The information may be used in animal and public health to inform and design disease prevention and control strategies.

Financial Support: USDA Award #-2019-38821-29149

Key Words: virulence, genes, goats, metagenomic, gut, *E. coli*

P128 - Intramuscularly administered combined acellular vaccine candidate SecVax prevented *Vibrio choleraeO1 El Tor* and ETEC H10407 intestinal colonization in an adult rabbit colonization model.

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Diarrheal diseases caused by *Shigella*, *Vibrio cholerae*, and enterotoxigenic *Escherichia coli* (ETEC) remain major public health burdens, causing over 500 million diarrhea episodes and approximately 400,000 deaths annually. Despite the significant global disease burden, there are currently no licensed vaccines for *Shigella* or ETEC. Although oral cholera vaccines (OCVs) are available, their protective efficacy remains limited among adults and little or none in very young children in endemic countries. Virulence heterogeneity and stimulation of strong immunity particularly in young children are the key obstacles to developing vaccines against *Shigella*, *Vibrio cholerae, and* ETEC. By applying a multiepitope fusion antigen (MEFA) vaccinology platform, we recently developed SecVax, a protein-based combined vaccine, which has demonstrated broad immunogenicity and in vitro cross-protective efficacy against *Shigella*, *V. cholerae*, and ETEC. To further evaluate SecVax preclinical efficacy against *V. cholerae* and ETEC colonization at small intestines, in this study, we intramuscularly immunized adult rabbits with SecVax, challenged rabbits with ETEC strain H10407 (CFA/I, LT, STa) and *V. cholerae* O1 *El Tor* (N16961), and examined prevention of bacteria intestinal colonization. Data showed that rabbits immunized with SecVax developed antibodies to both *V. cholerae* and ETEC toxins and target adhesins, and also significantly reduced ETEC H10407 and *V. cholera* O1 EI Tor strain bacterial colonization in rabbit small intestines. These results indicated that SecVax is cross-protects against adult rabbit challenges with ETEC H10407 and *V. cholera* O1 EI Tor, suggesting that SecVax can potentially be an effective broadly protective vaccine against *V. cholerae* and ETEC.

Key Words: *Vibrio cholerae*, enterotoxigenic *Escherichia coli* (ETEC), combined vaccine, intestinal colonization, rabbit model, diarrhea

P129 - Developing a vaccine against spotty liver disease caused by *Campylobacter hepaticus* in commercial layer chickens.

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Spotty liver disease (SLD), caused by *Campylobacter hepaticus*, is a recently emerged disease that causes substantial production losses in the commercial egg industry. Its impact has intensified with the growing shift toward antibiotic-free and cage-free production systems. This study aimed to develop an effective vaccine to prevent SLD. To achieve this, we utilized an attenuated avian pathogenic *E. coli* (APEC) strain as a live vaccine vector to deliver *C. hepaticus* mimotopes identified through phage display and reverse vaccine technology, with the overarching goal of providing protection against both SLD and *E. coli* egg yolk peritonitis, another major disease contributing to significant economic losses in laying hens. We constructed Δ*aroA* Δ*asd* APEC PSU078 strains engineered to express one of 14 *C. hepaticus* mimotopes, each cloned into the pYA3342 expression vector. Surface expression of the mimotopes on the recombinant APEC strains was confirmed by western blot analysis. A chicken vaccination–challenge trial was conducted using 9 groups of chickens. One group served as an unvaccinated challenged control, and another as an unvaccinated unchallenged negative control. Each experimental group received a mixture of 2 recombinant *E. coli* strains carrying different mimotopes at a dose of 1×10^9 cfu in 1 mL of PBS, administered orally twice at 16 and 18 wk of age. At 26 wk, chickens in all groups, except for the chickens in the unchallenged control group, were challenged with *C. hepaticus* (1×10^{10} cfu per chicken) by oral gavage. Two weeks after the challenge, all chickens were euthanized for necropsy to assess gross hepatic lesions associated with SLD. Bacterial isolation was performed on bile and liver samples to evaluate colonization. Isolated bacteria were confirmed as *C. hepaticus* using a validated PCR. The immunogenicity and efficacy studies demonstrated that 4 mimotopes, which mimic structural regions of the *C. hepaticus* flagellar proteins, induced robust immune responses and conferred significant protection against *C. hepaticus* colonization in both bile and liver. These findings support the feasibility of a mimotope-based subunit vaccine for SLD. Future work will focus on developing a multi-epitope vaccine incorporating multiple flagellar protein–derived mimotopes of *C. hepaticus* and evaluating this recombinant APEC vector for its immunogenicity and protective efficacy against both *C. hepaticus* and APEC.

Financial Support: This project was supported by Agriculture and Food Research Initiative Competitive Grant No. 2023-67015-40780 from the USDA National Institute of Food and Agriculture.

Key Words: *Campylobacter hepaticus*, colibacillosis, chickens, *Escherichia coli*, mimotopes, vaccine

P130 - Model systems to study Shiga toxin-producing *Escherichia coli* interactions with the bovine gastrointestinal tract.

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Cattle are the primary reservoirs for Shiga toxin-producing *Escherichia coli* (STEC) and a principal source of human infection. Developing effective preharvest control methods that limit STEC in cattle requires better understanding of STEC interactions with the animal. Toward this end, we developed 2 model systems that permit such evaluations while limiting animal usage. (1) In vivo model: The bovine rumen is the initial transit site for STEC before progressing through rest of the ruminant gastrointestinal tract (GIT). We developed an economical, novel, nonterminal and reusable rumen-fistulated animal model that permits in vivo evaluation of multiple STEC strains in the bovine rumen. Using this model system, we successfully evaluated STEC survival and determined proteins expressed in vivo in the rumen. Animals were fed contrasting maintenance (high fiber) and lactation (high energy-protein) diets to study dietary effects. STEC were contained within dialysis cartridges to prevent animal GIT contamination. To demonstrate the uniqueness of in vivo evaluations, parallel studies were conducted using extracted rumen fluid in vitro in flasks maintained under anaerobic conditions. Strain/serotype-dependent differences in O157 and non-O157 STEC recovery from the rumen fluid of cattle fed either diet was observed. Analysis of the STEC genes expressed in the rumen demonstrated a preference to survival over virulence. Differences in the adaptive responses to rumen fluid was influenced by the animal's diet and growth conditions. These new insights into the STEC responses could help formulate modalities to control STEC colonization of cattle. (2) In vitro model: The bovine GIT site at which O157 persists is the rectoanal (RAJ) junction. An in-depth understanding of O157-RAJ tissue interactions could help develop novel modalities to limit O157 in cattle. Here, we report the development of a complete RAJ-in vitro organ culture (RAJ-IVOC) system that represents both cell types of the RAJ and permits adherence studies with results as observed in vivo. Pieces of RAJ tissue, collected at cattle necropsies from unrelated studies, were assembled and tested variously to derive optimal conditions for assaying bacterial adherence on a viable IVOC. O157 strain EDL933 and *E. coli* K12 with known adherence differences were used to standardize the RAJ-IVOC adherence assay. Cell viability, structural cell markers and histopathology were used to determine tissue integrity, and adherence assessed via microscopy and culture. DNA fingerprinting verified recovered bacteria against the inoculum. Appropriately assembled RAJ-IVOC, in Dulbecco's Modified Eagle Medium, at 39°C, with 5% CO_2 and gentle shaking for 3 to 4 h, maintained tissue integrity and reproduced the expected adherence phenotype of the test bacteria. The RAJ-IVOC model system provides a convenient method to prescreen multiple bacteria-RAJ interactions before in vivo experiments, thereby reducing animal usage.

P131 - The carbon starvation-inducible lipoprotein (Slp) influences differential adherence of *Escherichia coli* O157:H7 at the bovine rectoanal junction.

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Shiga toxin-producing *Escherichia coli* O157:H7 (O157), a foodborne human pathogen, persists at the rectoanal junction (RAJ) of the bovine intestinal tract in asymptomatic cattle reservoirs. Identifying mechanisms used by O157 for initial adherence before persistence at the RAJ could help develop effective O157 control modalities. We recently established the role of carbon starvation-inducible lipoprotein (Slp) in initial adherence of O157 to Caco-2 cells, with the human polymeric immunoglobulin receptor (pIgR) protein as the Slp-receptor. Here, we evaluated the role of Slp in O157 adherence to the bovine RAJ using the RAJ squamous epithelial (RSE) cell- and RAJ-in vitro organ culture (IVOC)-adherence assays. The wild-type O157 strain EDL932 (EDL932-WT), it's isogenic *slp* deletion mutant (EDL932 Δ*slp*), and the *slp* complemented mutant (EDL932 Δ*slp*-p:*slp*) were tested with no bacteria controls. Adherence was verified by culturing and immunofluorescence (IF) staining of O157. Tissue integrity was determined using nuclear/cell staining dyes and histopathological examination. All test strains adhered in a diffuse-moderate pattern on RSE cells. However, differential adherence was observed on the RAJ-IVOC with the strains preferentially adhering to the columnar cells. Additionally, EDL932-WT and EDL932 Δ*slp*-p:*slp* strains adhered in slightly greater numbers than the EDL932 Δ*slp* strain to the RAJ-IVOC, causing disruptions primarily in the columnar region of otherwise intact RAJ-IVOC tissues. Furthermore, pIgR was predominantly detected by IF microscopy and RNAscope in situ hybridization at the columnar region of the RAJ-IVOC tissue. *In silico* modeling demonstrated the possibility of a bovine pIgR-bacterial Slp interaction. Hence, our observations support the role for Slp in the initial adherence of O157 to the columnar cells at the bovine RAJ, unlike the squamous cells where the loss of *slp* did not affect attachment. In addition, a possible mucosal immune-interference resulting from the bovine pIgR-Slp interaction could contribute toward long-term O157 colonization of cattle.

General Abstracts: PRRSV—Posters

P133 - Sex-dependent hypothalamic responses to gestational porcine reproductive and respiratory syndrome virus (PRRSV).

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Porcine reproductive and respiratory syndrome (PRRSV) infection during gestation induces maternal immune activation that can cross the placenta and influence fetal brain development. The hypothalamus plays a central role in regulating behavior, growth, reproduction, and stress, making it a key target of prenatal viral effects. The objective of this study is to assess how PRRSV infection during gestation alters hypothalamic gene expression in female and male piglets. Hypothalamic RNA-seq was performed on 3-wk-old female and male offspring (n = 64) from PRRSV-infected and control sows. Differential expression analysis was conducted using edgeR across PRRSV × sex contrasts to identify affected pathways and regulatory genes. Across PRRSV-by-sex contrasts, more than 560 genes were differentially expressed in control versus virally inoculated females and over 360 in control versus virally inoculated males. Disrupted pathways included steroid biosynthesis, fatty acid elongation, and immune signaling modules such as cytokine-chemokine interactions. Representative genes in these categories included CCL2, CCL5, CXCL10, CD3G, ISG15, and IDO1 (immune signaling) and CYP7B1, CYP3A2, and ELOVL7 (lipid and steroid metabolism). Transcription factors STAT1, STAT2, STAT3, and LHX8 were enriched, connecting immune and metabolic regulation to neuroendocrine processes that influence feeding and reproductive behavior. Gene network reconstruction highlighted opposite transcriptional shifts between females and males, with females showing relative downregulation and males relative upregulation in lipid metabolic pathways in pigs exposed to PRRSV. Gestational PRRSV infection enriches hypothalamic immune-metabolic networks in a sex-dependent manner. These molecular differences may contribute to disrupted immune responses, feeding behavior, and reproductive function in offspring. Our results emphasize the role of prenatal viral exposure in shaping long-term health and productivity in 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.

Key Words: immune-metabolic signaling, pigs, behavior, hypothalamus, gene expression

P134 - Determinants of porcine reproductive and respiratory syndrome virus tropism.

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Porcine reproductive and respiratory syndrome virus (PRRSV) is an important swine pathogen. The virus primarily infects swine macrophages, with CD163 as the main cellular receptor. Pigs are the only known natural host. Interestingly, nonsusceptible cell lines transfected with CD163 from various species, including humans, monkeys, mice, and dogs, become susceptible to PRRSV infection. This study aimed to assess the influence of CD163 sequence variation, expression levels, and the cellular background in which CD163 is expressed on susceptibility to PRRSV infection. To evaluate the effect of CD163 sequence variation, HEK-293T cells, a nonsusceptible cell line, were transduced with lentiviruses expressing CD163 from pigs, monkeys, mice, and humans, followed by inoculation with a field PRRSV isolate. Infection rate and virus yield were quantified at various time points after inoculation. To study the effect of CD163 expression levels, CD163-knockout MA-104 cells were transduced with lentivirus expressing monkey CD163. Susceptibility of wild-type MA-104 cells (expressing low levels of CD163) was compared with that of transduced cells. To assess the influence of cellular background, porcine CD163 was expressed in various cell lines, including PK-15, BHK-21, and Vero, and their susceptibility to PRRSV infection was evaluated. Transduction of HEK-293T cells with CD163 from pig, monkey, human, and mouse rendered the cells susceptible to PRRSV infection. However, susceptibility levels varied significantly, with cells expressing CD163 from pig and monkey exhibiting significantly higher infection rates than those expressing CD163 from human and mouse. MA-104 cells overexpressing monkey CD163 showed significantly higher infection rates than wild-type MA-104 cells, which expressed minimal levels of CD163. Finally, expression of porcine CD163 in different cell types led to variable infection rates and viral yields, indicating that cellular background also plays a critical role in susceptibility to PRRSV infection. PRRSV tropism is influenced not only by CD163 sequence and expression levels but also by the cellular background in which CD163 is expressed. These findings suggest that cellular factors beyond the CD163 receptor contribute to the susceptibility of cells to PRRSV infection.

Financial Support: This study was funded by the Agriculture and Food Research Initiative competitive grants 2023-67015-39657 and the Animal Heath Project 1020749 of the USDA National Institute for Food and Agriculture.

Key Words: PRRSV, tropism, CD163, swine viruses

P135 - Replication of rescued porcine circovirus type 3 (PCV3) in pigs.

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Porcine circovirus type 3 (PCV3), a recently discovered circovirus, is epidemiologically associated with reproductive failure, and respiratory distress in pigs. In infected pigs, PCV3 results in generalized inflammatory lesions which include vasculitis and myocarditis. Currently, little is known regarding the molecular pathogenesis of the PCV3 as laboratory culture of the virus is a challenge. Hence, the primary goal of this study is to develop a PCV3 infectious clone, and study the replication patterns of the rescued, recombinant PCV3 in pigs. To develop the PCV3 infectious clone, the entire genome of PCV3 strain USMN 2016 cloned into a shuttle vector. Recombinant PCV3 was rescued by transfection of PK-15 cells with the circularized PCV3 genome. The identity of the rescued virus was verified by sequencing and PCV3-specific monoclonal and polyclonal antibodies. Groups of conventional weaned 3-wk-old pigs (n = 5) were infected with the rescued PCV3 virus. Serum samples were collected every 2 weeks for the assessment of antibodies by ELISA and viral replication by qPCR. The presence of PCV3 antigen in lymphocytes was evaluated by flow cytometry. All pigs were euthanized 6 weeks after infection and gross and microscopic lesions due to PCV3 infection were evaluated. Sero-conversion and virus neutralizing antibody responses were observed in PCV3 infected pigs and correlated with the detection of viral DNA in serum. PCV3 viral antigen was detected in PBMCs by flow cytometry. PCV3 virus was re-isolated from the serum of infected pigs and was viable on serial passages. Significant gross pathological lesions were not detected. However, microscopic lesions included mild lymphoid hyperplasia. Thus, the recombinant PCV3 virus resembled natural isolates in its patterns of infectivity in pigs. The lack of overt clinical signs or pathology is consistent with experimental models of circoviral infections in conventional pigs. The availability of a functional PCV3 infectious clone enables further in-depth and well-controlled studies into PCV3 pathogenesis.

Financial Support: This study was funded by a USDA NIFA competitive grant award 2021-67015-34419

Key Words: swine, pigs, circoviruses, PCV3, infectious clone, reverse genetics

P136 - A conserved tyrosine-based endocytic signal in PRRSV glycoprotein 3 directs its intracellular trafficking and promotes viral replication.

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Porcine reproductive and respiratory syndrome virus (PRRSV) glycoprotein 3 (GP3) associates with GP2 and GP4 to form a trimeric complex essential for viral entry and assembly. Within GP3, we identified 2 conserved sequences, YAWL at positions 108 to 111 and YVDI at 136 to 139. These motifs resemble the tyrosine-based sorting signal YxxΦ, which adaptor protein complexes bind to direct cargo trafficking through secretory and endocytic pathways. This study aimed to determine whether these motifs regulate intracellular trafficking of GP3 and affect virion assembly. Site-specific mutations were introduced to the full-length PRRSV genome using our newly developed LOIPA (linear overlapping infectious polymerase amplicon) reverse genetics system, which reconstructs the entire PRRSV genome directly from overlapping PCR fragments and a synthetic regulatory linker. The PCR amplicons were co-transfected into BHK21 cells, and rescued mutants were propagated in porcine alveolar macrophages for characterization. Among 7 mutants, Y108A produced the most striking phenotype, displaying a complete loss of GP3 localization in the ER Golgi intermediate compartment (ERGIC), the cis and medial Golgi, and the trans-Golgi network. This indicates that tyrosine 108 is crucial for GP3 entry into the early secretory pathway and for subsequent delivery to the virion assembly site. In contrast, other substitutions within the YAWL and YVDI motif did not alter compartmental targeting, demonstrating that the YVDI motif does not act as a functional sorting signal and may instead play a secondary regulatory role. Quantitative RT-PCR confirmed that Y108A mutants retained infectivity but showed a significant reduction in genome copies compared with the wild-type virus with *P*-values below 0.05. Thus, Y108 is nonessential for infectivity but promotes efficient replication by enabling secretory entry. Although A109C and D138C also produced lower genome levels, their trafficking patterns remained unchanged, suggesting that their replication deficits arise through mechanisms distinct from the Y108A-mediated sorting defect. Collectively, these findings demonstrate that YAWL is the functional endocytic signal in GP3 and plays a key role in controlling intracellular trafficking and glycoprotein sorting during infection. Our study also highlights the utility of LOIPA system as a rapid and versatile platform for generating targeted PRRSV mutants without the need for bacterial cloning.

Financial Support: USDA-NIFA No. 2023-67015-39710

Key Words: tyrosine-based sorting signal, PRRSV, glycoprotein 3

General Abstracts: Salmonella—Posters

P137 - Association of major liver abscess pathogens' prevalence and load with liver abscess severity in feedlot cattle.

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Liver abscesses (LA) in feedlot cattle cause major economic losses, and the extent of the impact is dependent on the severity of abscesses. The primary pathogen is *Fusobacterium necrophorum* ssp. *necrophorum* (FNN), with *F. necrophorum* ssp. *funduliforme* (FNF), *Trueperella pyogenes* (TP), and *Salmonella enterica* (SE) implicated as secondary pathogens. This study aimed to compare pathogen prevalence and concentrations in purulent materials of abscesses sampled from livers with mild abscesses (1–3 small to medium abscesses, n = 134) and from livers with severe abscesses (multiple small to medium or one or more large abscesses with or without adhesions, n = 140), and in matched ruminal (n = 182) and colonic (n = 121) epithelial tissues collected at slaughter. Samples were homogenized and pathogens were detected by culture and quantitative real-time quantitative PCR (qPCR) and quantified by qPCR. Prevalence data were compared by Fisher's exact test, and bacterial concentrations were analyzed using *t*-tests on log-transformed values. *Fusobacterium necrophorum,* either of the 2 subspecies, was prevalent in >90% of abscesses, with prevalence in severe abscesses greater than in mild abscesses ($P = 0.04$). The prevalence of TP was greater ($P < 0.01$) in severe than in mild abscesses by culture (42% vs. 26%) and trended to be greater ($P = 0.06$) by qPCR. The concentrations (log cfu/g of tissue) of FNN, FNF, or TP were not different ($P > 0.14$) between mild and severe abscesses. The SE prevalence was not different between the 2 groups, and none of the samples had quantifiable concentrations. Co-occurrences of FN and TP (36.7% vs. 19.3%; $P < 0.01$) and FNN, TP, and SE ($P = 0.04$) were greater in severe than in mild abscesses. No difference in prevalence or load was evident in ruminal epithelial tissues from cattle with severe or mild abscesses. In colonic epithelial tissues, the FN and SE co-occurrence was significantly greater in cattle with severe abscesses than in cattle with mild abscesses. *Fusobacterium necrophorum* predominated in both mild and severe abscesses, but higher FN-TP co-occurrence in severe abscesses than in mild abscesses suggest potential synergistic contributions to disease severity. Increased FN-SE co-detection in colonic tissues provides evidence that the hindgut could also be a pathogen source. These findings emphasize that severe abscesses are caused by mixed infections, therefore, interventions to reduce severe abscesses should target both FN and TP.

Financial Support: Foundation for Food and Agriculture Research

Key Words: *Fusobacterium necrophorum*, *Trueperella pyogenes*, *Salmonella enterica*, pathogen prevalence, liver abscess severity, feedlot cattle

P138 - Microbiota modulation in chickens influences *Salmonella* colonization and host cecal gene expression.

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Early-life exposure of chicks to microbiota from adult birds can reduce *Salmonella* colonization, likely through competitive exclusion. This study evaluated the anti-*Salmonella* effects of 2 microbial interventions (a mature-bird cecal content group [CCG] and a defined 15-species bacterial consortium [DCG]) versus a sterile PBS control (CON). We also characterized how these treatments altered host cecal transcriptomes before and after challenge with *Salmonella enterica* serovar Heidelberg (SH2813). One-day-old White Leghorn chicks were randomly assigned to 3 groups and gavaged with DCG, CCG, or CON. After a week, a subset of birds from each group was euthanized for prechallenge cecal content and tissue collection. Remaining birds were challenged with 1×10^8 cfu SH2813 and sampled at 3, 14, and 21 dpi. At all time points, cecal contents were used for SH2813 enumeration and cecal tissue RNA was isolated for transcriptomic analysis. *Salmonella* colony-forming units were analyzed using Kruskal–Wallis tests and gene expression was analyzed with DESeq2. A 2-log10 reduction in SH2813 occurred in DCG relative to CON at 28 dpi (adj. $P < 0.05$). SH2813 counts in CCG birds remained below 10 cfu/g across all time points. Before *Salmonella* challenge, 37 and 676 genes were differentially expressed in CCG and DCG birds, respectively, versus CON. At 3 dpi, DCG and CCG showed differential expression of 181 and 142 genes. At later time points, CCG birds showed more differentially expressed genes (588 at 14 dpi; 838 at 28 dpi), whereas DCG birds showed fewer (290 at 14 dpi; 59 at 28 dpi). GO analyses indicated enrichment of immune-related pathways in CCG at 14 and 28 dpi, while transporter-related pathways were primarily enriched in DCG before challenge. Across treatments, differentially expressed genes included intestinal transporters, barrier-function, and immune-regulatory genes involved in host–microbiota interactions. This study demonstrates the capacity of commensal bacteria to reduce the duration and density of *Salmonella* colonization. The reduction in CCG birds and specific transcriptional responses produced by each microbial treatment highlight the role of microbial community members in shaping intestinal physiology and immunity. These findings support targeted microbiota-based strategies to improve poultry health, disease resistance, and production efficiency, and underscore the need for further research into microbe–host interactions during early-life colonization.

P139 - Detection of *Salmonella enterica* across the beef production chain with digital qPCR.

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Salmonella enterica ssp. enterica is one of the leading causes of foodborne illness in the United States. Beef products account for a relatively small proportion of cases, but there is increased interest among US federal agencies in reducing salmonellosis from all sources, including beef. Current methods for detecting *Salmonella* pre-harvest, such as culture and qPCR rely on enrichment methods to increase *Salmonella* growth, which can bias results. Microfluidic digital qPCR, an alternative approach, has been shown to be a more sensitive platform for pathogen detection than established methods, but it has yet to be applied to the detection of *Salmonella* in cattle. Therefore, the objective of this study was to develop digital qPCR protocol for the detection of *Salmonella* from cattle feces without the need for pre-enrichment. Fecal samples were collected from 80 bull calves enrolled in a separate study evaluating the impact of management and marketing decisions on long-term health outcomes. Twenty animals were randomly selected and all fecal samples from each of the following time points were processed: T1 (modified live viral (MLV) vaccination; age: ~107 d old), T2 (7 d after vaccination; age: ~114 d old), T3 (revaccination; age: ~183 d old), T4 (abrupt weaning; age: ~230 d old), T5 (arrival at backgrounding yard; ~213 d old), and T6 (end of backgrounding; ~258 d old). Fecal samples were transported to the VERO research laboratory in Canyon, TX and mixed with a 50:50 solution of glycerol and PBS for storage at −80 C. Fecal samples were then thawed, homogenized, and aliquots of 250 uL with and without pre-enrichment. Samples were pre-enriched in 2.5 mL of tryptic soy broth incubation for 3 h at 42 C. DNA was extracted with the DNeasy PowerSoil Pro kit on a Qiacube Connect DNA/RNA device. An additional aliquot was taken for *Salmonella* isolation. Digital qPCR was performed on a Quantstudio Absolute Q digital qPCR platform using primers for the *invA* and *pagC* genes. The *Salmonella* prevalence based on culture was 28%, whereas digital qPCR detected Salmonella in all animals. We observed no significant difference in *Salmonella* detection between pre-enriched and non-pre-enriched fecal samples, though *Salmonella* DNA concentration was higher in enriched samples. Therefore, we conclude that digital qPCR is a highly sensitive, effective, and rapid tool for detecting *Salmonella* in the feces of preharvest cattle.

Financial Support: This research was funded by the Texas Cattle Feeders Association.

Key Words: *Salmonella,* beef cattle, detection, PCR, quantification

P140 - Genomic analysis of *Salmonella* Dublin from bovine clinical cases: Insights from the NAHLN AMR program (2019–2024).

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Salmonella enterica serovar Dublin (*S.* Dublin) is a host-adapted pathogen primarily associated with cattle, causing severe disease, particularly in calves. Beyond its impact on animal health, *S.* Dublin has been linked to serious human infections and foodborne outbreaks. This serovar is frequently resistant to multiple antibiotics, complicating treatment in both veterinary and clinical settings. In this study, *S.* Dublin isolates from clinical animal cases, mostly bovine, collected between 2019 and 2024 through the National Animal Health Laboratory Network (NAHLN) Antimicrobial Resistance (AMR) Program underwent comprehensive genotypic analysis. The aim was to characterize AMR determinants, virulence traits, and phylogenetic relationships to publicly available isolates from diverse sources. A total of 126 *S*. Dublin sequences were analyzed, including 124 from cattle and 2 from nonbovine sources (a cat and a dog). Genotypic identification, serotyping, and de novo assembly were conducted using a custom pipeline. AMR determinants and plasmids were identified via AMRFinderPlus, ABRicate, and PlasmidFinder. *Salmonella* pathogenicity islands (SPIs) and virulence genes, including the *spv* operon, were detected using SPIFinder and the FDA SalmonellaVF Comparison Tool. Phylogenetic analysis was performed using SNP-based clustering to compare NAHLN isolates with 417 publicly available *S.* Dublin genomes from human, food, animal, and environmental sources. Most clinical isolates harbored multiple AMR genes, with a high prevalence of multidrug resistance (99.2%). Frequently detected AMR genes included those conferring resistance to aminoglycosides (*aph(3")-Ib* and *aph(6)-Id*), β-lactams (bla_{CMY-2}), sulfonamides (*sul2*), phenicols (*floR*), and tetracyclines (*tet*(A)). All isolates carried the *spvRABCD* virulence operon. Key SPIs, including SPI-1, −3, −5, −6, −9, −12, −14, and the CS54 island, were present in over 92% of isolates. Publicly available *S.* Dublin genomes were selected based on close phylogenetic clustering with NAHLN isolates and were part of the dominant SNP clade (PDS000028192.1212) identified in NCBI's Pathogen Detection Isolates Browser. Phylogenetic analysis is ongoing. This study highlights the widespread presence of AMR determinants and the *spv* operon in *S.* Dublin isolates and underscores the importance of continued genomic surveillance to monitor the emergence and spread of *S.* Dublin strains across animal and human populations.

Key Words: *Salmonella*, Dublin, antimicrobial resistance, surveillance, One Health

P141 - Prevalence of *Salmonella enterica* in feces and lymph nodes of beef-on-dairy finisher cattle.

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Salmonella enterica is a major foodborne pathogen, causing an estimated 1.35 million infections, 26,500 hospitalizations, and 420 deaths annually in the United States. In cattle, beef contamination occurs primarily through feces and *Salmonella*-positive lymph nodes. The practice of breeding dairy cows with beef semen to produce “beef-on-dairy” (BOD) crossbred calves for beef production has increased substantially, yet the prevalence and associated public health risks of *Salmonella* in these cattle remain unknown. We conducted a cross-sectional study to determine the prevalence in feces, subiliac (SLN), and mesenteric (MLN) lymph nodes. A total of 663 samples were collected from 221 BOD finisher cattle at slaughter from 2 Texas feed yards. *Salmonella* was isolated via culture and confirmed by agglutination and PCR (*invA* and *pagC* genes). The overall prevalence was 17.5% (116/663), with the highest prevalence in SLN (28.1%; 62/221), followed by MLN (17.2%; 38/221) and feces (7.2%; 16/221). Prevalence was significantly higher in feedyard 2 (22.8%) than in feedyard 1 (10.6%). A generalized linear mixed model indicated that sample type was a significant predictor of positivity. Compared with feces, the odds of detecting *Salmonella* were 3.5 times higher in MLN ($P = 0.0002$) and 7.5 times higher in SLN ($P < 0.0001$). The model also revealed significant clustering by lot (variance = 1.795), with some lots exceeding 60% prevalence. In contrast, the sex of the animal (steer vs. heifer) had no significant effect on prevalence (OR = 0.89, $P = 0.82$). Dominant serotypes were Montevideo (45.7%) and Anatum (42.2%); Typhimurium and Newport were detected in lymph nodes (4.9%). Antimicrobial susceptibility testing revealed high resistance to streptomycin (49.1%), tetracycline (45.7%), chloramphenicol (42.2%), and ampicillin (37.9%). These findings identify SLN as a key reservoir for *Salmonella* in BOD cattle and underscore the significant effect of lot- and feedyard-level factors on prevalence. The common antimicrobial resistance observed heightens the public health concern. Ongoing studies focus on molecular characterization to further elucidate ecology and transmission dynamics.

Financial Support: Research coordinated by the National Cattlemen’s Beef Association, a contractor to the Beef Checkoff

Key Words: *Salmonella*, beef-on-dairy cattle, feces, lymph nodes, nontyphoidal salmonellosis, foodborne infections

P142 - Phage therapy as a promising approach against multidrug-resistant *Salmonella*.

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The growing emergence of multidrug-resistant (MDR) *Salmonella* strains has hindered effective treatment, emphasizing the demand for antibiotic alternatives. Bacteriophage (phage) therapy has emerged as a safe and effective alternative that can target *Salmonella* while preserving the beneficial gut microbiota. In our study, we isolated phages that exhibited potent inhibitory effects on the growth and biofilm formation of MDR *Salmonella*. To obtain and purify phages with strong lytic activity, effluent water samples (n = 36) were enriched in 2× tryptone soya broth (TSB) supplemented with targeted *Salmonella* isolates and incubated at 37°C with shaking for 12 h. Supernatants were centrifuged and screened for lytic activity using the double agar overlay method. Phages with clear plaques were serially diluted and purified by repeated plaque assays to a single phage population. The selected phages demonstrated broad lytic activity against MDR and extensively drug-resistant (XDR) *Salmonella* strains recovered from necropsied cattle. To assess phage efficacy, time-kill assays were carried out over 210 min at multiplicities of infection (MOIs) of 1, 10, and 100 using 3 candidate phages (Sal-Φ1, Sal-Φ29, and Sal-Φ8). All tested phages produced a significant reduction in bacterial growth compared with the control. Phage Sal-Φ1 produced the strongest inhibition, achieving 93.1%, 95.5%, and 96.5% reduction at MOIs of 1, 10, and 100, respectively. Sal-Φ29 also demonstrated high activity, with growth inhibition of 90.5%, 93.2%, and 93.7% across the same MOIs. Sal-Φ8 showed slightly lower efficacy, with reductions of 87.2%, 86.0%, and 82.8% in the respective groups. Phage Sal-Φ1 remains active across a pH range of 2–10 and maintains viability from − 20°C to 50°C for 24 h. Groups were analyzed using *t*-tests or one-way ANOVA ($P < 0.05$), and all assays were performed in triplicate. Ongoing studies are assessing the efficacy and stability of the selected phages. These results demonstrate that phages have promising potential as targeted biocontrol agents against *Salmonella* infections. The findings suggest that the selected phages can inhibit *Salmonella* populations, supporting further studies on their efficacy and safety for broader use.

Financial Support: Center for Biomedical Research Excellence (COBRE), and Center for Pharmaceutical Research and Innovation (CPRI, NIH P20 GhdsbM130456) and U.S. Department of Agriculture (USDA) National Institute for Food and Agriculture (NIFA) (grant number 2022-09086)

Key Words: bacteriophage, extensively drug-resistant (XDR), multidrug-resistant (MDR), *Salmonella*

P143 - Comparative analysis of AMR determinants and phylogenetic relationship of *Salmonella* Typhimurium isolates from humans and cattle.

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Salmonellaenterica serovar Typhimurium is a leading foodborne pathogen with zoonotic potential and is frequently associated with antimicrobial resistance (AMR), a global health threat. Understanding the genetic determinants of AMR across human and animal reservoirs helps to limit the transmission and guide stewardship. The objectives of this study were to characterize plasmids and integrons in S. Typhimurium isolates from humans and cattle, to assess the association of integrons with AMR genes, and to evaluate the genetic relatedness of isolates across hosts using core-genome phylogenetics. Human (n = 88) and bovine (n = 32) isolates were analyzed for integrons identification using PCR followed by Sanger sequencing, and for whole-genome sequencing on Illumina MiSeq. After de novo assembly in SPAdes, AMR genes were identified using MEGARes 3.0, and plasmids were detected with PlasmidFinder. The locations of integrons and AMR genes were determined through BLAST analyses of whole-genome assemblies. Phylogenetic relatedness was assessed using core-genome analysis, and statistical associations were evaluated using Chi-squared and Fisher's exact tests ($\alpha = 0.05$). Integrons of 1,000 and 1,200 bp occurred more frequently in cattle isolates, which also carried a higher proportion of integron-associated AMR genes than human isolates. Shared plasmids [Inc.FIB(S), Inc.FII(S)] among isolates from both species highlighted the potential for horizontal AMR transfer, whereas host-specific plasmids suggested niche-driven adaptation. AMR genes associated with integrons were closely mapped with integrons on both plasmids and chromosomes. Core-genome phylogeny revealed close relatedness between cattle and human isolates, possibly due to shared environment, while tighter subclades indicated intraspecies maintenance of clones. It was concluded that cattle may act as reservoirs for integron-mediated AMR dissemination. Mapping of integron-associated AMR genes near integrons supports their role in resistance spread. Although human and cattle isolates were genetically related, resistance determinants were host-specific, likely due to environmental pressures or microbial niches. Therefore, countries should prioritize AMR stewardship programs to address environment driven resistance.

Financial Support: United States Department of Agriculture (USDA) Animal Health & Disease Funds: Project No. COLV 2021-03

Key Words: antimicrobial resistance, *Salmonella* Typhimurium, integrons and plasmids, whole-genome-sequencing, transmission

P144 - Age difference in T-cell proliferation dynamics in cattle following *Salmonella* Dublin stimulation.

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Salmonella Dublin (*S*. Dublin) poses a significant risk of infection to neonatal calves during the first 2 mo of life, due to the potential for intrauterine transmission and bacterial shedding through feces and colostrum. This susceptibility can be attributed to their immature immune systems, which result in a delayed immune response against pathogens compared with those of adult cows. These differences impair critical immune functions, including T-cell proliferation, Th1 polarization, and IFN-γ production, which are essential for *S.* Dublin clearance. The lack of baseline knowledge about these age-specific differences represents a challenge to interpreting vaccine responsiveness in calves. To address this, a mechanistic and age-optimized monitoring of the T-cell proliferation assay is needed. The objective of this study was to characterize the temporal dynamics of T-cell proliferation in calves and cows at multiple time points following stimulation with *S*. Dublin. We hypothesized that calf T-cell proliferation would be significantly delayed compared with adult cows after stimulation with *S*. Dublin, reflecting their immature immune status. Blood samples were collected from 1-wk-old calves (n = 5) and adult mid-lactation cows (n = 5). The newborn calves came from *S*. Dublin free farms. Peripheral blood mononuclear cells were isolated using a density gradient centrifugation method. The isolated cells were stained with 0.3 µ*M* of CFSE and then exposed to heat-inactivated wild-type *S*. Dublin, Concanavalin A as a positive control, or media alone as a negative control, with each condition set up in duplicate. Culture and cell stimulants were partially replaced every 3 d, and the proliferation patterns of the cells were observed on d 5, 7, and 9. The cells were labeled with antibodies against CD3 and efluor660 viability dye and subsequently analyzed via flow cytometry. For each sample, 5,000 events were recorded within the CD3+ gate. Statistical comparisons between age groups were performed using a one-way ANOVA with multiple comparisons, with significance set at $P < 0.05$. Data from these experiments are currently being analyzed and will be presented at the conference. By tracking T-cell proliferation dynamics between calves and cows, we anticipate demonstrating the delayed proliferative response of young cattle. Establishing these baseline patterns will provide a foundation for accurately evaluating vaccine-induced proliferation in future calf studies.

Key Words: adaptive immunity, bovine, calves, correlates of protection

P145 - Evaluation of the antibiotic potentiation ability of phytochemicals against *Salmonella* Typhimurium and ESBL *E. coli*.

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Gram-negative pathogens such as ESBL *E. coli* and *Salmonella Typhimurium* are contributors to the rise in antimicrobial resistance (AMR). This further exacerbates the global impact of $400 million in direct medical costs due to AMR. With few antibiotic classes in development, the identification of new compounds and the potentiation of existing antibiotics is imperative. In this study, we evaluated the antibiotic-potentiating ability of 2 phytochemicals, Methyl trans-cinnamaldehyde (MTC) and Piceatannol (PIC). Initially, various combinations of different antibiotics and the phytochemicals were screened using the broth dilution method, resulting in the identification of promising synergistic combinations with antibiotics. Using the Bliss independence model, checkerboard assays were conducted to evaluate the synergy between the phytochemicals and antibiotics. Different concentrations of tetracycline (0.87–900 µ*M*) and MTC (12.5–800 µ*M*) were tested against *S.* Typhimurium (ATCC 13311). Additionally, ceftriaxone (0–800 µ*M*) and PIC (0–800 µ*M*) were tested against ESBL *E. coli* (ATCC BAA 196). Following that, for MTC, an in vitro adhesion and invasion assay was performed using a monolayer of Caco-2 cells with a seeding density of 106 cells/well in a 12-well plate and *S.* Typhimurium concentration of 107 cfu/well, giving a multiplicity of infection 1:10. The MTC concentrations used were 200 µ*M*, 400 µ*M*, 800 µ*M*, and 1,200 µ*M* per well. Synergy data were statistically analyzed using the Bliss independence model for multiple time points, and adhesion and invasion assays were analyzed using ANOVA in GraphPad PRISM. Results showed that, among the combinations of tetracycline and MTC, 53.24% showed a highly synergistic interaction (>0.25), while the rest, 46.76%, showed moderate synergy (0–0.25). However, a high synergy of 74% observed in the case of ceftriaxone and PIC, whereas 26% showed either low synergy or antagonism (<0). Further, during the adhesion and invasion assay, we observed a reduction in adhesion by 38%, 77%, 85%, 89%, and invasion by 92%, 95%, 100%, 100% for the MTC concentrations of 200, 400, 800, and 1,200 µ*M*, respectively, when compared with the cell control. Moreover, the basal respiration of the combination was lower than that of the control group. Overall, both the phytochemicals provided significant synergistic interactions with the antibiotics, and MTC was able to reduce the *S.* Typhimurium adhesion and invasion in Caco-2 cells.

Key Words: AMR, piceatannol, methyl trans cinnamaldehyde, synergy, *S*. Typhimurium, ESBL *E. coli*

P146 - Multi-omics analysis of *Salmonella* ArtAB toxin to determine its role in host-pathogen interactions.

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Salmonella is a significant cause of foodborne illness worldwide and is often transmitted from agriculturally important animals to humans. It also causes disease in livestock, reducing animal welfare and productivity. This high-priority pathogen produces numerous virulence factors that enable it to evade host immune defenses. Among these are AB_5 toxins, a family of multi-subunit proteins that are well-known virulence factors in other gram-negative bacteria. AB_5 toxins have been identified in many *Salmonella* serovars; however, their cellular and molecular roles in pathogenesis remain poorly understood. We previously purified an AB_5-like toxin, ArtAB, and its B_5 subunit (ArtB) from *Salmonella* Typhimurium and observed morphological effects in tissue culture. In this study, we evaluated the cytotoxicity of ArtAB and ArtB on Chinese hamster ovary (CHO) cells. Based on these results, we treated CHO cells with equimolar concentrations of ArtAB (10 µg/mL) and ArtB (7.25 µg/mL) for 4 h, followed by mRNA sequencing and whole-cell proteomics to assess differential gene and protein expression. Both data sets show that ArtAB downregulates pro-inflammatory signaling and alters cytoskeletal organization and cell-cell contacts. In contrast, ArtB behaved similarly to the buffer control (1× PBS + 5% glycerol) and did not significantly modulate gene or protein expression. To examine the distribution of AB_5 toxins across hosts, we analyzed sequenced *Salmonella* genomes from cattle, chickens, and pigs. We found that 33% of cattle, 14% of chicken, and 10% of pig genomes contained consensus B-subunit homologs. Within each host, these homologs exhibited an average sequence identity of ~70% to 80% to ArtB. These findings suggest strong evolutionary conservation of *Salmonella* B-subunits across host-adapted serovars and potential functional redundancy. This conservation may parallel the biological roles of other enterotoxin B-subunits, such as cholera toxin B (CTB) and heat-labile toxin B (LTB), which serve as both vaccine antigens and protein adjuvants. Defining the signaling pathways induced by *Salmonella* AB_5 toxins, along with their genomic prevalence, is essential for understanding their role in pathogenesis and may guide the development of new livestock vaccines to reduce zoonotic transmission.

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Key Words: ArtAB, AB_5 toxin, *Salmonella enterica*, omics, pathogenesis, One Health

P147 - Prevalence of *Salmonella* and multidrug-resistant (MDR) enteric bacteria in backyard poultry flocks in Texas: Evidence of a commensal reservoir of MDR.

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The recent surge in backyard poultry (BYP) ownership has created a growing public health interest, particularly regarding the potential for transmitting antimicrobial resistance (AMR) at the community-animal interface. Surveillance for AMR in these noncommercial, mixed-species flocks is notably limited. This study was designed to determine the prevalence of cephalosporin-resistant (CefR) and multidrug-resistant (MDR) enteric bacteria in BYP across Texas to assess this critical AMR risk. We sampled a total of 53 BYP flocks, owned by 34 households across 11 Texas counties. Cloacal swabs (n = 81) were collected from 81 birds (approximately 10% of the bird population of 942), subjected to isolation of *Salmonella* and quantification of total aerobic and cefR bacteria. A collection of 105 representative CefR isolates underwent antibiotic susceptibility testing (AST) against >12 antibiotics from 7 classes; MDR was defined as resistance to ≥3 classes. A survey was administered to document flock management and antibiotic use. The survey established minimal antimicrobial selection pressure: only 1 owner reported antibiotic use in their flock (28/29 respondents, or 96.6%, reported no use). Nevertheless, CefR bacteria were highly prevalent, isolated from 75/81 swabs (92.6%). These CefR bacteria represented 19% (2.18 × 10E9 cfu) of the total aerobic counts (9.37 × 10E9 cfu), indicating a significant presence in the commensal gut flora. Notably, of the 105 representative CefR isolates tested, 90 (85.7%) were classified as MDR. MDR was pervasive, with high resistance observed across most species, including chickens (87% MDR), and other nonchicken species such as ducks, quails, turkeys, guineas (range = 78% to 100% MDR), and geese (33% MDR). Furthermore, BYP households included vulnerable populations, with high-risk caretakers (≤5 or ≥ 65 yr) present in 11 households. None of the samples tested positive for *Salmonella.* The high prevalence of CefR bacteria and the MDR observed (85.7%) across BYP flocks in Texas is striking, particularly given the near-absence of reported antibiotic use. These findings suggest the BYP gut microbiota harbors a substantial and persistent reservoir of MDR likely driven by mobile genetic elements rather than current selection pressure. This widespread MDR in commensal bacteria may pose a significant One Health risk for transmission within the community, necessitating targeted surveillance and public education for high-risk caretakers.

Financial Support: TTU-SVM and Judy Calder Foundation

Key Words: *Salmonella*, backyard poultry, antibiotic resistance, *Salmonella*

P148 - Detection of multidrug-resistant nontyphoidal *Salmonella enterica* in poultry, humans, and the environment: Evidence from Northwestern Ethiopia.

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Nontyphoidal *Salmonella* are significant foodborne zoonotic pathogens closely linked to poultry and poultry products. Despite their public health importance, limited data are available on the epidemiology and antimicrobial resistance patterns of nontyphoidal *Salmonella* in poultry production systems in Ethiopia. This cross-sectional study aimed to estimate the prevalence, identify risk factors, and assess the antimicrobial resistance profiles of *Salmonella enterica* from poultry farms in Bahir Dar city, northwestern Ethiopia. Standard bacteriological methods, PCR-based detection, and serotyping were used to investigate the presence of *Salmonella* in chicken (n = 126), environmental (n = 198), and human (n = 45) samples collected from 22 poultry farms. Antimicrobial susceptibility profiles were determined using the Kirby–Bauer disk diffusion method. Data from questionnaires, and Fisher's exact tests were used to identify risk factors associated with *Salmonella* occurrence. Nontyphoidal *Salmonella* species were detected on 18.1% (4/22) of the farms. *Salmonella enterica* was recovered from 3.1% (6/198) of environmental boot samples, 3.2% (4/126) of cloacal swabs, and 4.4% (2/45) of human stool samples. Two *Salmonella* serotypes were identified from among 12 *Salmonella* isolates: *S.* Enteritidis (41.6%, 5/12) and *S.* Typhimurium (16.6%, 2/12). All *Salmonella* isolates demonstrated complete resistance to ampicillin (100%) and tetracycline (100%) and exhibited multiple drug resistance patterns, with a high multiple antibiotic resistance index ranging from 0.45 to 0.55. The prevalence of *Salmonella* was significantly associated with the absence of foot baths ($P = 0.0096$), and the presence of other animal species on the farm ($P = 0.026$). The demonstrable emergence of multidrug-resistant *Salmonella* Enteritidis and *Salmonella* Typhimurium serotypes, alongside key factors driving the prevalence of nontyphoidal salmonellosis on poultry farms in northwestern Ethiopia, underscores the need for improved intervention strategies and ongoing large-scale One Health genomic surveillance to accurately monitor temporal dynamics of *Salmonella* infections and mitigate the rise of multidrug resistance.

Key Words: antimicrobial resistance, nontyphoidal *Salmonella*, prevalence, One Health, risk factors

P149 - Quantitative risk assessment of *Salmonella* contaminated mechanically recovered poultry meat imported into South Africa.

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Salmonella has been identified as a pathogen of public health concern as it has been linked with foodborne disease outbreaks globally. In this study, a quantitative risk assessment approach was used to quantify the likelihood of importing mechanically recovered poultry meat contaminated with *Salmonella* into South Africa. The approach has been used before to evaluate the risk associated with the import or export of animal commodities. A quantitative risk analysis model was developed in Microsoft Excel. The Monte Carlo simulation was run at 10,000 iterations using a commercial software. Sensitivity analysis was performed to identify which factors had a significant effect on the overall likelihood of importation of contaminated recovered poultry meat. The overall likelihood of importing mechanically recovered poultry meat contaminated with *Salmonella* into South Africa was calculated as 5.88×10^{-4} (5th = 5.886×10^{-5} to 95th = 5.73×10^{-3}). The prevalence of *Salmonella* in the exporting country ($\rho = 1.00$), the sensitivity of the culture ($\rho = 0.05$) and PCR ($\rho = 0.05$) test, the within consignment prevalence ($\rho = 0.02$), state decision ($\rho = 0.03$) were positively correlated with the probability of importing a contaminated mechanically recovered poultry meat consignment, whereas the number of consignments imported into the country was negatively correlated ($\rho = -0.01$) with the probability of importing a contaminated consignment. A quantitative risk assessment model shows that the likelihood of importing mechanically recovered poultry meat consignment contaminated with *Salmonella* species was low. Therefore, caution must be exercised, and continuous vigilance must be implemented, as a previous study on the prevalence of *Salmonella* species in mechanically recovered poultry meat consignment has reported the presence of zoonotic *Salmonella* serotypes.

Key Words: mechanically recovered poultry meat, *Salmonella*

General Abstracts: Tick-Borne Diseases—Posters

P151 - An ecological analysis of ticks in the country of Georgia using ten years of surveillance data.

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Ticks serve as vectors for numerous animal and human pathogens, making their ecological characterization essential for disease control measures. Within the country of Georgia, multiple ticks of veterinary and public health importance are prevalent, yet comprehensive studies on tick community composition and biogeographic patterns remain limited. We analyzed 720 tick occurrence records representing 21 species collected across Georgia from 2013 to 2023, integrating these data with environmental covariates. Species distribution models were developed using boosted regression trees, while bioregions were constructed using spatially constrained hierarchical clustering. Co-occurrence networks were constructed to identify co-occurring species. Biodiversity metrics were calculated to characterize community structure. Tick diversity was high across Georgia (Shannon's H' = 2.40, Gini-Simpson = 0.89) with moderate evenness (Pielou = 0.79) and low dominance (Berger-Parker = 0.17). The species with the most presence records were *Ixodes ricinus* (17.2% of records), *Haemaphysalis punctata* (16.4%), and *Dermacentor marginatus* (14.6%). Three distinct bioregions were identified, with temperature being the most influential feature driving tick distribution and bioregionalization. Nine significant positive co-occurrence relationships were detected between species pairs, with no negative associations observed. Species distribution models revealed high niche overlap across all 7 modeled species (mean Schoener's D = 0.91, Warren's I = 0.74), with southeastern Georgia showing the highest predicted suitability for most species. Our findings highlight strong biodiversity, overlapping distributions, and the importance of temperature in influencing tick ecology in Georgia. These results underscore the need for integrated multi-species vector control and the value of ecological frameworks for guiding vector-borne disease prevention in the region.

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Key Words: ticks, *Ixodes ricinus*, vector ecology, machine learning, biogeography, biodiversity

P152 - Susceptibility of *Anaplasma marginale* to tetracycline in cell culture and red blood cells.

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Bovine anaplasmosis, caused by *Anaplasma marginale*, is a production-limiting tick-borne disease of cattle. Prophylactic use of tetracycline is one of the few preventatives. The anecdotal reports of outbreaks despite tetracycline use raise questions about how *A. marginale* responds to tetracycline and if there is variation in susceptibility among strains. This variation could occur through variations in efflux pumps, as *A. marginale* has no annotated tetracycline resistance genes. To address these questions, we measured the response of *A. marginale* to tetracycline in cell culture, including transcript levels of selected efflux pumps. We then compared the response of 2 strains of *A. marginale* to tetracycline in infected red blood cells. DAE100 cells were infected with the St. Maries strain of *A. marginale* and allowed to replicate for 5 d. One to 100 µg/mL of tetracycline was then added for 3 d. For red blood cell assays, blood was collected from an infected calf and exposed to 1, 0.1, and 0.01 µg/mL tetracycline for 5 d. Following this treatment, transcript levels for *rpoH* or the efflux pumps *Am167*, *Am171*, *Am738*, and *Am1229* were measured using real-time qPCR. Protein levels for Msp5 were measured using western blotting and densitometry. For efflux transcript analysis, $2^{-\Delta\Delta CT}$ was calculated using *rpoH* as a reference gene, and a one-way ANOVA and Dunnett's multiple comparison test were performed. There was a dose-dependent decrease in *A. marginale* transcript and protein in cell culture from 2 to 25 µg/mL, and little difference in response to doses between 25 and 100 µg/mL. *Am1229* transcript was significantly increased ($P = 0.047$) in response to tetracycline. In red blood cells, the Kansas 6DE strain had no significant reduction in *A. marginale rpoH* transcript after exposure to 1 ug/mL tetracycline, whereas the St. Maries strain had a 9-fold decrease in *rpoH* transcript after the same exposure. Establishing doses and responses in cell culture will allow for a better understanding of how *A. marginale* responds to tetracycline and direct comparison among strains. The increase in transcript of *Am1229* suggests upregulation of an efflux pump in response to tetracycline. These data demonstrate differences in susceptibility to tetracycline between strains.

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

Key Words: *Anaplasma marginale,* tetracycline, *Dermacentor andersoni*, tick cell culture

P153 - Unraveling structural features of the sexual-stage *Babesia bigemina* HAP2 fusogen protein using computational approaches.

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Tick-borne apicomplexan parasite *Babesia* (*B.*) *bigemina* is one of the causative pathogens of bovine babesiosis, which causes enormous economic losses in the cattle industry worldwide. Currently, several control measures are available to tackle this disease, but each has its own shortcomings, and novel control options are urgently needed. As *B. bigemina* has a dixenic life cycle and completes its sexual stage at the midgut of the tick vector, blocking this inevitable stage can prevent the transmission of this parasite. In the sexual stage of *B. bigemina*, HAP2 is an indispensable protein without which gamete fusion of this parasite can't succeed. Structural studies on HAP2 revealed that it has 3 extracellular domains, designated as DI, DII, and DIII. It is a transmission-blocking vaccine candidate of *Babesia bovis* and *Plasmodium spp*. Previous studies showed that blocking HAP2 domains of this protein can result in the blockade of gamete fusion of *Plasmodium* spp. to different extents. For this reason, this study focused on determining the structure of this protein. Our study compared the AlphaFold-predicted *Babesia bigemina* HAP2 with the crystal and predicted structures of HAP2 of *Chlamydomonas reinhardtii*, *Arabidopsis thaliana*, *Plasmodium* spp., and *Babesia* spp. Though the predicted structures were not cross-validated with experimental data due to the lack of experimental structure of *Babesia* spp. or related parasites, we compared this predicted HAP2 structure with the experimental structures of HAP2 of *C. reinhardtii* and *A. thaliana*. We used ChimeraX for the structural alignment and analysis for this study. We observed that 3 domains of *B. bigemina* HAP2 overlapped with the domains of those HAP2 proteins that showed significant structural similarity, despite having no significant sequence similarity. In addition, we performed structural analysis using HAP2 from multiple strains of *B. bigemina*, and we also discovered that they were remarkably similar. Also, we found that disulfide bonds are strictly conserved across the 3 domains. Defining the sequence of each HAP2 domain allowed us to generate domain-specific antibodies. The domain-specific antibodies will be validated using an in vitro sexual stage induction assay. This study defined the structure and sequence of the domains of *B. bigemina* HAP2, which provides a novel avenue for developing a transmission-blocking vaccine by targeting the HAP2 domains.

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Key Words: bovine babesiosis, *Babesia bigemina*, HAP2, HAP2 domain, transmission-blocking vaccine

P154 - A conserved motif in the piroplasmid RAP-1 and the *Babesia* RAP-1 related antigen (RRA) molecules is partially exposed on the surface of merozoites and provides clues to the potential function of the protein.

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All piroplasmid organisms sequenced to date contain variable copies of genes encoding for the rhoptry-associated protein-1 (RAP-1) family, which appear to be unique to this order. RAP-1 proteins are candidates for the development of diagnostic methods and potential components for subunit vaccines. Also, *Babesia* parasites, but not *Theileria*, contain genes encoding a RAP-1-related antigen (RRA). Unique sequence features of this protein family include signal peptide and RAP-1 domains, which consist of an approximately 300 amino acid long sequence containing 4 conserved cysteine residues and a 15-amino acid motif (15-mer), along with several shorter amino acid motifs. We hypothesize that the conservation of the 15-mer holds biological and immunological significance. AlphaFold-3 was used to predict surface structures of RRA from *B. bovis*, *B. bigemina*, and *B. ovata.* BLASTP was employed to identify protein residue similarities. Antibodies against the *B. bovis* RAP-1 and RRA 15-mer were generated in rabbits. Surface exposure was assessed using immunofluorescence (IFA) on permeabilized and non-permeabilized extracellular *B. bovis* merozoites. BlastP revealed the presence of the 15-mer motif in 2 distinct adenylate cyclase type 10-like proteins as well as in other ATP-binding proteins, such as dynein, an ATP-binding cytoskeletal motor protein. AlphaFold predicted identical structures for the region, including the 15-mer motif in the RRA of *B. bovis*, *B. bigemina* and *B. ovata*, revealing similar globular shapes among the RRA, adenyl-cyclase and dynein molecules, with surface exposure of amino acid residues of the 15-mer sequences. Consistently, anti-RAP-1 and anti-RRA 15-mer antibodies reacted with the surface of aggregated extracellular *B. bovis* merozoites in IFA analysis performed on non-permeabilized extracellular merozoites. Protein structural comparisons and motif conservation suggest a possible ATP-binding function for current or ancestral members of the RAP-1 family. The partial surface exposure of the conserved 15-mer motif, along with its previously demonstrated recognition by antibodies in protected cattle, suggests that it may be a candidate for inclusion in future multiepitope peptide-based vaccines.

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Key Words: bovine babesiosis, rhoptry associated protein, anti-*Babesia* subunit vaccine

P155 - High-resolution melt analysis for differentiating virulent *Theileria orientalis* Ikeda infections from Buffeli and Chitose genotypes.

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Theileria orientalis is an economically important hemoparasite of cattle transmitted by *Haemaphysalis longicornis* (Asian longhorned tick). The virulent *T. orientalis* Ikeda genotype causes clinical disease marked by anemia, anorexia, and abortions. Although it is rarely fatal, subclinical infections with the Ikeda genotype are common. The coexistence of the virulent Ikeda with the less virulent Chitose and the non-virulent Buffeli genotypes complicates diagnostic efforts. This study aimed to develop a molecular diagnostic method using high-resolution melt (HRM) analysis to differentiate Ikeda infections from Chitose and Buffeli. We developed a green fluorescent DNA-binding real-time PCR assay (Green-rtPCR) targeting a -bp fragment of the major piroplasm surface protein (MPSP) gene, specific to the Ikeda, Chitose, and Buffeli genotypes. Plasmids containing the -bp amplicon from Ikeda, Chitose, or Buffeli were used to standardize the post-amplification HRM analysis. HRM analysis was performed using the Precision Melt Analysis Software (Bio-Rad). Pairwise sequence comparison of the -bp MPSP fragment from the Ikeda genotype with the Chitose and Buffeli genotypes revealed 8 and 4 single nucleotide polymorphisms, respectively. HRM analysis of the -bp MPSP fragment from Ikeda, Chitose, and Buffeli revealed distinct melting temperatures of 82.8°C, 82.2°C, and 81.8°C, respectively, which were effectively differentiated using relative fluorescence unit plots. Sensitivity evaluation demonstrated that the Green-rtPCR-HRM assay detected between 106 and 10^{1} copies of the -bp MPSP fragment of the Ikeda genotype and between 106 and 10° copies for the Chitose and Buffeli genotypes. Additionally, the Green-rtPCR-HRM assay demonstrated high specificity by accurately identifying the Ikeda, Chitose, and Buffeli genotypes without cross-amplifying DNA from other cattle pathogens, including *Theileria parva*, *Theileria annulata*, *Anaplasma marginale*, *Babesia bovis*, and *Babesia bigemina*. Collectively, these findings provide strong support for evaluating cattle field samples to enable differential molecular detection of the *T. orientalis* Ikeda genotype in regions where the Chitose and Buffeli genotypes are also present. We developed a highly sensitive and specific assay based on HRM analysis to differentiate between infections caused by *T. orientalis* Ikeda from those caused by the Chitose and Buffeli genotypes.

Financial Support: USDA-ARS CRIS# 2090–32000-044-000-D

Key Words: *Theileria orientalis*, Ikeda genotype, Chitose genotype, Buffeli genotype, high-resolution melt analysis

P156 - *Theileria orientalis* and *Haemaphysalis longicornis* in Oklahoma. Current status, future options.

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Oklahoma cattle production is currently ranked as the fourth state in the country based on cattle inventory, therefore, the health of Oklahoma livestock herds needs to be constantly monitored and informed of new threats. During August 2024, the discovery of *Haemaphysalis longicornis* tick in the state created an alert for cattle producers due to the potential losses that the tick can cause in Oklahoma livestock. In cattle, this tick can transmit *Theileria orientalis*, an intracellular protozoan parasite that causes anemia, weakness, reduced weight gain, abortion, and sometimes death, with no treatment available. On physical examination infected animals have pyrexia, icterus, and elevated heart and respiratory rates; these clinical signs can be mistaken for anaplasmosis, caused by the intracellular pathogen *Anaplasma marginale,* which infects red blood cells and is endemic in the state of Oklahoma. *Theileria orientalis* comprises 11 genotypes, with the genotype Buffeli native to the United States and considered non-pathogenic. With the introduction of *H. longicornis* in the US, the pathogenic Ikeda genotype has been described in several eastern states, reporting animal and economic losses. Due to the overlap in clinical signs between the endemic *A. marginale* in Oklahoma and the emergent *T. orientalis* in Eastern states, it is important to understand the status of *Theileria* sp. in Oklahoma cattle to provide proper diagnosis, preventive measures, and treatment when available. Therefore, the purpose of this study was to evaluate the status of *T. orientalis* in cattle beef herds from Oklahoma. Between November 2024 and October 2025, we collected blood from 342 animals from 16 different cattle herds from Oklahoma and utilized the blood samples to test and characterize *Theileria* species and *Anaplasma marginale*. Additionally, ticks were also collected from animals and areas near cattle herds to survey for the presence of *H. longicornis* ticks. No additional findings of *H. longicornis* were detected; however, 30 animals were detected as positive for *T. orientalis* by PCR, with 13 (3.8%) belonging to the Chitose genotype, 16 (4.6%) to the Buffeli genotype, and 1 (0.29%) to the Ikeda genotype. Additional data analysis is underway. The results from this study provide preliminary information addressing the distribution of this *T. orientalis* disease and its vector in the state of Oklahoma.

Financial Support: College of Veterinary Medicine, OSU

Key Words: *Theileria orientalis*, *Anaplasma marginale*, cattle, ticks, Oklahoma

P157 - Synergistic interaction between Buparvaquone and ELQ685 in inhibiting *Babesia bovis* in vitro.

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Babesia bovis is a highly pathogenic and globally distributed tick-borne parasite responsible for bovine piroplasmosis, a disease that causes significant economic losses in the livestock industry. The development of effective and safe therapies is urgently needed to improve global disease control strategies. This study aimed to evaluate the in vitro efficacy of ELQ-685 in combination with buparvaquone (BPQ), 2 compounds that target distinct sites within the cytochrome bc1 complex of the parasite's mitochondrial respiratory chain, against *B. bovis*. Parasites were incubated with varying concentrations of both drugs in combination ratios (ranging from 25 to 600 nM) over 3 consecutive days, starting with a parasitemia level of 1%. The half-maximal inhibitory concentrations (IC50) for each drug alone were determined, and the fractional inhibitory concentrations (FIC) for the combinations were calculated to assess potential interactions. Parasitemia levels were monitored daily using microscopic examination, and data were analyzed using nonlinear regression models. The results showed that the combination of ELQ-685 and BPQ significantly inhibited the growth of *B. bovis* ($P < 0.05$), exhibiting a synergistic effect. By disrupting mitochondrial respiration—a vital pathway for parasite survival—ELQ-685 highlights its potential as a highly effective antiparasitic agent. These findings support further in vivo evaluation of ELQ-685, alone or in combination with BPQ, as a promising and safer therapeutic option for the treatment of bovine babesiosis.

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Key Words: in vitro, Apicomplexa, endochin-like quinolones, cytochrome bc1

P158 - Report of three cases of dogs naturally infected with *Ehrlichia canis* in Chiapas, México.

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Ehrlichia canis is an obligate intracellular gram-negative bacterium transmitted by the bite of hard ticks (e.g., *Rhipicephalus sanguineus*). In dogs, this bacterium causes a disease called canine monocytic ehrlichiosis (CME). Clinical signs caused by E. canis can present in acute, subclinical, and chronic forms. Diagnosis of CME is based primarily on antibody detection and identification of the bacteria in cytoplasmic inclusions or through the identification of certain genes. Case 1: A dog exhibiting lethargy, apathy, petechiae, weight loss, and anemia. The dog tested positive on blood smear (identification of monocytes with morulae), positive for antibodies (Anigen Rapid CaniV-4, Bionote), and positive for the 16S rRNA gene of *E. canis* using PCR. Case 2: Dog with lethargy, apathy, petechiae, weight loss, and anemia. The dog tested positive on blood smear, positive on PCR, and negative for antibodies. Case 3: Dogs with apathy and weight loss. The dog tested negative on blood smear and PCR but positive for antibodies. Because canine patients arrive at the clinic with the disease at different stages, it is important to use diagnostic tools to support field clinicians in accurately diagnosing CME to establish control programs that safeguard animal health.

Key Words: *Ehrlichia canis*, PCR, *Rhipicephalus sanguineus,* blood smear, ehrlichiosis

P160 - A comparative study of the prevalence and molecular diagnosis of ehrlichiosis in eastern areas of Alabama: A One Health concern for human and canine populations.

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Ehrlichiosis is a tick-borne disease caused by bacteria of the genus *Ehrlichia*. The pathogen is a gram-negative, obligate intracellular bacterium, which specifically targets and infects leukocytes, especially monocytes and neutrophils, in both humans and animals. In the United States, the southeast and central areas are the primary locations for ehrlichiosis cases. This study analyzed 25 ticks collected from dogs from August 2023 to August 2024 from Macon County, Lee County, and Montgomery, AL, in comparison to 25 ticks from dogs of Macon County from the early 2000s. Comparison was performed by collecting ticks within the 3 counties, taking ticks collected during the earlier period and were preserved in 70% ethanol, extracting the DNA from the individual ticks, and performing molecular detection of *Ehrlichia* spp. using the PCR assay along with DNA sequencing. A 2-tailed *t*-test was done to determine the statistical significance of the 2 prevalence rates. We have found that the pathogen is highly prevalent ($P < 0.000001$) in the ticks of more recent populations compared with that of the early 2000s. The 16S rRNA target gene was used as a genetic marker to identify *Ehrlichia* presence. The prevalence of *Ehrlichia* DNA for our data set was 6 out of 25 ticks (24%) from 2006 to 2008 and 23 out of 25 ticks (92%) from 2023 to 2024. All the sequenced PCR products were matched to the *Ehrlichia* spp. We analyzed the 16S rRNA target gene for the *Ehrlichia* species of interest sequences using the NCBI database. The NCBI BLAST and MAFFT multiple sequence alignment were utilized and yielded a result of 90% to 100% similarity in sequences between our samples and *Ehrlichia* spp. reference sequences. As infected ticks serve as sentinels or warnings to indicate the presence of pathogenic *Ehrlichia* in the eastern Alabama area, continuous data collection and analysis would be an essential tool in assessing and adopting tick prevention efforts. These preliminary data show that Macon, Lee, and Montgomery County residents are potentially at an increased exposure to ticks carrying *Ehrlichia* pathogens. To reduce tick-borne disease in humans and animal populations, educational programs on preventative measures, signs of ehrlichiosis, and appropriate treatment protocols are needed. This knowledge can help prevent tick bites, reduce infection cases, and increase timely pursuit of medical intervention as human-animal-environment interactions, a One Health concern, become more interconnected.

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Key Words: *Ehrlichia*, 16SrRNA, tick-borne disease, PCR, Alabama

P161 - Prevalence and molecular detection of Rocky Mountain spotted fever in ticks from animals in east central Alabama.

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Rocky Mountain spotted fever (RMSF) is a life-threatening tick-borne disease caused by the gram-negative, intracellular bacteria Rickettsia rickettsii that infects vascular endothelium cells. The RMSF is the most common rickettsial disease in the United States and mostly affects the Southeast. This study analyzed 50 tick samples collected from dogs and pigs between May 2023 and July 2024 from different counties in the east-central area of Alabama. Individual ticks were processed for DNA extraction and molecular detection of *Rickettsia* spp. using the PCR method. The outer membrane protein A (ompA), outer membrane protein B (ompB), and high-temperature requirement A (htrA) gene were used as genetic markers. All rickettsial genes were positive in ticks from dogs and pigs. The prevalence of incidence of *Rickettsia* spp., including RMSF, was 15 out of 50 (30%) tick samples from the animals. Most of the sequenced PCR products were homologous to *R. rickettsii* and *Rickettsia* spp. We analyzed the DNA sequences using the NCBI Blast software program and found 94% similarity between our samples and other *Rickettsia rickettsii* spp. Additionally, our samples displayed 93% similarity with other *Rickettsia* spp. in the spotted fever group. Infected dogs serve as sentinels to indicate the presence of infected ticks in the east-central area of Alabama. Macon, Lee, Bullock, and Montgomery County residents are at considerable risk of exposure to the SFG, including RMSF. With this exposure risk, we can form a brief curriculum that covers preventative measures, signs for both humans and animals, and a plan of action for each stage of infection. Making this knowledge common among residents will help decrease the number of tick bites and the severity of infections as we catch them in earlier stages and begin the proper treatment.

Financial Support: Research Support: NIH/NIMHD RCMI grant # U54MD007585 and NIH T35OD010432 Student Support: DHHS/HRSA D34HP00001-35-00 and NIH T35OD010432

P162 - Impact of environmental conditions on survival and pathogenicity of *Francisella tularensis* ssp. *holarctica* live vaccinal strain (LVS).

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Francisella tularensis, the causative agent of tularemia, poses critical biodefense concerns due to its aerosolization potential and tick vector transmission from soil environments. Understanding environmental survival mechanisms is essential for bioterror preparedness. This study investigated environmental effects on bacterial survival and pathogenicity using a novel soil-based culture model. The *F. tularensis* ssp. *holarctica* live vaccinal strain (LVS) was cultured in variable conditions of nutrient availability, temperature, and atmosphere. Soil containing media representing a nutrient-limiting environment or rich media with CO_2 comparable to animal tissues at ambient or 37°C. The *F. tularensis* grew under all media types, atmospheres, and temperatures except for a single culture combination of soil media incubated at 37°C with CO_2. Changing any of these growth parameters restored bacterial growth, indicating complex environmental sensitivity by *Francisella*. The ability of bacteria grown under the variable conditions was compared in pathogenicity using an in vitro model of intracellular infection. Mouse macrophages (RAW 264.7) were infected with the various bacterial cultures, and bacterial uptake, intracellular viability, and multiplication were observed over 48 h. Two-way ANOVA revealed significant effects of media growth condition ($P < 0.0001$) at all stages of intracellular infection. All pre-grown bacteria were internalized at 2 h; however, low-temperature soil media with CO_2 resulted in a 100-fold reduction in bacterial uptake compared with soil without CO_2. All bacterial culture conditions produced similar intracellular survival and replication, except for a significant delay in replication by *F. tularensis* grown in 30°C CO_2 soil media. By 48 h, intracellular viability decreased because of macrophage death for all conditions except 30°C with CO_2. In conclusion, environmental conditioning significantly influences *F. tularensis* survival potential with important biodefense implications. Combining elevated temperature and atmospheric CO_2 in soil environments suggests natural containment mechanisms. Notably, infection outcomes for soil-conditioned bacteria remained comparable to rich media-grown bacteria, indicating pathogenicity preservation despite environmental stress. Future investigations will examine transcriptional-level pathogenicity changes and elucidate molecular mechanisms underlying temperature-CO_2 growth inhibition in soil matrices.

Key Words: *Francisella tularensis*, environmental survival, biodefense, pathogenicity, soil ecology, tularemia

P163 - Integrating conventional and high-throughput sequencing techniques for surveillance of tick-borne pathogens in Central Appalachia.

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Tick-borne diseases are a growing concern for health professionals in the United States, especially in regions such as Central Appalachia, where the ranges of northern and southern tick populations converge. Overlapping tick distributions create potential hotspots for pathogens by increasing opportunities for co-infection, pathogen spillover, and exposure of naïve animal hosts to novel tick species and microbes. Our study had 2 objectives: (i) assess pathogen presence in regional tick populations using conventional PCR and Sanger sequencing; and (ii) compare those results to a relatively new molecular diagnostic method using hybridization capture technology and high-throughput, next-generation sequencing. Our dataset included 4 species of adult female ticks: *Ixodes scapularis* (n = 76), *Dermacentor variabilis* (42), *Amblyomma americanum* (58), and *Rhipicephalus sanguineus* (64). Specimens were collected between 2017 and 2020 from sites across Kentucky, Tennessee, and Virginia. Ticks were field-collected while questing, except for *R. sanguineus*, which is an endophilic, kennel-associated species. First, we tested ticks for veterinary-relevant tick-borne pathogens, including *Ehrlichia*, *Babesia*, and *Rickettsia*, using conventional PCR methods, published primers, and Sanger sequencing. Preliminary results show the presence of *Babesia odocoilei* (n = 13; 5.4% of 240 ticks), *Ehrlichia ewingii* (1; 0.6% of 161 ticks), and *Rickettsia* spp. (16; 6.7% of 240 ticks), including *R. amblyommatis* (9; 3.8% of 240 ticks). In addition to these pathogens, we sequenced DNA from tick endosymbionts, including *Coxiella*, *Rickettsiella*, *Pantoea*, and *Arsenophonus*. The presence of these microbes warrants further study into their role in vector competence and pathogen persistence. Our next objective is to subject these tick samples to a hybrid capture technique (i.e., EctoBaits) before high-throughput sequencing. We expect to simultaneously confirm tick species while identifying pathogenic microbes and previous host blood meals. These results are forthcoming and will be presented with our conventional PCR results to evaluate sensitivity and specificity. Hybrid capture may offer improvements in detection breadth and cost efficiency, while allowing surveillance efforts to include bigger data sets across veterinary and public health sectors. This work contributes to molecular diagnostic workflow developments that are scalable, standardized, and improve detection of tick-borne pathogens.

Key Words: Ixodid tick, tick-borne pathogen, Sanger sequencing, hybridization capture, next-generation sequencing

P165 - Enhancing plague surveillance in wildlife: Expansion of a bead-based immunoassay beyond canid species.

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Plague, caused by the bacterium *Yersinia pestis*, occurs in many wild mammals across North America. The *Y. pestis* is classified as a Category A priority pathogen due to its high morbidity and mortality, as well as its potential use in bioterrorism. Elevated human risk of *Y. pestis* infection has been linked to a rise in seropositive wildlife, particularly rodent-eating carnivores. We recently developed a bead-based immunodiagnostic assay called the F1-LPA for semi-quantitative assessments of host antibodies targeting the F1 capsular antigen of *Y. pestis* in coyotes (*Canis latrans*). This assay was more sensitive and specific compared with traditional methods for plague serodiagnosis. Here, we evaluate a pan-species variant of the F1-LPA for serodiagnosis of *Y. pestis* exposure in other wildlife susceptible to plague. To facilitate detection, this assay uses conjugates of protein A and protein G in lieu of species-specific secondary antibodies. Samples were classified as detected if their MFI was at least 1.6× higher than a validated standard and had a signal-to-noise (S/N) ratio of 10 or more relative to background. This 2-step threshold approach enhances the accuracy of detection by combining normalized signal intensity with background noise, providing clear and consistent results. In a multi-species study, the F1-LPA results closely matched PHA results in both detection and non-detection. A total of 113 samples (55 positives, 58 negatives) from 7 different species, the sensitivity of F1-LPA was 100% for California ground squirrel (n = 20), 100% for deer mouse (n = 6), 100% for American black bear (n = 20), 100% for yellow-pine chipmunk (n = 20), 90.90% for shadow chipmunk (n = 20), 90.90% for lodgepole chipmunk (n = 20), and 100% for Douglas squirrel (n = 7). Data from sylvatic plague vaccine (SPV), a poxvirus-based vaccine containing F1 and LcrV, experiments demonstrate that the assay works. A naïve animal becomes F1 seropositive after SPV consumption. This was shown in pen trials and in the field. For pen trials, a group of 7 prairie dogs shows seroconversion at an average of 44× the baseline. In field trials, SPV application increased anti-F1 seropositivity rate on a specific chunk of BLM land by 17.9%. Across all samples (including those for routine monitoring and surveillance), there are very clear negative animals and very clear positive animals. Thus, F1-LPA offers a reliable and sensitive tool for the detection of *Y. pestis* in a variety of wild mammals.

General Abstracts: Vaccinology—Posters

P167 - Inulin acetate nanoparticles as a novel TLR4-based adjuvant for influenza vaccination in pigs.

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Influenza A virus remains a major threat to swine health and global agriculture, causing respiratory illness and economic losses. This study explores inulin acetate nanoparticles (InAc-NPs) as a novel TLR4-based adjuvant and delivery system for swine influenza vaccination. Hemagglutinin (HA) and matrix protein extracellular domain (M2e) from A/California/04/2009 (H1N1) were encapsulated into InAc-NPs using a double emulsion solvent evaporation method. Nanoparticles were characterized for size, charge, morphology, antigen loading, and stability using dynamic light scattering, scanning electron microscopy, RP-HPLC, and SDS-PAGE. InAc-HA-NPs and InAc-M2e-NPs had mean diameters of 361 ± 0.52 nm and 327 ± 0.30 nm, with near-neutral surface charges, indicating uniform particle formation. Antigen loading was 2.42 ± 0.20 µg HA and 2.07 ± 0.28 µg M2e per mg of nanoparticles. Lyophilized InAc-NPs preserved the structural integrity of the antigen (HA) for over 12 mo at 4°C, whereas HA in solution exhibited complete degradation. In vitro, InAc-NPs showed higher uptake by porcine alveolar macrophages (n = 3 biological replicates) compared with PLGA-NPs, mediated through TLR4 recognition. In vivo, vaccination studies were conducted in 3-wk-old littermate piglets with mixed gender (n = 5 per group), and InAc-NPs elicited robust antigen-specific antibody responses, with anti-HA and anti-M2e IgG titers increased 4.7- and 1.7-fold over saline controls, respectively. The results were expressed as mean ± SD. The variance between groups was compared using one-way ANOVA followed by Bonferroni's post hoc multiple comparison test. Data were analyzed in GraphPad Prism software (CA), and $P < 0.05$ was considered statistically significant. Antibody levels were comparable to those induced by the commercial adjuvant AddaVax, highlighting the potential of InAc-NPs as a stable and effective vaccine delivery platform. InAc-NPs not only generated higher antibody titers but also produced antibodies with high avidity with an affinity rate constant (KD) of 3.49×10^{-9}. This study shows that InAc-NPs act as an effective adjuvant and delivery system in pigs. The InAc-NPs induced strong, high-affinity IgG responses, supporting their potential as a vaccine platform for swine influenza and broader veterinary and human applications.

Financial Support: This research was fully supported by USDA-NIFA Agriculture and Food Research Initiative Competitive Grant no. 2016-09475 and the Haarberg-3D-center (pilot grant).

Key Words: influenza, vaccine, adjuvant, hemagglutinin, TLR4

P169 - EpiCC-guided vaccine candidate selection improves T-cell epitope coverage against circulating European swine influenza A virus strains.

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Swine influenza A virus (sIAV) causes substantial economic losses in the swine industry. Although current vaccines offer some protection, antigenic mismatches between vaccine strains and circulating field viruses can reduce their efficacy. Traditional vaccine selection methods that use serology and phylogenetics fail to address the role of T-cell immune responses, which are essential for comprehensive protection. This study used EpiCC (T-Cell Epitope Content Comparison) to evaluate European sIAV isolates. EpiCC is a computational pipeline that compares shared putative T-cell epitope content between vaccine strains and field isolates, generating quantitative metrics for assessing vaccine candidates and identifying superior vaccine candidates with improved epitope coverage. This study included 448 European sIAV HA sequences from subtypes H1N1 (n = 112), H1N2 (n = 126), H3N1 (n = 10), and H3N2 (n = 200) reported by Henritzi (2020) or obtained from GISAID that were analyzed using the PigMatrix algorithm to identify putative T-cell epitopes. EpiCC quantified the sharing of putative epitopes between the field isolates and 2 commercial European sIAV vaccines (one monovalent and one trivalent), generating coverage scores for each comparison. We performed 2 main comparisons: (1) We assessed whether the commercial vaccines' epitopes covered the epitopes of field isolates, and (2) each isolate was evaluated as a potential vaccine candidate based on EpiCC scoring. The commercial vaccines demonstrated median T-cell epitope coverage ranging from 33% to 74% against field isolates (>67% is considered likely to be protective) within the same subtypes. H1 isolates formed 2 distinct subclusters, as reflected by EpiCC scores and epitope coverage patterns. Furthermore, several field isolates demonstrated superior EpiCC scores and epitope coverage compared with existing vaccines. The best vaccine candidate isolates showed increases in median epitope coverage ranging from 2.2% for H3N1 to 24.1% for H1N1 as compared with the coverage of commercial vaccines. These results demonstrate the value of EpiCC for evaluating vaccines and successfully identify multiple field isolates as promising candidates with superior T-cell epitope coverage compared with commercial formulations. A broader and more targeted screening process, especially for H3 isolates, will likely reveal candidates with greater immunological potential, leading to enhanced protection against swine influenza.

Key Words: EpiCC, T-cell epitopes, swine influenza, vaccine selection, vaccine evaluation

P170 - Evaluation of a mucosally delivered subunit vaccine against bovine tuberculosis in a calf vaccination-challenge model.

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Bovine tuberculosis (bTB), caused by *Mycobacterium bovis*, remains one of the most damaging livestock diseases globally, leading to substantial economic losses and posing a significant zoonotic threat—particularly due to persistent wildlife reservoirs. Control efforts are hampered by limited vaccine options. Although the Bacillus Calmette–Guérin (BCG) vaccine has been used, a recent meta-analysis of ~1,300 studies estimated its direct protective efficacy in cattle at only ~25%, showing notable reductions in lesion severity but minimal effects on infection prevention or clearance. To address this gap, we evaluated a mucosal subunit vaccine utilizing *Bacillus subtilis* spores engineered to display conserved *M. bovis* antigens (Ag85B, CFP10, and ESAT-6). Holstein calves were vaccinated via both oral and intranasal routes and then challenged with *M. bovis* embedded in Matrigel, allowing localized assessment of bacterial growth. Longitudinal blood samples were collected to monitor systemic immune responses, and Matrigel was recovered at necropsy for microbiological culture, histological analysis and transcriptomic analysis. Systemic responses will be further characterized through the transcriptional profiling of Matrigel and peripheral blood mononuclear cells (PBMCs), along with serum cytokine analyses. Bacterial burden analysis revealed that spore-vaccinated calves harbored ~10^5 cfu at the challenge site, compared with ~10^6 to 10^7 cfu in both unvaccinated and BCG-vaccinated calves—a 1.5-log reduction ($P < 0.001$ vs. unvaccinated; $P < 0.01$ vs. BCG). Notably, BCG did not reduce bacterial burden relative to controls. Additional analyses—including histopathology (H&E, acid-fast staining), multiplex cytokine profiling, and RNA-Seq of Matrigel and PBMCs—are underway to elucidate immune correlates of protection. These findings demonstrate that the mucosal *B. subtilis* spore subunit vaccine conferred superior protection compared with BCG under the tested conditions. Ongoing studies aim to define the immune mechanisms driving this enhanced efficacy.

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Key Words: bovine tuberculosis, *Bacillus subtilis*, spore subunit vaccine, Matrigel, *Mycobacterium bovis*, RNA-Seq

P171 - Development and testing of *Mycobacterium avium* ssp. *paratuberculosis* DIVA vaccines in ruminants.

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Johne's Disease is caused by *Mycobacterium avium* ssp. *paratuberculosis* (MAP) and is a significant economic problem in the dairy industry worldwide. We successfully generated live-attenuated strains that can differentiate vaccinated from infected animals (DIVA) and demonstrated that DMAP52 and DMAP56 marked and unmarked mutants are pro-apoptotic in macrophages. We conducted experiments for MAP K-10 wild-type (WT) and all mutants. For growth rate comparisons, MAP cultures with an initial optical density (OD) at 600 nm of 0.05 were grown standing in MOADC at 37°C for 28 d taking OD readings and colony forming units (CFUs) after 5 weeks of incubation. Results showed that strains had similar growth properties in broth cultures to each other which is a desirable characteristic for candidate vaccine mutants. For in vitro sensitivity to oxidative stress, strains were cultivated for 3 h in hydrogen peroxide. There were significant differences to hydrogen peroxide at 5 m*M* (DMAP56 unmarked) and 20 m*M* (DMAP52 marked and unmarked, and DMAP56 unmarked) compared with the WT. To evaluate sensitivity to low pH, the strains were incubated at pH levels (4.5 and 6.5) for 30 d taking OD readings and plating colony counts at d 0 and every 10 d after. All mutants were susceptible to the low pH of 4.5 compared with the WT. We performed confocal microscopy to determine the fate of MAP strains (MOI of 20:1 bacilli per cell) in bovine monocyte derived macrophages. Colocalization within endosomes at early (Rab5), transition (Rab5 and Rab7), and late (Rab7) stages was assessed. The results showed that DMAP52 marked and unmarked, but not for DMAP56 derivatives, had significantly more endosome colocalization at all stages than the WT, a characteristic of attenuated strains. To determine cytokine responses, we infected peripheral blood mononuclear cells, supernatants were collected and were measured using the Milliplex® Bovine Cytokine/Chemokin Magnetic Bead Multiplex Assay. There were no significant differences between the WT strain and the mutants, therefore the MAP mutants are immunogenic. We are currently constructing 2 double mutants (DMAP52 unmarked DMAP56 marked and DMAP52 marked DMAP56 unmarked) that we plan to test for further attenuation, unmarking, and DIVA capabilities to serve as a vaccine candidates. In conclusion, we have demonstrated the attenuation of DMAP52 unmarked in broth cultures and primary mononuclear cells.

Financial Support: This research was funded by the United States Department of Agriculture (USDA) National Institute of Food and Agriculture (NIFA) competitive program Agriculture and Food Research Initiative, grant number 2020-67015-31416 (NEB 39-185).

Key Words: paratuberculosis, DIVA vaccine, bovine, macrophage, cytokine profiling, double mutant

P173 - Induction of trained immunity in foals with poly-*N*-acetyl-glucosamine.

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Rhodococcus equi is an intracellular bacterium that causes severe pneumonia in foals worldwide for which no vaccine is commercially available. Gavage with live virulent *R. equi* has been shown to induce trained immunity and protect foals against *R. equi* pneumonia after intrabronchial challenge, but that strategy is not acceptable for use at farms. Poly-*N*-acetyl glucosamine (PNAG) is a highly-conserved microbial surface polysaccharide found on *R. equi* and many other bacteria. Our laboratory has previously shown that either transfusing foals with PNAG hyperimmune plasma or vaccinating mares with PNAG pre-foaling protected foals against pneumonia following intrabronchial challenge, but vaccinating pregnant mares does not ensure transfer of PNAG antibodies to foals, and plasma transfusion is expensive and can be risky for foals. Although foals do not mount a robust immune response to vaccines, we aimed to test a new formulation of PNAG using the viral particle Qbeta as a carrier (PNAG-Qbeta), which showed improved immunogenicity in animals. Therefore, our objectives were to determine (1) whether the PNAG-Qbeta vaccine would be immunogenic in foals and protect against *R. equi* pneumonia and (2) whether the PNAG-Qbeta vaccine would induce trained immunity by generation of trained circulating monocytes. Healthy Quarter Horse foals (n = 12) were randomly assigned to receive either PNAG-Qbeta vaccine (1 mL of 125 mg PNAG-Qbeta formulated with the adjuvant Montenide [10%]; n = 6) or control saline (1 mL; n = 6) intramuscularly at ages 2, 14, and 26 d. At age 30 d foals were challenged intrabronchially with 2×10^6 *R. equi* and monitored for the development of clinical pneumonia until age 64 d. Serum samples were collected at ages 2, 14, 26, and 56 d and tested by ELISA for anti-PNAG IgG1. Fresh peripheral blood mononuclear cells (PBMCs, which include circulating monocytes) were isolated from whole blood on d 30 and submitted for single-cell RNA-sequencing. Preliminary results indicate foals were neither effectively immunized nor protected by PNAG-Qbeta vaccination. Trained immunity results are pending but will be presented at the conference. Collectively, our study will provide evidence as to whether PNAG vaccine is immunogenic in foals and a trained immunity inducer, possibly through ficolin receptors. These results will guide our efforts for the development of PNAG-Qbeta as a vaccine for either mares or foals.

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Key Words: foal, poly-*N*-acetyl glucosamine-Qbeta, vaccination, antibody, *Rhodococcus equi*, infectious disease

P174 - Optimized *Shigella* MEFA: A multivalent vaccine candidate against shigellosis.

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Shigella, being a leading cause of diarrhea in children and a common etiology of diarrhea in travelers, has no licensed vaccines against it. To develop a cross-protective *Shigella* vaccine, our previous pulmonary lethal challenge study of a polyvalent protein immunogen, which contains conserved immunodominant epitopes of *Shigella* invasion plasmid antigens (Ipa) and Shiga toxins, demonstrated a robust protective immune response. The study involved in silico–predicted epitopes from genes *IpaB*, *IpaD*, *VirG*, and *GuaB* and Shiga toxin, all of which were fused to a backbone protein (IpaD) using an epitope- and structure-based multiepitope fusion antigen (MEFA) vaccinology platform. Based on the above data, an upgraded and optimized *Shigella* MEFA was created, featuring a total of 11 functional epitopes (2 from *IpaD*, 3 from *IpaB*, *VirG*, *Stx1A*, *Stx2A*, and *Stx2B*). We have mapped each gene (*IpaB*, *IpaD*, *VirG*, *Stx1*, and *Stx2*) to identify the most functional epitope of each gene. Total (approx. 10 sequences) epitopes were predicted for each target gene in silico and subsequently tested in mice for their optimal functional capacity employing respective functional assays with serum. This functional epitope listing helped us to develop an upgraded *Shigella* MEFA with top-ranking functional epitopes on the backbone of *IpaD*. Results so far indicate a successful purification of the optimized *Shigella* MEFA, as evidenced by an optimal Western blot. The pulmonary lethal challenge study shows ~99.00% protective efficacy of the optimized MEFA protein against *Shigella flexneri* 2a and *Shigella sonnei*. Undergoing work involves analyzing samples collected for ELISA and functional assays to draw future conclusions.

Key Words: protein, vaccine

General Abstracts: Virology—Posters

P179 - Functional assessment of swine *ANP32* variants on avian influenza virus polymerase activity using *in vitro* models.

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Domestic swine are key intermediate hosts in the generation of mammalian-adapted avian influenza viruses (AIV). The ability of swine to be infected by AIV has been attributed to multiple host factors. Specifically, unique substitutions within the swine acidic leucine-rich nuclear phosphoprotein 32 family member A (*ANP32A*) protein have been shown to increase its proviral capacity for AIV compared with other mammalian orthologs. Despite extensive characterization of interspecific differences, a survey of intraspecific variation in swine *ANP32* genes and effect on AIV permissiveness has not been reported. Therefore, the overall goal of this research is to identify natural variants within swine *ANP32A/B* genes that influence AIV pro-viral capacity using high-throughput parallel sequencing and in vitro functional assays. Targeted sequencing of *ANP32A* from 12 DNA pools representing multiple genetic lines and domestic breeds (n ≈ 120) was performed using the Oxford Nanopore MinION platform. Variant calling and visualization of aligned reads revealed limited variation within the *ANP32A* coding region. Additional comparison of publicly available sequences, representing diverse European (n = 67) and Asian (n = 59) domestic breeds and wild boar (n = 45), corroborated the limited variation observed within the *ANP32A* coding region as well as *ANP32B*. However, visualization of RNA sequencing alignments from wild boar (n = 5) and domestic pigs (n = 11) revealed at least 3 *ANP32A* isoforms, one of which has not been previously reported and is distinct from any known human isoform. CRISPR-Cas9 gene editing of the newborn pig trachea (NPTr) cell line was used to generate an *ANP32A* knockout clone for functional assessment of the novel swine isoform using the Influenza minigenome reporter system. Preliminary testing in wildtype and knockout NPTr cells with or without exogenous *ANP32A* expression detected clear differences in viral polymerase activity between treatment groups, demonstrating successful implementation of the minigenome system. The outcomes of this research have the potential to benefit swine producers directly by improving animal welfare, but also human health by limiting the ability of swine to serve as AIV reservoirs.

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Key Words: swine, *ANP32*, host genetics, pro-viral, avian influenza virus, minigenome

P180 - Sex-specific effects of obesity on fecal microbiota during acute low- and high-dose influenza A virus infection in mice.

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Obesity is associated with alterations in gut microbiomes, which affect influenza pathogenesis. The objective of this study was to determine whether biological sex and obesity impact the fecal microbiota alterations during influenza A virus (IAV) infection. Diet-induced non-obese and obese male and female mice were infected either with a high-dose () or a low-dose ($10^{1.5}$ $TCID_{50}$) of A/California/04/2009 H1N1 IAV. Fecal pellets were collected before infection (i.e., baseline) and at 3 d post-infection (dpi), and the V3-V4 region of the 16S rRNA gene was amplified and sequenced (Illumina MiSeq). Raw reads were processed using QIIME2, and taxonomy was assigned (SILVA database). First, we compared the sex-specific effects of obesity. Before virus infection, obese males had significantly higher *Firmicutes* and F/B ratio; lower *Actinobacteria*; and different β diversity compared with non-obese males. In females, obesity was associated with significantly lower *Actinobacteria*, higher *Verrucomicrobiota*, and different β diversity. After a high-dose infection, no significant phylum-level changes from baseline were observed at 3 dpi in males. In non-obese females, *Actinobacteria* were significantly decreased; Shannon diversity increased, and β diversity differed. However, in obese females, *Proteobacteria* were significantly decreased. After a low-dose infection, in non-obese males, *Firmicutes* showed a higher trend at 3 dpi, whereas obese males had significantly decreased *Actinobacteria* and F/B ratio, and increased *Verrucomicrobiota*. We also compared biological sex differences. At baseline, only β diversity was significantly different between non-obese males and females. However, obese males had increased *Firmicutes* and *Actinobacteria*; lower *Bacteroidota* and *Verrucomicrobiota*; and different β diversity compared with the obese females. After a high-dose infection, at 3 dpi, non-obese males only showed significant differences in β diversity compared with non-obese females, whereas no such differences were observed in obese males and females. After a low-dose infection, at 3 dpi, fecal microbiota were comparable between non-obese males and females. However, obese males, compared with females, had significantly increased *Firmicutes*, *Actinobacteria*, and F/B ratio; reduced *Verrucomicrobiota*; and different β diversity. Our data suggest that both sex and obesity influence alterations in gut microbiota during the acute phase of low- and high-dose IAV infection.

Key Words: diet-induced obesity, influenza A virus, gut microbiota, sex difference

P181 - Determining the prevalence of influenza A in bovine species through competitive enzyme-linked immunosorbent assay and polymerase chain reaction methods.

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Recent outbreaks of H5N1 highly pathogenic avian influenza A virus (HPAI) in ruminant species, especially dairy cows in the United States have caused various public and veterinary health concerns. Infection in dairy cows can result in severe diseases, and the virus may infect many other ruminant species. This study investigated the seroprevalence of antibodies against H5N1 in ruminants submitted for diagnostic testing through the Kansas Veterinary Diagnostic Laboratory. A total of 1,237 ruminant serum samples were collected between 2023 and 2024 including 641 samples from dairy cows and 596 from goats, sheep, and deer. The test was performed using 2 commercial competitive enzyme-linked immunosorbent assay (ELISA) testing kits, A and B. Additionally, polymerase chain reaction (PCR) tests for HPAI targeting the M-gene were performed on milk and nasal swabs from dairy cows. Among all serum samples, 0.40% (5/1,237) tested positive on test A, while 0% were positive on test B. Of the dairy cows, 0.31% (2/641) were positive on test A; none tested positive on test B. Among goats, sheep, and deer, 0.50% (3/596) were positive on test A, and 0% on test B. In PCR testing, 0.08% (3/3,941) of samples from dairy cows returned positive results. The results demonstrate that HPAI virus can be detected in ruminants by PCR and antibodies to influenza A can be determined by ELISA in different samples. With the 2 ELISA testing kits, a difference in sensitivity was found, highlighting the importance of using a test with an adequate sensitivity rate for surveillance testing. The difference in the PCR results and the ELISA results indicate a higher prevalence of active influenza infection verses animas that have seroconverted. This highlights the importance for continuing surveillance for HPAI in ruminant species, particularly dairy cows, as viral adaptations could increase zoonotic potential and increase spread of the virus.

Financial Support: Boehringer Ingelheim Veterinary Scholars Program, IDVET, NAHLN, VD

Key Words: avian influenza A, highly pathogenic avian influenza A virus, bovine, seroprevalence, enzyme-linked immunosorbent assay, polymerase chain reaction

P182 - Determining the prevalence of influenza A in feline species through competitive enzyme-linked immunosorbent assay and polymerase chain reaction methods.

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Unanticipated outbreaks since March 2024 of H5N1 highly pathogenic avian influenza virus (HPAI) in feline species in the United States has caused various veterinary and public health concerns. Infections result in a high mortality rate in felines, also facilitating mutations and possible cross-species transmission. This study aimed to investigate the seroprevalence of antibodies against H5N1 and presence of viral RNA in feline species submitted for diagnostic testing through the Kansas Veterinary Diagnostic Laboratory. A total of 617 feline serums were collected in 2023 to 2024 and analyzed for the presence of Avian Influenza A antibodies. The test was performed using 2 commercial competitive enzyme-linked immunosorbent assay (ELISA) testing kits, A and B. In addition, polymerase chain reaction (PCR) tests for Avian influenza A virus targeting the M-gene were performed on 225 feline brain tissue samples submitted due to neurological signs between 2023 and 2024. Of the 617 feline samples, 3 tested positive on test A (0.49%) and 1 tested positive on test B (0.16%). In the test detection of M-gene, 10 out of 225 (4.44%) showed a positive result. The results demonstrate that HPAI can be detected in feline species by both ELISA and PCR. The findings highlight the importance of continuing surveillance, especially due to the virus's high mortality rate in felines and increased risk of zoonotic transmission. Moreover, the higher detection rate by PCR compared with ELISA supports the hypothesis that many cats may succumb to infection before seroconversion is able to occur.

Financial Support: Boehringer Ingelheim Veterinary Scholars Program, IDVET, NAHLN, VDL

Key Words: avian influenza A, highly pathogenic avian influenza virus, feline, seroprevalence, enzyme-linked immunosorbent assay, polymerase chain reaction

P184 - Assessing SARS-CoV-2 spike variation on ACE2 species' tropism and implications for spillover.

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During the most severe acute respiratory syndrome cornavirus-2 (SARS-CoV-2) pandemic, secondary spillover of from humans to animals and back to humans was reported between domestic mink and mink farm workers. With recent studies indicating a high prevalence of SARS-CoV-2 in North American white-tailed deer populations, there is a growing concern that this now endemic virus will accumulate mutations and transmit into additional animal reservoirs or spill back over into humans, potentially evading current COVID-19 vaccines and therapeutics. Therefore, understanding how species tropism shifts arise during spillover events is critical to vaccine design and disease management. Here, we apply our laboratory's unique in vitro pseudovirus entry platforms to test a panel of SARS-CoV-2 variants and permutations across ACE2 orthologs derived from common wildlife species found across North America and elsewhere, and show that RBD variation affects cell entry efficiency with ACE2 from different species. We perform side-by-side comparisons with classical entry assays and an alternative variation of the assay we have designed for improved sensitivity and scalability. Our results show the alternative assay performs similarly or better than the previous approach, and will subsequently be used in larger screens of permutations in the receptor binding domain predicted using rationally-developed machine-learning methodologies. Results from this work identifying species shifts in SARS-CoV-2 species receptor tropism will inform One Health approaches for tackling the threat of virus spillover, including the development of broad vaccination strategies driven by contact patterns among diverse wildlife and human hosts.

Key Words: coronavirus, virology, spillover, wildlife, tropism

P185 - Persistent circulation of extinct SARS-CoV-2 variants of concern in white-tailed deer.

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Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) has demonstrated an incredibly broad host range with the ability to infect many animal species. Among the susceptible species, white-tailed deer (WTD) seem to play an important role in the ecology and epidemiology of the virus potentially serving as a new wildlife reservoir for SARS-CoV-2 in North America. We have previously shown that WTD can carry SARS-CoV-2 variants of concern (VOC) such as α and gamma for up to 6 mo following their last detection in humans. Here we expanded the timeline of our previous cross-sectional study (conducted in the 2020–2021 [Season 1] and 2021–2022 [Season 2] hunting seasons) and tested lymph node samples collected from WTD in the state of New York during the 2022–2023 (Season 3, n = 2,668) and the 2023–2024 seasons (Season 4, n = 2,735). All samples were tested initially by SARS-CoV-2 RT-PCR, with virus RNA being detected in 5 samples from Season 3 (0.18%) and in 105 samples (3.83%) from Season 4. Complete genome sequence analysis revealed the circulation of several of the Omicron VOC sublineages circulating in humans around the same time frame in New York. Notable, 3 WTD samples collected during Season 4 were typed as Alpha (n = 1) or Delta (n = 2) VOCs, which are SARS-CoV-2 VOCs that have been extinct from the human population for almost 2 years. Importantly, the Alpha VOC sequence detected in Season 4 was collected in November 2022 and was closely related to an Alpha VOC sequence that we detected in WTD in New York during Season 2, in July 2021. These 2 sequences were collected in Windsor and Sherburne counties, respectively, which are 50 miles apart and located in the southern tier region of New York. These results suggest continuous long-term circulation of SARS-CoV-2 in WTD populations in the United States, underscoring the importance of this animal species as a reservoir for the virus in North America.

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Key Words: SARS-CoV-2, white-tailed deer, reservoir, epidemiology

P186 - Phylodynamic analysis of bovine viral diarrhea virus in the Republic of Korea.

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Bovine viral diarrhea virus (BVDV) is a major pathogen of cattle industry worldwide, responsible for diarrhea, respiratory distress, immunosuppression, and reproductive abnormalities, leading to significant economic losses. However, to date, no studies have investigated the molecular epidemiology and evolutionary pattern of BVDV in the Republic of Korea (ROK). Therefore, we conducted a comprehensive study to assess the prevalence, circulating subtypes, and evolutionary dynamics of BVDV in cattle. A total of 5,286 samples, including serum, whole blood, and diarrheic feces, were collected from cattle across the ROK during 2021–2022. Samples were obtained through passive surveillance conducted by regional animal health laboratories. Viral RNA was detected using real-time RT-PCR targeting the conserved 5′UTR region. The associations between sample type and BVDV positivity were evaluated using chi-squared tests with Bonferroni correction. Among 5,286 samples collected in the ROK, 1,584 samples (29.9%) tested positive. The 3 subtypes were identified: BVDV-1a (16.5%), BVDV-1b (53.6%), and BVDV-2a (29.9%), with BVDV-1b being predominant. The serum showed the highest detection rate (36.9%), followed by feces (12.3%) and whole blood (9.6%) ($P < 0.001$). The evolutionary rates of BVDV-1 and BVDV-2a were estimated at 3.99×10^{-3} and 4.0×10^{-3} substitutions per site per year, respectively, using BEAST v1.7.2 with an uncorrelated lognormal relaxed clock model. Maximum clade credibility (MCC) trees were constructed in TreeAnnotator v1.7.2 and visualized using FigTree v1.4.4. The MCC tree based on the 5′UTR indicated that the most recent common ancestors of BVDV-1 and BVDV-2 in the ROK likely emerged around 1988 and 1995, respectively. Bayesian skyline plot analysis demonstrated a marked increase in the viral population of BVDV-1 around 2018, whereas BVDV-2a exhibited a more gradual expansion beginning around 2016. This study provides the first comprehensive evolutionary dynamics of BVDV in the ROK, revealing the predominance of BVDV-1b, the diagnostic advantage of serum, and evidence of rapid viral evolution. These results emphasize the need for continuous molecular surveillance and control strategies in the Korean cattle industry.

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Key Words: bovine viral diarrhea virus, evolutionary rate, phylodynamic analysis, subtype, Republic of Korea

P187 - Mucosal infection of CAST/EiJ mice with monkeypox virus.

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Mpox, a zoonotic disease caused by monkeypox virus (MPXV), primarily replicates in rodents and small mammals with occasional spillovers to humans. Historically, human-to-human transmission was limited and mainly occurred through close contact and respiratory droplets. However, during the 2022 global outbreak of MPXV clade IIb, sexual transmission, particularly among men who have sex with men, emerged as the predominant transmission route. Experimental studies investigating MPXV infection via rectal and genital mucosal routes are limited, and the need for a small animal model to better understand MPXV pathogenesis is critical. Here, we aim to investigate the susceptibility of CAST/EiJ mice, which are highly permissive to MPXV infection, to MPXV clade IIb infection via different mucosal routes. CAST/EiJ mice (mixed sex, n = 5 male, 5 female) were infected with 2×10^5 PFU of MPXV clade IIb via intraperitoneal or mucosal (intranasal, rectal, or genital) routes. Mice were monitored daily for clinical signs and weight changes, and swabs (oropharyngeal, rectal, and urogenital) were collected to assess viral shedding. At the end of study, mice were euthanized and necropsied, and various tissues and blood were collected for virological and histopathological analyses. Intraperitoneal infection of mice with MPXV produced non-lethal, mild clinical disease, particularly in male mice, which showed slight body weight loss from 5 to 10 d post-infection, corresponding to increased clinical scores marked by ruffled fur and subdued behavior; viral DNA was also detected in oropharyngeal swabs during this period. In contrast, mucosal inoculation via intranasal, rectal, or genital routes did not induce obvious clinical signs, as mice remained largely asymptomatic with no skin lesions and no to minimal body weight loss. However, viral DNA was consistently detected for at least 8 d in oropharyngeal, rectal, and genital swabs in the respective inoculation groups, except in male mice infected via the preputial route. The sustained recovery of viral DNA at the mucosal inoculation sites suggests active viral replication in the associated mucosal tissues. Histopathology, serology and analyses for infectious virus or viral DNA in collected tissues and organs will provide further insights into viral replication and dissemination throughout the animals following mucosal inoculation.

Financial Support: This study was supported by grant USDA ARS NACA #58-3022-3-004.

Key Words: monkeypox virus (MPXV), Mpox, mucosal infection, genital and rectal inoculation, CAST/EiJ mice

P188 - Experimental transmission of Japanese encephalitis virus by North American *Culex tarsalis* mosquitoes in a domestic swine model.

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Japanese encephalitis virus (JEV), a mosquito-borne flavivirus, is the leading causative agent of viral encephalitis in Asia and western Pacific countries. The virus persists in an enzootic cycle between mosquitoes and vertebrate hosts, primarily swine and ardeid birds. Swine play a critical role in JEV epidemiology due to their capacity to develop high viremia leading to significant mosquito infection and subsequent viral amplification. The Australian outbreak of JEV in 2022 demonstrated how swiftly the virus can establish in naïve regions when competent vectors and susceptible hosts are present. This emphasizes the concern that JEV introduction in North America could threaten public health and cause substantial economic losses in the swine industry. This work examines whether North American *Culex tarsalis* (*Cx. tarsalis*) mosquitoes can serve as a competent vector of JEV using a domestic swine model. Three sequential experiments were conducted to reproduce the complete transmission cycle. First *Cx. tarsalis* mosquitoes were fed artificial blood meal spiked with JEV genotype II and sampled to assess infection and dissemination. By 14 d post infection (DPI), infectious virus was detected in approximately 20% of salivary glands, indicating *Cx*. *tarsalis* can potentially transmit the virus. Next, JEV-fed mosquitoes were allowed to feed on naïve pigs, all of which developed viremia between 2- and 3 DPI. Lastly, naïve mosquitoes were allowed to feed on viremic pigs on 2- and 3 DPI. After 14 d, this resulted in an average infection rate of 22.5% and 58% of mosquitoes that fed from pigs at 2- and 3-DPI, respectively. Our findings demonstrate that North American *Cx*. *tarsalis* mosquitoes can acquire, maintain, and transmit JEV GII through domestic swine. These results provide direct experimental evidence of vector competence in a widespread North American mosquito species and susceptibility of native hosts. This highlights risk for JEV emergence in the United States, where competent vectors and amplifying hosts coincide and establishes a valuable transmission model for future studies of JEV dynamics.

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P189 - Japanese encephalitis virus: Potential for establishment in North America.

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Japanese encephalitis virus (JEV) is a mosquito-borne arbovirus belonging to the family *Flaviviridae*. The transmission cycle involves multiple species of mosquito vectors and vertebrate hosts. In endemic regions, JEV is typically maintained in a cycle between *Culex tritaeniorhynchus* and amplifying hosts such as avian and swine species, with potentially fatal infections in dead-end hosts including humans and equines. Over 2 billion people live in the endemic area, with an estimated 20,000 deaths occurring despite the widespread use of highly efficacious vaccines. In addition to the endemic vector *Cx. tritaeniorhynchus*, other *Culex* species mosquitoes such as *Culex quinquefasciatus* and *Culex pipiens* have also been demonstrated to be capable of transmitting JEV. Similar to West Nile virus, another member of the JEV serocomplex, JEV has an exceptionally wide host range and competent vector species. If introduced into non-endemic areas, it would likely be almost impossible for disease eradication following the establishment of an enzootic transmission cycle between asymptomatic amplification hosts and competent vectors. Our research has demonstrated the susceptibility of North American mosquitoes to be infected with JEV, their ability to transmit the virus, and the susceptibility of both domesticated and feral phenotype American pigs to infection with JEV. Here we discuss the potential for the introduction and establishment of JEV into new regions where competent mosquito vectors and susceptible vertebrate hosts occur.

Financial Support: State of Kansas National Bio and Agro-Defense Facility Transition Fund awarded to Dana L. Vanlandingham

Key Words: arbovirus, Japanese encephalitis virus, swine

P190 - Genotypic diversity of VP4 and VP7 in rotavirus A, B, and C detected in US swine.

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Rotavirus A, B, and C (RVA, RVB, RVC) remain significant contributors to enteric disease in swine. To define the current molecular landscape of these viruses in US herds, we analyzed 5,280 VP4 (P type) and VP7 (G type) gene sequences from diagnostic submissions to the Iowa State University Veterinary Diagnostic Laboratory. The data set included 1,070 VP4 sequences (344 RVA, 161 RVB, 565 RVC) collected between 2021 and 2024 and 4,210 VP7 sequences (1,413 RVA, 535 RVB, 2,262 RVC) collected from 2014 to 2024. Alignments were performed using MAFFT, followed by manual trimming in Geneious. Phylogenetic trees were constructed in IQ-TREE using model testing and ultrafast bootstrapping. Genotypes were assigned based on clustering with reference strains curated from NCBI GenBank and the USDA Swine Pathogen Database. The RVA strains were dominated by P[13] & P[23] (VP4) and G9 & G4 (VP7), RVB by P[4] and G16 & G20, and RVC by P[5] and G6. Based on the analyzed sequences, RVA was distributed across age groups, with a modest increase in grow-finish pigs; RVB was most common in suckling pigs but also present in neonates and older stages; and RVC was concentrated in suckling and neonatal pigs. Temporal trends revealed no evidence of genotype replacement. RVA P[13] increased from 38.7% to 50.4% between 2021 and 2024, whereas P[23] decreased slightly and stabilized. RVB P[4] increased from 60% to 80%. RVC P[5] remained stable (73%–78%). From 2014 to 2024, G9 remained the dominant RVA genotype with fluctuating proportions (38%–58%), and G4 showed modest gains. RVB G16 increased from 34.5% to 57.8%, and RVC G6 consistently exceeded 60% prevalence. Phylogenetic trees showed well-supported clustering by genotype, with multiple lineages co-circulating across time. These results define a contemporary genomic baseline for porcine RVA, RVB, and RVC in US swine herds. The observed diversity has direct implications for molecular assay performance and vaccine composition, as certain genotypic shifts may affect diagnostic sensitivity or antigenic match. Expanding surveillance to include whole-genome data and epidemiological metadata will help monitor evolutionary dynamics and inform precision diagnostics and vaccine updates.

Key Words: rotavirus, swine, phylogenetics, genomic surveillance, molecular diagnostics

P191 - Evaluation of innovative emulsion and polymer adjuvants for inactivated H5N8 HPAI virus vaccine.

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This study investigates strategies for vaccinating against Highly Pathogenic Avian Influenza (HPAI), a major threat to the poultry industry. We evaluated the safety, immunogenicity, and efficacy of an inactivated reverse genetics-based H5N8 vaccine utilizing various adjuvant technologies. The H5N8 vaccine was tested using Montanide ISA 78 VG, Montanide ISA 71R VG (water-in-oil emulsion adjuvants), and Montanide GEL P PR (a polymer-based aqueous adjuvant), comparing them to a commercial vaccine (oily formulation). Four-week-old influenza antibody-negative chickens were inoculated subcutaneously with a 0.5 mL dose of the H5N8 vaccine, formulated with either ISA 78, ISA 71 R, GEL P, the commercial vaccine, or the antigen alone. Safety monitoring involved tracking clinical signs and weight for 35 d. Immunogenicity was assessed by measuring hemagglutination inhibition (HI) antibody titers on d 0, 7, 14, 21, and 28 post-vaccination. Efficacy was determined by challenging the vaccinated chickens with an H5N1 field virus isolate 35 d post-vaccination, followed by a 10-d observation for survival and viral shedding. All adjuvanted formulations were found to be safe, with no local or systemic reactions observed. Chickens vaccinated with ISA 78, ISA 71R, and GEL P developed significantly higher HI antibody titers compared with the control group ($P < 0.0001$). The ISA 78 group showed superior immunogenicity on d 21 and 28 compared with GEL P ($P = 0.0002$; $P < 0.0001$), ISA 71 R ($P = 0.0075$; $P < 0.0001$), and the commercial vaccine ($P < 0.0001$). Post-challenge, all vaccinated groups achieved 100% survival and demonstrated reduced viral shedding compared with the control group. In conclusion, the H5N8 vaccines formulated with Montanide ISA 78 VG, ISA 71 R VG, and GEL P PR elicited substantial humoral immune responses. Montanide ISA 78 VG, in particular, induced the highest antibody titers, highlighting its potential as a potent adjuvant candidate for HPAI vaccines. These results confirm that both oil- and polymer-based adjuvants are efficacious for HPAI vaccine formulation.

Key Words: poultry, influenza, vaccine, adjuvant

P192 - Evaluating the distribution and clustering of SARS-CoV-2 antibodies in dogs across the United States.

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SARS-CoV-2 has been detected in various animal species, including dogs. Although human-to-animal transmission has been documented, the extent and distribution of SARS-CoV-2 exposure in dogs across the United States remain unclear. To address this need, we investigated the seroprevalence of SARS-CoV-2 antibodies in dogs in the United States and scanned for spatial and temporal clusters of high seroprevalence. Serum samples from 953 dogs from 37 states were screened by serological assays, and 58 (6.09%) samples tested positive for SARS-CoV-2 antibodies. The lowest seropositivity was detected in the third quarter of 2021 (1.16%), and the highest was in the third and fourth quarters of 2024 (23.26%). Maps visualized the distribution of seroprevalence, and the Pacific Northwest and Florida had high seroprevalence, whereas the Northeast and Midwest United States had low seroprevalence. These regional differences were statistically significant and confirmed by Poisson regression and spatial scan statistics, with Washington and parts of California showing elevated seroprevalence compared with the Northeast and Midwest. Two significant space-time clusters were identified: the primary cluster in areas in the state of Washington in October 2024, and the secondary cluster in Florida in March 2022. These findings highlight the importance of surveillance to understand SARS-CoV-2 epidemiology in companion animals and its implications for public health.

Key Words: SARS-CoV-2, dog, seroepidemiologic study, clustering

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