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&
Presentation Abstracts

103rd Conference of Research Workers in Animal Diseases

January 22-24, 2023

Chicago Marriott Downtown Magnificent Mile

Chicago, IL

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CRWAD 2023 President's Message

January 2023

Welcome to the 103rd Conference of Research Workers in Animal Diseases! It is my great honor and privilege to serve as this year's CRWAD President. Like you I look forward to participating in this year's outstanding Conference.

Despite the ongoing challenges of the COVID pandemic, I am very pleased that we can again meet in person in Chicago. Seeing and talking with old friends and making new acquaintances is an important part of the Conference. I am also pleased that we can again offer a virtual meeting option for those who are unable to attend in person. I remind you that the full content of the onsite program, and the virtual abstract presentations, are available for all registered attendees to access from now until June 2023.

I want to thank the CRWAD Council members, and especially the program committee, for organizing this year's excellent program. Special thanks to Dr. Paul Morley, CRWAD Executive Director, Jennifer Stalley (Midwest Solutions), and our program committee co-chairs Dr. Brandy Burgess and Dr. Heather Wilson for their tireless efforts to organize the program you will enjoy during the next few days. This year's meeting features 450 abstracts, by scientists from 22 different countries, on a wide array of topics relevant to animal and human health.

This year's Conference is of special significance to me, as it will be the 40th consecutive year that I have attended CRWAD. When I joined the faculty of the new UW-Madison School of Veterinary Medicine in 1983, my Chair, Dr. Ron Schultz (2018 CRWAD Dedicatee), encouraged me and my colleagues to attend. This began an ongoing relationship with CRWAD, and its attendees, which has been of critical professional importance to me. I am grateful for the professional connections that I made, collaborative relationships that were established, and friendships that were forged over the years.

Year after year I find that CRWAD offers terrific value as a venue for animal health researchers. CRWAD brings together a diverse group of researchers from academia, industry, and government agencies in a unique forum at which one can learn about the latest animal health research findings and discuss their potential impact on prevention and regulation of animal diseases. In addition, CRWAD provides an invaluable opportunity for students, post-doctoral trainees, and junior scientists to present their research findings, and begin to network with other scientists. Students have the opportunity to give a talk at CRWAD and receive feedback on their work; an invaluable experience that is increasingly rare for students at most scientific conferences.

As my academic career draws to a close it has been my distinct pleasure to serve as CRWAD President and offer my perspectives on the organization and this year's Conference. I hope you find this year's Conference, and what I hope will be many future CRWAD meetings, to be both valuable and enjoyable.

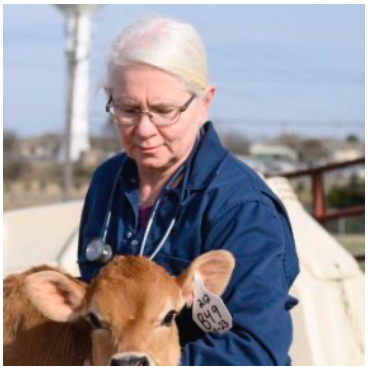
Chuck Czuprynski, PhD, AVCM Honorary Diplomate
CRWAD President

CRWAD 2023 Featured Speakers

"Adrift in the Viroshere: animal health in the age of viral metagenomics"
Tony L. Goldberg, DVM, PhD
CRWAD Keynote Speaker
Department of Pathobiological Sciences
University of Wisconsin – Madison
Sunday, 1/22/2023 2:00 PM

"Veterinary epidemiology and animal health economics: the way we were"
Tim Carpenter, PhD, MS
AVEPM Calvin Schwabe Award
University of California, Davis
Sunday, 1/22/2023, 8:30 AM

"Development of "intelligently-designed" vaccines based on understanding of bacterial pathogenesis and host response"
Thomas Inzana, PhD
ACVM Distinguished Microbiologist
Long Island University
Monday, 1/23/2023, 2:00 PM



"Applied research to enhance resistance to respiratory disease in livestock"
Amelia Woolums, PhD, MS, DVM
AAVI Distinguished Veterinary Immunologist
Mississippi University
Tuesday, 1/24/2023, 8:30 AM

CRWAD 2023 Featured Speakers

"The Impact of COVID on Agricultural Research"
COVID's Impact on Agricultural Research Special Symposium
G. Cliff Lamb, MS, PhD
North American Meat Institute
Sunday, 1/22/2023, 3:00 PM

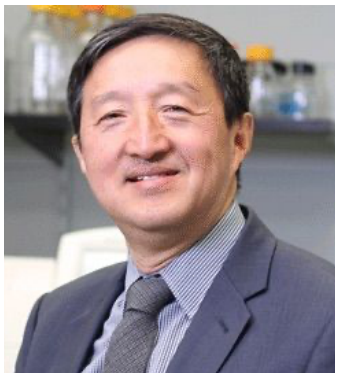
"What Happened? COVID-19 and the Meat Industry"
COVID's Impact on Agricultural Research Special Symposium
KatieRose McCullough, PhD, MPH
North American Meat Institute
Sunday, 1/22/2023, 4:15 PM

"Conducting research during a global pandemic: musings of an early career scientist"
Noelle Noyes, MA, DVM, PhD
Precision Agriculture and Animal Health Special Symposium
University of Minnesota College of Veterinary Medicine
Sunday, 1/22/2023 5:00 PM

CRWAD 2023 Featured Speakers

"Inflammation and innate immune tolerance in the pathogenesis of bovine respiratory disease and Mycoplasma bovis pneumonia"
Jeff Caswell, DVM, PhD, DVSc
ACVM Featured Speaker
Ontario Veterinary College
University of Guelph
Monday, 1/23/2023, 2:45 PM

"Strategies for Preventing Rhodococcal Foal Pneumonia"
Noah Cohen, PhD, MPH, VDM
AAVI Featured Speaker
Texas A&M
Tuesday, 1/24/2023, 10:30 AM



"A new era of vaccinology – COVID and beyond"
Shan Lu, MD, PhD, MHA
Vaccine Network Featured Speaker
Department of Medicine
University of Massachusetts Medical School
Monday, 1/23/2023, 8:30 AM

"Immunomodulation strategies to prevent and reduce bovine respiratory syncytial virus infection in preweaned calves"
Jodi McGill, PhD, MS
ACVM Featured Speaker
College of Veterinary Medicine
Iowa State University
Monday, 1/23/2023, 5:00 PM

CRWAD 2023 Featured Speakers

"3D organotypic cell cultures – an alternative ex vivo infection model for animal research"
Rahul Nelli, BVSc & AH, MVM, PhD
AAVI Featured Speaker
Iowa State University
Tuesday, 1/24/2023, 9:15 AM

"Time-release microneedle-based vaccine platforms"
Thang Nguyen, PhD
Vaccine Network Featured Speaker
Institute of Materials Science
University of Connecticut
Monday, 1/23/2023, 9:15 AM

"Challenges on developing a vaccine for protection against Mycoplasma bovis respiratory disease in cattle"
Jose Perez-Casal, PhD
ACVM Featured Speaker
Western College of Veterinary Medicine
University of Saskatchewan
Monday, 1/23/2023, 4:15 PM

"The state of play of veterinary economics: where we have been, where we are, and where we still need to go"
Karl Rich, PhD, MS
AVEPM Featured Speaker
Oklahoma State University
Sunday, 1/22/2023, 9:15 AM

CRWAD 2023 Featured Speakers

"Emerging technology and potential application in animal health"
Michael Roof, PhD
Vaccine Network Featured Speaker
State of Iowa Bioscience Initiative
Monday, 1/23/2023, 10:30 AM

"Highly pathogenic avian influenza in the US and approaches to control"
Erica Spackman, PhD, MS
AAVI Featured Speaker
USDA ARS
Tuesday, 1/24/2023 11:15 AM

"Veterinary epidemiology: A vision of the future"
Mark Stevenson, PhD, MPH
AVEPM Featured Speaker
Melbourne School of Design
Sunday, 1/22/2023 11:15 AM

"Spatial Epidemiology: Pushing the Boundaries"
Michael Ward, BVSc (Hons 1), MSc, MPVM, PhD, FACVSc, DVSc
AVEPM Featured Speaker
University of Sydney
Sunday, 1/22/2023 10:30 AM

CRWAD Fellows

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

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

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

CRWAD is pleased to announce the 2023 Fellow Inductees:

David Benfield, MS, PhD
Emeritus Professor Dept of Animal Sciences, Center for Food Animal Health, Ohio State University

Carol Chitko-McKown, PhD
AHGRU, U.S. Meat Animal Research Center, ARS-USDA, Clay Center, Nebraska

Robert Ellis, MS, PhD, CBSP, DACM, DACVM (Hon.)
Emeritus Professor, Colorado State University

Laurel Gershwin, DVM, PhD, DACVM
Dept of Pathology, Microbiology, and Immunology, University of California, Davis

Joan Lunney, PhD
USDA-ARS Beltsville, MD

David Renter, DVM, PhD
College of Veterinary Medicine, Kansas State University

Patricia Shewen, DVM, Msc, PhD
Professor Emerita, University of Guelph, Ontario

Subramaniam Srikumaran, BVSc, MS, PhD
Professor Emeritus, College of Veterinary Medicine, Washington State University

Thomas Wittum, MS, PhD
Ohio State University, College of Veterinary Medicine, Dept of Veterinary Preventive Medicine

2023 CRWAD Fellows Inductees

David Benfield, MS, PhD
Emeritus Professor Dept of Animal Sciences, Center for Food Animal Health, Ohio State University

Carol Chitko-McKown, PhD
AHGRU, U.S. Meat Animal Research Center, ARS-USDA, Clay Center, Nebraska

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Dept of Pathology, Microbiology, and Immunology, University of California, Davis

Joan Lunney, PhD
USDA-ARS Beltsville, MD

David Renter, DVM, PhD
College of Veterinary Medicine, Kansas State University

Patricia Shewen, DVM, Msc, PhD
Professor Emerita, University of Guelph, Ontario

Subramaniam Srikumaran, BVSc, MS, PhD
Professor Emeritus, College of Veterinary Medicine, Washington State University

Thomas Wittum, MS, PhD
Ohio State University, College of Veterinary Medicine, Dept of Veterinary Preventive Medicine

Please visit https://crwad.org/fellows_directory/ for biographical information about 2023 CRWAD Fellows Inductees and the past Inductees.

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1 - Veterinary epidemiology and animal health economics: the way we were

T. Carpenter
University of California Davis. tecarpenter@ucdavis.edu
Session: AVEPM - Schwabe Symposium, 01/22/2023, 08:30 - 09:15

Veterinary epidemiology and animal health economics have progressed much in the last 50 years. In this session, we will present a retrospective look at the "state of the art", beginning in the 1970s and conclude with a prospective look into where we might be going in these fields. Our focus in veterinary epidemiology will be limited to epidemiologic modeling and spatial epidemiology. In this specific presentation, I will focus on my personal research over the past five decades, constraints facing us and limitations we were able to overcome, in an attempt to push the existing boundaries of science.

The 1970s were a decade fraught with challenges, conflicts, and seemingly boundless dreams. The United States had recently succeeded in sending a man to the moon, using computing power far inferior to today's ubiquitous smartphone. Later in that decade and into the early 80s, personal computers, including portable computers, "loaded" with spreadsheet and word processing software, were enabling individuals to be free of constraints associated with mainframe computers. Epidemiologic modeling was in its infancy and very much following advances made in weather forecast modeling. Spatial epidemiology additionally benefited greatly from the availability of precise satellite spatial data, sparking the genesis of mapping programs capable of displaying and quantifying complex epidemiologic data. In the meantime, animal health economics was legitimized after the first meeting of what was to be known as the International Symposium on Veterinary Epidemiology and Economics in 1976. However, it would be many years before research involving animal health economics would be welcomed into the scientific literature.

Despite the challenges facing researchers in veterinary epidemiology and economics, advances were made using, what today would be considered, relatively limited resources. Also, along the way, researchers have made notable contributions by combining the apparently disparate disciplines of modeling, spatial analysis and economics. In this presentation, I will highlight some of these contributions, in an attempt to demonstrate what has been done until now. Later in this session, you will hear what scientific breakthroughs in veterinary epidemiology and animal health economics might occur in the future.

Notes:

2 - The state of play of veterinary economics: where we have been, where we are, and where we still need to go

K. Rich
Ferguson College of Agriculture, Oklahoma State University. karl.rich@okstate.edu
Session: AVEPM - Schwabe Symposium, 01/22/2023, 09:15 - 10:00

Veterinary economics has evolved markedly over the past several decades, aided by both the growing collaboration of veterinarians with social scientists and enhancements in computational power in computers. Pioneers such as Dr. Tim Carpenter, who pushed the concepts and intuition of economic cost-benefit analysis and the early use of spreadsheet techniques, have been instrumental in spearheading this increased collaboration between technical and social science, leading towards a process of better, more comprehensive analyses and ostensibly more effective disease control strategies in practice. At the same time, while economic contributions to veterinary science have evolved from an analytical perspective, more work remains on framing the nuances associated with animal disease outbreaks, specifically from the standpoint of understanding stakeholder decision making and behavior which often undermine control efforts in non-intuitive ways. This presentation will walk through a roadmap of the evolution of advances in veterinary economics and highlight an agenda for both researchers and practitioners to utilize much of the untapped potential of economics and social science in unpacking the complexities and subtleties associated with behavioral drivers of animal disease.

Notes:

3 - Dietary oligosaccharides modulate microbiome, enteric glia, and epithelial barrier function in suckling pigs

A. Ziegler[1], C. Deck[1], M. Caldwell[1], A. Sheridan[1], S. Erwin[1], B. Wieland[1], A. Hattenhauer[1], C. Mariant[1], L. Van Landeghem[1], J. Odle[1], A. Blikslager[1]
[1]North Carolina State University. amanda_ziegler@ncsu.edu
Session: General Health & Physiology 1, 1/22/2022, 08:30 - 08:45

Objective
In our pig intestinal ischemia model, a neonatal defect in epithelial barrier restitution can be rescued by direct application of mucosa of weaned pigs which contains a more mature network of enteric glial cells (EGC), known drivers of restitution. EGC network maturation is driven by microbial colonization which can be modulated with dietary prebiotic fiber. Therefore, we hypothesized that dietary oligosaccharide supplementation accelerates postnatal microbial colonization and EGC network maturation, thus enhancing restitution.

Methods
Suckling piglets were fed control (n=8) or prebiotic-supplemented (n=8) formula and samples were collected for 16S rDNA sequencing, western blot, imaging, and EGC culture. Migration abilities, calcium responses to ATP, paracrine effects on IPEC-J2 cell restitution, and protein secretome were assessed in colonic EGC cultures.

Results
Colonic microbial taxa changed in a time- and diet-dependent manner with the prebiotic-fed taxa clustering by day 7 and becoming progressively more tightly clustered over time (P<.050). Surprisingly, prebiotic-fed colon had lower levels of the EGC markers glial fibrillary acidic protein (GFAP) and S-100B at day 21 (P<.050), and subjectively reduced density of GFAP+ and S-100B+ subepithelial EGC in preliminary volume imaging of prebiotic-fed colonic mucosa at days 14 and 21. Furthermore, EGC from prebiotic-fed colonic submucosa showed decreased chemotactic motility toward sterile filtered colonic contents (P=.010), and decreased intracellular calcium response to ATP (P=.0075). However, prebiotic-fed porcine colonic glia enhanced IPEC-J2 restitution versus IPEC-J2 cells alone (P=.032). In addition, prebiotic-fed colonic submucosal EGC differentially secreted 13 proteins of interest versus control-fed.

Conclusions
Dietary oligosaccharides in neonates exert important effects on colonic EGC network development and phenotype, and on epithelial restitution *in vitro*. Ongoing work to understand microbiome-EGC-epithelial interactions during postnatal development may lead to novel management practices to improve health in suckling pigs.

Financial Support
U.S. National Institute of Child Health and Human Development; U.S. Department of Agriculture, National Institute for Food and Agriculture; U.S. National Institute of Diabetes and Digestive and Kidney Diseases; U.S. National Institutes of Health Office of Research Infrastructure Programs

Notes:

4 - Effect of commingling and dietary fiber interventions on select performance metrics of suckling and weaned pigs

T.N. Gaire[1], T. Wehri[1], D. Brown[1], C. Snow[1], J.C. Hsu[2], I.B. Slizovskiy[1], O.J. Lopez[1], G.R. Diaz [1], M. Meneguzzi[1], J.G. Young[1], C.F. De Abreu[1], A. Canturri[1], F. Pena Mosca[1], M. Medrano[1], L. Miller[1], S. Li[1], M. Ruch[1], M. Schwartz[1], R. Singer[3], M. Pieters[1], N.R. Noyes[1]
[1]Department of Veterinary Population Medicine, University of Minnesota, [2]Center for Animal Health and Food Safety, University of Minnesota, [3]Department of Veterinary and Biomedical Sciences, University of Minnesota. tgaire@umn.edu
Session: General Health & Physiology 1, 1/22/2022, 08:45 - 09:00

Objective
As part of a multi-year study, this study aimed to understand whether commingling and dietary fiber would also impact important production outcomes.

Methods
A total of 84 sows were included in this 2x2 factorial design randomized control trial (commingled/ MSCC) vs. not commingled/ CONV and high- (HF) vs. low-fiber (LF) creep feed). From each litter, ten piglets were randomly selected and enrolled. At enrollment, each piglets were tagged, weighed, and then given a single dose of Excede (CCFA, at 100mg/mL, 0.5 mL IM/piglet, Zoetis Animal Health) and sampled by inserting a sterile swab into the rectum at 0-12h hpb and then daily until 2-4 days post-birth (dpb). After processing, MSCC treatment was initiated to enrolled litters within a room, where piglets were allowed to move freely between the farrowing crates, while piglets in the CONV group remained with their own sow. On days 17-18 of age, all enrolled piglets received Baytril (Enrofloxacin, 100 mg/mL, 0.35 mL IM/piglet, Elanco US Inc), after which creep feed was initiated, either standard feed or supplemented with potato starch depending on pre-allocated treatment group. At 23dpb, all piglets were weaned and moved to the nursery facility, where they were weighed upon arrival.

Results
A total of 84 sows and 833 piglets were enrolled in the study. Average body weight of enrolled piglets was not significantly different between MSCC and CONV groups at birth (MSCC: 1.4±0.3 kg, CONV: 1.5±0.3 kg, P=0.12) and at weaning (MSCC: 5.9±1.5 kg, CONV: 6.2±1.6 kg, P=0.09) with no significant interaction between commingling and dietary treatment on average weaning weight (P= 0.29). Average piglet mortality rate was 14% with the MSCC/HF group had the lowest mortality (~10%) while MSCC/LF and CONV/LF both had the highest mortality (both ~15%).

Conclusions
Overall for the larger study, 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 antibiotic exposures, while supporting overall performance in production pigs.

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

Notes:

5 - Rapid rumen microbiome change driven by weaning strategy on beef calves

G.R. Diaz [1], T.N. Gaire[1], M. Daneshi[1], P. Ferm[1], I.B. Slizovskiy[1], T. Goldsmith[1], L. Caixeta[1], J. Armstrong[2], N.R. Noyes[1]
[1]Department of Veterinary Population Medicine, University of Minnesota, [2]University of Minnesota Extension.
diazo005@umn.edu
Session: General Health & Physiology 1, 1/22/2022, 09:00 - 09:15

Objective
Several management practices along the productive cycle of beef cattle may impact the development of the microbial community (microbiome) of the rumen, especially those at early stages. Thus, this study assessed the impact of different castration and weaning strategies over the rumen microbiome of beef calves until weaning.

Methods
We conducted a longitudinal study with 32 beef calves randomly assigned to 2 different weaning (fence-line or truck weaning) and 4 different castration strategies (Castration at birth, turn-out, pre-weaning or weaning) in "2x4" factorial design. Rumen fluid samples were collected at 3 time-points: 1 month before weaning (pre-weaning), at weaning and 2 days after weaning (post-weaning). DNA was extracted and submitted for shotgun metagenomic sequencing to study the rumen microbiome composition in terms of: (1) bacterial relative abundance and (2) bacterial diversity within (alpha-diversity) and between (beta-diversity) calves' rumen. The association between weaning and castration strategies and changes in the rumen microbiome were tested using a mixed-effect model.

Results
Our results showed that: (1) Overall, the most abundant phyla across calves along time were *Bacteroidetes* (40.8% ± 8.4), *Firmicutes* (28.6% ± 8.98), *Proteobacteria* (10.8% ± 1.53) and *Fibrobacteres* (9.06% ± 5.51). After weaning, *Bacteroidetes* and *Fibrobacteres* increased while *Firmicutes* decreased, especially evidenced in the truck-weaned calves. (2) Decreasing in alpha diversity indices (Shannon and Pielou's) was significantly associated to weaning strategy ($p<0.01$), especially evidenced 2 days after weaning in the truck-weaned calves. (3) Rumen microbiome diversity between calves (beta-diversity) was mainly explained by collection day (43.2%, $p<0.01$) and weaning strategy (39.4%, $p<0.01$).

Conclusions
These results may suggest that weaning strategy, rather than castration, has a bigger potential impact on the rumen microbiome of calves, with a particular low-diverse composition in calves weaned by truck. The long-term effects of our findings need to be further addressed in a longer follow-up period.

Financial Support
University of Minnesota; Minnesota Beef Council; Fulbright; CONCYTEC Peru

Notes:

6 - Behavioral and physiological changes associated with poor performance in late-fall toxic fescue grazing steers

I.M. Llada[1], J.M. Lourenco[1], M.M. Dycus[1], J.M. Carpenter[1], N.S. Hill[1], N.M. Filipov[1]
[1]University of Georgia. ignacio.llada@uga.edu
Session: General Health & Physiology 1, 1/22/2022, 09:15 - 09:30

Objective
Fescue toxicosis (FT) is the most important pasture-related pathology in US beef cattle caused by an ergot alkaloid (EA)-producing endophyte *Epichloë coenophiala*. The EAs impair thermoregulation and alter behavior in the summer leading to decreased productivity. However, the nature of toxic fescue grazing-climate interaction and its impact on animal behavior and performance during late fall is unknown and the main objective of the current research.

Methods
Eighteen steers were randomly placed on pastures containing nontoxic (NT), toxic (E+) and endophyte-free (E-) fescue (E-) for 28 days. Rectal temperature (RT) and body weights (BW) were measured at different time points. Surface (base of the tail) temperature (ST) was recorded continuously with a temperature sensor (iButton®) and animals' activity was recorded with sensors (IceTag®) attached to their hind limb. Environmental conditions were collected using data loggers (HOBO®) placed on the paddocks.

Results
Across the 28 days, steers on E+ gained about 60% less weight than the other two groups. Overall, E+ steers had higher RT and lower ST (39.5, 29.5; both in °C), than E- (39.3, 29.6), or NT (39.3, 30.9); E+ grazing steers also spent more time lying, less time standing and took more steps than the other groups. This activity pattern was most evident at sunrise, early afternoon, and sunset. However, during the coldest hours (

Conclusions
These data suggest that late fall E+ grazing impairs core and surface temperature regulation and it increases non-productive lying time, which may be partly responsible for the decreased weight gains. Interestingly, grazing NT fescue appears to be associated with improved thermoregulatory capacity. To gain new insights into FT pathogenesis across different grazing environments, we plan to integrate these data with multi-compartment metabolomics and microbiome responses to toxic fescue.

Financial Support
U.S. Department of Agriculture

Notes:

7 - Weather impacts cattle behavior during disease challenge

E. Rients[1], C.E. Franco[2], S. Hansen[1], J.L. McGill[2]
[1]Department of Animal Science, Iowa State University, [2]Department of Veterinary Microbiology and Preventive Medicine, Iowa State University. erients@iastate.edu
Session: General Health & Physiology 1, 1/22/2022, 09:30 - 09:45

Objective
Bovine respiratory disease (BRD) contributes nearly 3 billion dollars annually in losses in the beef industry and weather may contribute. In a study evaluating the effects of intranasal (IN) Zn and Vitamin A (VA) treatments on BRD progression conditions created an opportunity to evaluate how weather contributes to behavior during BRD challenge.

Methods
Angus crossbred steers (n = 48; 333 ± 4.2 kg), split into 2 groups (n = 24) with similar starting weights, were used in a 14 d BRD challenge study. Group 1 experienced average temperatures from 0.2 to 17°C and 13.9 cm of precipitation and group 2 experienced 8.8 to 24.7°C and 7.9 cm of precipitation. Steers had an ear motion detector tag (CowManager) to gather activity and ear surface temperature data. On d -1, steers were trucked for 8 hours. Steers were challenged on d 0 via aerosol inoculation with 10^4 $TCID_{50}$ BRSV followed by $5x10^8$ CFU of *Mannheimia hemolytica* on d 5. On d 4, steers received IN treatments of Zn, VA, Zn and VA, or control with no intranasal treatment.

Results
Steer activity and ear surface temperatures were not affected by IN treatments ($P \geq 0.41$) so group and day effects are presented (group × day for all measures $P \leq 0.01$). Total not active minutes increased over time and at a greater rate in group 2 ($P = 0.01$). Total minutes of high activity decreased over time ($P < 0.01$) with group 1 having 342 and 220 high active minutes on d 0 and 12, respectively and group 2 having 375 and 290 high active minutes on d 0 and 12, respectively. Group 1 had more changes in total ruminating minutes, peaking on d 5 and 10 at 330 minutes per day, while group 2 remained between 195 and 270 minutes per day. Environmental temperature increased ear surface temperature in group 2 ($P < 0.01$).

Conclusions
Steers will behave differently based on weather conditions in a disease challenge. In future development of management technologies for BRD detection, weather conditions may be important considerations for behavioral changes.

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

Notes:

8 - Effects of regional limb perfusion technique antibiotic levels achieved at the target site: a meta-analysis

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Session: General Health & Physiology 1, 1/22/2022, 09:45 - 10:00

Objective
Intravenous regional limb perfusions (RLP) are widely used in equine medicine to treat distal limb infections. RLPs are generally deemed successful if the peak antibiotic concentration (Cmax) in the sampled synovial structure is at least 8–10 times the minimum inhibitory concentration (MIC) for the bacteria of interest. Despite widespread clinical use, the optimal technique for performing a successful perfusion remains unclear. The objective of this meta-analysis was to examine the effect of technique on synovial concentrations of antibiotic and to assess under which conditions Cmax:MIC >10.

Methods
A literature search including the terms “horse”, “equine”, and “regional limb perfusion” between 1990 and 2021 was performed. Cmax and measures of dispersion were extracted from studies and Cmax:MIC was calculated for sensitive and resistant bacteria. Other extracted variables included synovial structure sampled, antibiotic dose, tourniquet location, tourniquet duration, general anesthesia versus standing sedation, perfusate volume, tourniquet type, and the concurrent use of local analgesia. Mixed effects meta-regression was performed, with Cmax:MIC as an outcome variable. Sensitivity analyses were performed to assess the robustness of our findings.

Results
Thirty-six studies with 123 arms (permutations of dose, route, location and timing) were included. Cmax:MIC ranged from 1 to 348 for sensitive bacteria and 0.25 to 87 for resistant bacteria. Summary values (θ) of 42.8 x MIC and 10.7 x MIC were generated for susceptible and resistant bacteria, respectively, but these were not considered reliable because of high heterogeneity among studies. On meta-regression, the only variables producing statistically significant differences in outcome were the type of tourniquet and the concurrent use of local analgesia.

Conclusions
Our results suggest that wide rubber tourniquets and concurrent local analgesia should be strongly considered for use in RLP and that adequate therapeutic concentrations are often achieved across a variety of techniques for susceptible but not resistant pathogens.

Notes:

9 - Diversity and community composition of the shell microbiome of American lobster (*H. americanus*) in Atlantic Canada

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Session: Aquaculture, 1/22/2022, 08:30 - 08:45

Objective
Due to current knowledge gaps regarding the shell microbial community – proposedly a key factor in proliferation of the fast-spreading epizootic shell disease (ESD) – of American lobster (*H. americanus)* in Atlantic Canada, this study aims to describe and analyze the shell microbiome of lobsters sampled from several locations in New Brunswick, Nova Scotia and Prince Edward Island.

Methods
More than 150 lobster shell swab samples and associated data on biotic and abiotic factors were collected from four lobster fishing areas. Novel long-read, next-generation 16S rDNA amplicon sequencing (PacBio) of cuticle samples followed by bioinformatic analyses in Qiime2 identified the shell associated bacteria to species level. Diversity indices such as Shannon, Pielou, Berger-Parker and Chao1 assessed the microbial composition and diversity. Furthermore, multivariate analyses were applied to detect any patterns in microbial species' abundances, composition or distribution based on biotic and abiotic factors.

Results
Two bacterial taxa, *Aquimarina* and *Thalassobius* which are thought to play a causative role in the development of ESD, were detected shell microbiome across all sampling events. The analyses of both alpha and beta diversities showed that the overall microbial composition differed by sampling location and sampling time but not by lobster sex. A Bray-Curtis ordination demonstrated that the shell microbial composition is more similar between samples from the same sampling location.

Conclusions
This study provides novel insights on the factors influencing the lobster shell microbiome which are important to predict the risk of ESD outbreaks in Atlantic Canada and in turn will encourage the development of suitable fisheries management strategies in the future.

Financial Support
Ocean Frontier Institute; Atlantic Veterinary College Research Fund

Notes:

10 - Boosting copper toxicity towards *Saprolegnia parasitica*

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Session: Aquaculture, 1/22/2022, 08:45 - 09:00

Objective
Saprolegnia parasitica (*Sp*) is a devastating oomycete pathogen that is responsible for significant losses in the production of freshwater fish. *Sp* is a major threat in the hatchery where outbreaks can lead to high mortality rates of fish embryos. Catfish, salmon and trout are particularly susceptible to *Sp* infection because of their longer hatching times. Alternative strategies and methods to combat *Sp* outbreaks in the hatchery are needed to boost production yields. Previous studies have identified copper sulfate as an effective agent in curving *Sp* growth and development. Encouraged by these observations, this study sought to investigate the ability of copper-binding ionophores to increase the antimicrobial activity of copper sulfate towards *Sp*.

Methods
We developed a screening platform using chemically defined media to assess, *in vitro*, the fidelity of select copper-binding agents to inhibit *S. parasitica* growth.

Results
Our screen has identified a group of small molecules including tetraethylthiuram disulfide (TDD), ciclopirox olamine (CLP), 2-mercaptopyridine N-oxide (MPO), 5-chloro-8-hydroxy-7-iodoquinoline (CHI), 5,7-dichloro-8-hydroxyquinoline (DHQ) and 8-quinolinol (8QN) which display antimicrobial activity that is dependent on extracellular copper concentration. These small molecules are effective at inhibiting *Sp* in the nM to low µM concentration range. We observe that MPO and CLP display the most robust antimicrobial activity with the inability to support *Sp* growth below 1 µM. However, the addition of the extracellular copper chelator, Bathocuproinedisulfonic acid, rescues this growth defect and restores *Sp* growth behavior.

Conclusions
In conclusion, our group has identified a selection of small molecules displaying robust antimicrobial activity whose mode of action is dependent on the level of bioavailable extracellular copper. Our current research directions are aimed at determining the mode of action of our identified ionophores to inhibit *S. parsitica* growth *in vitro* and to assess the ability of copper and ionophores to inhibit pathogen proliferation on catfish embryos.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture; Texas State University; National Science Foundation

Notes:

11 - Exploring the role of T3SS *sctV* gene in virulence of the oyster pathogen *Vibrio coralliilyticus*

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Session: Aquaculture, 1/22/2022, 09:00 - 09:15

Objective
Vibrio coralliilyticus RE22 is a pathogen of the eastern oyster *Crassostrea virginica* that causes high mortality rates in oyster hatcheries. We sequenced the RE22 genome and identified a wide array of virulence factors, including hemolysins, proteases, biofilm, and T1, T2, T3, and two T6 secretion systems. It is currently unknown if the RE22 T3SS plays a role in virulence against oyster larvae. To examine this, mutants targeting the *sctV* gene, the export gate of the T3SS system, were constructed and tested for virulence against oyster larvae.

Methods
Production of bacterial mutants: The T3SS of RE22 was annotated and structural genes were selected for mutation. Mutants containing a non-functional copy of *sctV* in pDM5 were prepared using Gibson Assembly and were transformed into *E. coli* Sm10. The donor strains with the construct were mated with RE22. Merodiploids were isolated on antibiotic plates and verified by PCR.
Oyster challenge assay: RE22 *sctV* mutant and wild type strains were grown overnight in marine medium at 27°C with shaking. Each strain was tested in triplicate with oyster larvae that were 5-7 days old. Larvae (5 mL in artificial seawater; 20 larvae/mL) were challenged with wild type or mutant strains (10^5 CFU/mL); sterile artificial seawater was used as a negative control. Oysters were fed an algal diet and assessed for survival after 20-24 hours.

Results
Over the course of 3 larval trials, oyster survival increased an average of 75% when challenged with the RE22 *sctV* mutant as compared to the wild type strain.

Conclusions
T3SS plays a significant role in the pathogenicity of RE22, as demonstrated in the reduction of virulence observed in the *sctV* knock-out strain. Interventions that target T3SS systems may help mitigate the impact of *Vibrio* outbreaks in aquaculture facilities. Ongoing efforts are examining the importance of three additional T3SS genes in RE22: *sctE* (major translocon), *sctA* (needle tip), and *sctC* (secretin ring).

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

Notes:

12 - Elucidation of virulence-associated proteins encoded by *Flavobacterium covae*, an important channel catfish pathogen

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Session: Aquaculture, 01/22/2023, 09:15 - 09:30

Objective
Flavobacterium covae is an important Gram-negative bacterial pathogen of channel catfish. *F. covae* had been recognized as *Flavobacterium columnare*-genomovar II but was recently separated out as separate species. The goals of this study are to identify *F. covae* virulence mechanisms and evaluate vaccine candidates through sequence analysis and mutagenesis studies.

Methods
This was done by: 1. Sequencing representative F. columnare genomes from different geographical locations and genetic groups using Nanopore methods. 2. Deleting critical genes in selected *F. covae* virulence-associated pathways.
This was done using PCR-based recombinational cloning of target gene flanking regions into the suicide vector pLYL001, transferring it into F. covae 94-081 by conjugation and passage to lose the vector.

Results
We sequenced 70 different whole genomes of the original *Flavobacterium columnare* group including 20 *F. covae* isolates. The isolates were provided by MSU diagnostic laboratory, Thomas Loch (Michigan State University), Ben Lafrentz (USDA-ARS), and Esteban Soto (UC Davis). Each sequence has over 100x coverage and represents 4-5 contigs. Comparative genomics and network analysis revealed cysteine and valine-leucine-Isoleucine biosynthesis pathways and *mazF-pemK*, *RelE-ParE* and zeta toxin are unique to virulent *F. covae* 94-081. To target these pathways for deletion production, we established suicide plasmids constructs that target the 23 genes representing these pathways. Conjugation transfers of these constructs have demonstrated the production of recombinant *F. covae*.

Conclusions
The sequenced genomes will be evaluated using artificial intelligence to help functionally identify putative genes and evaluate unique and shared sequences among the isolates. The virulence factor knock-out mutants will be evaluated for attenuation and ability to induce protection. Further, putative genes will be assessed to identify potential protective antigens using reverse vaccinology and tested by cloning the genes into expression systems and evaluated for the ability to induce protection in vaccinates.

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

Notes:

13 - Microcystin-LR exposure predisposes channel catfish to bacterial diseases

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Session: Aquaculture, 1/22/2022, 09:30 - 09:45

Objective
Cyanobacteria are common in catfish ponds and produce toxins that affect fish. The hepatoxin, microcystin-LR (MC-LR), is among the most common. Given the importance of the liver in the immune system, we hypothesize MC-LR exposure predisposes channel catfish to infectious diseases.

Methods
Catfish were given an IP dose of 500ng/g bw MC-LR and histopathology, and serum chemistry were compared to saline-injected controls over a 6-day period. In ex-vivo studies, catfish leukocytes were exposed to 0, 10, or 100 ng/ml of MC-LR for 6 hours and evaluated for phagocytic ability. In the third part, the survival of fish injected with 500 ng/g bw MC-LR exposed and to an LD20 dose of a virulent strain of *Aeromonas hydrophila* (vAh) was compared to fish exposed to MC-LR or vAh alone. In another trial, they were exposed to *Edwardsiella piscicida* (Ep).

Results
The MC-LR exposed fish appeared normal but stopped eating. Serum AST and ALT levels were elevated from 6 hours through 96 hours post-injection (HPI) and histology confirmed diffuse hepatic injury in the treated fish and substantial recovery by 144 HPI. In immunohistochemistry, strong diffuse MC-LR-specific staining was present in the liver early and the staining became more focused to isolated cells during later periods. In leukocyte studies, MC-LR exposure decreased the number of cells that endocytosed dextran and that phagocytosed bacteria. In the vAH- MC-LR challenge study, fish that received MC-LR experienced 67% mortality after vAh exposure compared to 22 % mortality in fish that were given saline injections and vAh exposure. In the Ep- MC-LR challenge study, fish that received MC-LR experienced 96% mortality after Ep exposure compared to 0 % mortality in fish that were given saline injections and Ep exposure, MC-LR only or the saline-only injected fish.

Conclusions
MC-LR exposure causes transient hepatic changes, suppresses phagocyte function, and makes channel catfish more susceptible to bacterial pathogens. This suggests that managing cyanobacterial blooms may reduce disease outbreaks in aquaculture.

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

Notes:

15 - Development of a modified live attenuated influenza virus vaccine against H9N2 for poultry

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Session: Vaccinology 1, 1/22/2022, 08:30 - 08:45

Objective
Influenza A virus (FLUAV) of the H9N2 subtype is enzootic in poultry in parts of Asia, the Middle East, Europe, and Africa, causing significant economic losses to the poultry industry. Vaccination is widely used to control disease and reduce transmission. Inactivated whole virus vaccines are being extensively used, though they confer limited protection. In contrast, live attenuated influenza virus (LAIV) vaccines mimic a natural infection, inducing greater immune responses through better humoral, mucosal, and cellular immunity. Therefore, we aim to generate efficacious and safe LAIVs carrying molecular markers and immunomodulators.

Methods
We used reverse genetics to generate LAIVs based on genome rearrangement where the open reading frame of M2 was introduced downstream PB1. Additionally, multiple stop codons were introduced in the M segment to prevent the expression of M2 from segment 7. Furthermore, molecular markers in the HA segment and immunomodulators in the NA segment were incorporated. The stability and growth properties of the viruses were analyzed *in vitro* whereas safety, immunogenicity, and efficacy were evaluated in two-week-old chickens.

Results
LAIV candidates were stable and grew to similar levels in comparison to wild-type viruses. Studies *in vivo* showed that LAIVs are safe and immunogenic, generating similar levels of anti-influenza NP and neutralizing antibodies. In addition, the inclusion of immunomodulators enhanced the generation of neutralizing antibodies, suggesting a role in the host immune response. The decrease in viral loads post challenge demonstrated the protective effect of LAIV vaccines. The inclusion of immunomodulators improved the protection conferred in comparison to the LAIV without immunomodulators.

Conclusions
This work provides novel insight into the development of vaccines against FLUAV carrying molecular markers and immunomodulators. The LAIVs generated are stable and safe, generate antibodies that protect against homologous challenge, and the inclusion of immunomodulators enhances the immune response.

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

Notes:

16 - Optimization of an intrauterine (i.u.) vaccine against porcine epidemic diarrhea virus (PEDV) in piglets

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Session: Vaccinology 1, 1/22/2022, 08:45 - 09:00

Objective
To optimize intrauterine (i.u.) vaccination at breeding to protect suckling piglets against PEDV by encapsulating PEDVS1 antigen with different adjuvants into PLGA -nanoparticles (NP)

Methods
PLGA NP (1-4) formulations encapsulating PEDV S1 antigen were combined with 4 different adjuvants (A1, A2, A3 and A4) respectively, using emulsification solvent evaporation method. The vaccines were tested in *in-vitro* to assess their effects on boar sperm viability using computer-assisted sperm analysis (CASA). Experimental gilts (n=4) in estrus were bred with live semen with PLGA NP1 vaccine. The same group of gilts received an intramuscular (i.m.) dose a month later (i.u/i.m.). Another group of gilts received only i.m. vaccine after 30 d post-breeding. Blood samples taken on Day 1, 30, 60, 100 and 125, colostrum collected on farrowing day and post-slaughter uterine tissue harvested on Day 125 were used to measure the immune responses. Piglets (3-d age) from sows were challenged with the PEDV and assessed for clinical, weight loss and survival scores post-challenge In subsequent trials, PLGA NP vaccines will be first tested in weaner piglets to reduce costs and increase flow through. Weaner piglets (n=4 per group) will be given i.m. vaccine and boosted 21 days later. Blood samples will be used to quantify PEDVS1 specific antibody, neutralizing antibody titers and cytokine secretion from T-cells. The formulation that promotes the best immune response will be used for a separate trial in gilts administered via the i.u. route and booster i.m. vaccine.

Results
CASA data showed that PLGA NPs are not spermicidal. Both i.u./i.m. and i.m. vaccinated groups showed a significant increase in PEDVS1 specific IgG and IgA in serum and in uterine tissue. Anti-PEDVS1 colostral IgG titres were significantly higher relative to the negative control sows. However, piglets born to either group lacked sufficient neutralizing antibodies to protect them against PEDV

Conclusions
NP vaccine administered via i.u. and i.u./i.m. route induced significant anti-PEDVS1 immunity in systemic and mucosal sites but did not provide passive protection in piglets

Financial Support
Saskatchewan Agriculture Development Fund; Dechra Development LLC Collaborative Research Agreement; Innovation Saskatchewan and the Ministry of Agriculture, Canada Foundation for Innovation through the Major Science Initiatives

Notes:

17 - A PRV vectored PCV2/CSFV-sub vaccine protects pigs against lethal CSFV challenge and failed to replicate in TG

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Session: Vaccinology 1, 1/22/2022, 09:00 - 09:15

Objective
Pseudorabies viruses (PRV), classical swine fever virus (CSFV) and porcine circovirus type 2b (PCV-2b) are devastating viral disease of pigs causing huge economic loss. Though PRV and CSFV eradicated from commercial swine industry in the United States, there is a constant threat for PRV spillover from wild pig and accidental CSFV introduction. Vaccination remains the most effective approach for preventing and controlling viral diseases. Therefore, we aimed to develop a novel, safe and protective PRV vectored CSFV and PCV2b subunit vaccine to protect pigs from all three deadly diseases.

Methods
A triple gene-deleted mutant PRV (PRVtmv) vector (Δthymidine kinase/Δglycoprotein E/ ΔgG) expressing chimeric PCV2b-capsid, CSFV-E2, and Erns-fused with porcine granulocytic macrophage-colony stimulating factor (Erns-GM-CSF) trivalent vaccine was constructed, designated as PRVtmv+. Immunogenicity and protective efficacy of PRVtmv+ against lethal wildtype (wt) CSFV challenge was determined at 7- and 28 day-post immunization (dpi), respectively in group of 5 pigs each. In addition, we determined the latency-reactivation and nasal virus shedding property of the PRVtmv+ in three immunized pigs.

Results
PRVtmv+ vaccinated pigs were completely protected against fatal infection upon lethal wt CSFV challenge at 28 dpi. Few pigs developed mild diarrhea and pyrexia at 5-8 day-post challenge (dpc). However, all pigs were totally recovered by 10 dpc. In contrast, all mock control and 7-dpi vaccine group pigs developed severe form of fatal CSFV infection with nervous signs and euthanized at 6 dpc. Upon dexamethasone-induced reactivation from latency, only the PRV wt-infected pigs shed virus in the nasal secretions with memory B cell response, but not the PRVtmv+. Though PRVtmv+ vaccine virus reactivated, late protein (glycoprotein C) was not detected in the TG neurons.

Conclusions
Our trivalent PRVtmv+ vaccine is efficacious and single dose confers protection against lethal wt CSFV challenge at 28 dpi. The PRVtmv vector is safe, does not replicate in TG neurons and has potential to use for various subunit vaccines.

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

USDA

Notes:

18 - Evaluation of local innate immune responses induced by novel cyclic polyphosphazene and other adjuvants in pigs

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Session: Vaccinology 1, 1/22/2022, 09:15 - 09:30

Objective
Adjuvants induce local innate immune responses, which in turn influence the development of antigen-specific immune responses. The objective of this study is to evaluate the capacity of the novel adjuvant cyclic polyphosphazene (CPZ) to stimulate potent innate immune responses in pigs and compare these results to other adjuvants.

Methods
Five groups of 4-week-old piglets (n=6/group) will be assessed in this experiment. All groups will be immunized intradermally (ID). Prior research indicated that at 100 µg/100 µl, PCEP ID induced significantly greater IFN-α, IL-12 and IL-1β relative to intramuscular administration of PCEP at the same dosage or control PBS. Each pig will be injected four times on their limbs (for a total of 16 injections), with either 100 µl endotoxin free, sterile PBS, 1µg/µl of PCEP in 100 µl PBS, 1 µg/µl of cyclic polyphosphazene in 100 µl of PBS, 100 µl Montanide 61 or 100 µl Montanide 660. Day of euthanasia will be termed day 0 and animals will be immunized on day -7, day -4, day -2 and day -1 using a separate limb each day of injection. Punch biopsies (8 mm) will be collected from the injection sites. Each biopsy will be collected for a particular assay: PCR, histology, ELISA, and kinome analysis. Genes and cytokines to be measured include but are not limited to: CCL2, CCL5, IFN-γ, and IL-1β. Blood and sera will be collected for observing systemic changes. Analysis will be completed Oct 2022.

Results
We expect to observe significant recruitment of innate immune cells induced by adjuvants relative to PBS. These cells will be detected directly and indirectly using histology and cytokine ELISAs respectively. We will also quantify differences in cell signaling by kinome analysis to better characterize the adjuvants' mechanisms of action.

Conclusions
This experiment will inform the mechanisms surrounding novel adjuvant cyclic polyphosphazene in stimulating innate immune effector mechanisms. From these conclusions we plan to establish a baseline to inform future studies about how CPZ contributes to the innate immune response- and overall, how adjuvants work mechanistically.

Financial Support
Natural Sciences and Engineering Research Council of Canada; Innovation Saskatchewan and the Ministry of Agriculture, Canada Foundation for Innovation through the Major Science Initiatives

Notes:

19 - Employing artificial intelligent, computational simulations, and molecular docking for developing PRRSV vaccine

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Session: Vaccinology 1, 01/22/2023, 09:30 - 9:45

Objective
Porcine reproductive and respiratory syndrome (PRRSV) is the most devastating pathogen that threatens the sustainability of the swine industry. PRRSV is a single-stranded positive-sense RNA virus first isolated in 1990 in North America. It composes of four major species and thousands of known and unrecognized strains. Developing an effective vaccine against PRRSV is a big challenge. Here we used a novel computational experiment for T cell epitope mapping against PRRSV N protein.

Methods
In this work, the in silico and computational-based tools have been used to predict epitope mapping against > 50 SLA-I, -II and -III alleles and for the evaluation of antigenicity, solubility, as well as proteolytic properties of the epitopes. The predicted epitopes were compared to 1000 PRRSV strains, and the most conserved regions were selected for conducting homology modelling, protein-peptide docking, and protein-peptide affinity evaluations. Ten strong epitopes were selected for generating the subunit-based vaccine construct that joined to each other through YYA, GGGGGG and KK linkers. The B-cell epitopes were mapped through selecting the most conserved regions and highly antigenic domains of the protein. The conformation of the vaccine construct was modeled and docked against pig toll-like receptor and the SLA alleles.

Results
All ten epitopes have shown to have strong antigenicity, solubility, and effective binding (i.e. > -70 kj/mol) against SLA proteins including but not limited to SLA-1*04:01:01, SLA-1*06:02, SLA-1*04:04, SLA-1*04:02, SLA-1*11:10, SLA-1*23:01, SLA-1*08:09, SLA-1*08:05, SLA-1*07:03. The vaccine construct was a stable protein with strong binding towards toll-like receptor.

Conclusions
Our structure-based vaccine design and immunoinformatics approaches can help to find conserved regions within antigenic proteins and to select the epitopes while addressing the huge diversity of pig MHC to improve the development of the immunogenic yet safe and effective vaccine.

Financial Support
Innovation Saskatchewan and the Ministry of Agriculture, Canada Foundation for Innovation through the Major Science Initiatives

Notes:

21 - Kinome analysis reveals age-dependent mechanisms of TLR7/8 induced signaling in porcine gamma-delta T cells.

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Session: Immunology 1, 1/22/2022, 08:30 - 08:45

Objective
With its high frequency of gamma-delta (γδ) T cells, pigs are potential model species for the study of γδ T cell responses. However, the mechanisms of antigen recognition by porcine γδ T cells remain largely unknown. Our previous experiments demonstrated a co-stimulatory effect of TLR7/8 ligand R848 on γδ T cells in an age and microenvironment-dependent manner. However, the signaling pathways behind this observation remained unclear. Thus, we employed kinome analysis to gain insight into the cellular kinase activity.

Methods
After purifying blood-derived γδ T cells from 7-week-old pigs and sows, they were cultured under 4 different conditions, including R848 and IL-2/IL-12, for 3 days. Cellular kinase activities within these cells were quantified with a porcine-specific peptide kinome microarray representing 282 unique phosphorylation events with nine technical replicates of each peptide.

Results
In the older age group, the activation of various STAT proteins mainly occurred under IL-2/IL-12 stimulation and was intensified by the addition of R848. Additionally, differences in phosphorylation of IRAK1, IKKγ and IKKβ under co-stimulation with R848 were observed. R848 alone resulted in differentially phosphorylated peptides involved in the MyD88-dependent pathway, potentially indicating a specific response to TLR7/8 stimulation. The responses in the younger age group showed a higher degree of variability. Only TAB1 was differentially phosphorylated in all 5 samples in response to R848. Interestingly CREB showed the biggest difference between young and old pigs. CREB was consistently involved in the responses of young but not adult γδ T cells. In contrast, increased IL-4 induced signaling was observed in all adult but not in young γδ T cell samples under co-stimulation with R848.

Conclusions
We confirmed that responses of γδ T cells to IL-2/12 stimulation with and without R848 have both conserved and age-specific responses. Overall, adult γδ T cells showed a more consistent response to stimulation with pro- and anti-inflammatory pathways involved.

Financial Support
Saskatchewan Agriculture Development Fund; Natural Sciences and Engineering Research Council of Canada; Innovation Saskatchewan and the Ministry of Agriculture, Canada Foundation for Innovation through the Major Science Initiatives

Notes:

22 - Regional specialization of intraepithelial T cells and microbiota in the pig intestinal tract

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Session: Immunology 1, 1/22/2022, 08:45 - 09:00

Objective
Intraepithelial T lymphocytes (T-IELs) contribute to immune defense at the intestinal barrier and are in close association with microbes that modulate their functions. Understanding how T-IELs provide immune defense against enteric disease in pigs is limited, as little is known of cellular phenotypes comprising T-IEL populations in the porcine intestine. However, identification of different T-IEL phenotypes can be used to infer biological functions. Parallel analysis of both T-IEL and microbial diversity in porcine intestine has also not been completed but could inform on potential relationships between T-IELs and microbiota. Therefore, the objective of the study was to perform parallel assessment of community structures for T-IELs and microbiota across intestinal locations in pigs.

Methods
T-IELs and microbial swabs were collected from jejunum, ileum, and cecum of 5- and 7-week-old pigs. T-IEL phenotypes were assessed via flow cytometry. Microbial diversity was assessed via 16S sequencing.

Results
T-IELs with innate-like phenotypes were found in higher proportions in distal intestine, including discovery of CD8aa+ ab T-IELs (CD3e+gdTCR-CD4-CD8a+CD8b-) that comprised 2-15% of total T-IELs in cecum. While age-specific shifts to T-IEL communities occurred in jejunum and ileum, age-specific shifts to microbial communities occurred only in cecum.

Conclusions
Findings indicate different functions of T-IELs based on intestinal location, where distally-located T-IELs have phenotypes associated with innate-like functions. Though we find T-IEL and microbial communities stabilize at different rates in different regions of the intestinal tract, further work is still required to determine if causational interactions exist between intestinal T-IELs and the microbiota in pigs. Nonetheless, results emphasize the intestinal tract is a regionally specialized tissue system in regards to both immune cells and microbiota. The context of our findings pertaining to intestinal regional specialization should be considered in disease research, as specific pathogens target different intestinal regions for invasion.

Financial Support
U.S. Department of Agriculture

Notes:

23 - An alternative cell culturing method to improve antigen-specific IFNγ responses to *Lawsonia intracellularis* protein

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Session: Immunology 1, 1/22/2022, 09:00 - 09:15

Objective
An extensive review of the literature suggested that pig PBMCs respond to antigens with very low-level interferon gamma (IFNγ). These very low IFNγ titres make it difficult to determine if a vaccine antigen is inducing a cell-mediated immune (CMI) response. We anticipate that supporting the growth of T cells prior to *ex vivo* restimulations with antigens will facilitate antigen-specific IFNγ production. Our objective is to optimize the PBMC preculturing conditions to amplify the antigen-specific IFNγ responses against *Lawsonia intracellularis*.

Methods
We obtained PBMCs from pigs vaccinated with killed *L. intracellularis*, and we previously established that *L. intracellularis* flagellin protein (FliC) induced a CMI response with low level IFNγ titres. We precultured the PBMCs for 7 days with: recombinant GM-CSF and IL-2, which are immunostimulants that promote proliferation and differentiation of monocytes and T-cells; molecular biology-grade *E. coli* with or without a plasmid induced to express FliC gene and resulting in increased recombinant FliC production. After 7 days, the precultured PBMCs were reisolated and then unstimulated in media alone or incubated for 2 days with 5 µg recombinant FliC or a mitogenic combination of 1 ng phorbol myristate acetate plus 100 ng ionomycin, which established that the cells were healthy and able to produce IFNγ. Control PBMCs from the same vaccinated pigs were stimulated with rFliC or mitogen but without the preculturing conditions. IFNγ was quantified by ELISA.

Results
Preculturing the PBMCs with the immunostimulants +/- *E.coli* prior to stimulation with FliC significantly increased the Flic-specific IFNγ secretion relative to the non-precultured PBMCs. Preculturing the PBMCs with immunostimulants alone ($p = 0.038$), or with the immunostimulants plus the *E.coli* enriched for FliC ($p = 0.005$) significantly augmented the FliC-specific IFNγ response.

Conclusions
Preculturing the PBMCs augmented the FliC-specific IFNγ response, and this adaptation to the IFNγ assay may assist us in identifying a repertoire of T-cell antigens from the *L. intracellularis* proteome.

Financial Support
Dechra Development LLC Collaborative Research Agreement; Innovation Saskatchewan and the Ministry of Agriculture and from the Canada Foundation for Innovation through the Major Science Initiatives

Notes:

24 - Differential expression of CD45RO and CD45RA in bovine T cells

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Session: Immunology 1, 1/22/2022, 09:15 - 09:30

Objective
Effective vaccination induces memory T cells capable of mounting protective immune responses during pathogen re-infections. Currently, two isoforms of CD45 tyrosine phosphatase, CD45RO expression plus CD45RA exclusion (CD45RO+/CD45RA-), are exclusively used for detecting bovine memory T cells; however, some published reports have challenged these markers. In this research, we evaluated if CD45RO+/CD45RA- are genuine markers for identifying memory T cells in cattle.

Methods
Purified bovine peripheral blood mononuclear cells (PBMCs) from the healthy Wye Angus cattle were stimulated with an activation cocktail or brefeldin A (BFA) for 4 hours before detection of interferon gamma (IFNγ)- and interleukin 4 (IL4)-producing memory T cells; subsequently, their expression of CD45RA and CD45RO (CD45RA/RO) were analyzed in CD4+, CD8+ and γδ T cell subtypes from the same cattle.

Results
20% of the examined cattle did not express CD45RO on their T cells without significantly affecting the IFNγ and IL4 inductions. In cattle expressing CD45RO, more than 80% of the CD45RO+ cells did not produce IFNγ and IL4. Instead, CD45RA/RO expression was found closely related to their distinct T cell subtypes: while CD45RO expression was always highest (> 90%) in gamma-delta (γδ), followed by CD4+ (~ 60%) and CD8+ T cells (~ 40%), CD45RA expression was exactly in the opposite trend, lowest in γδ (< 20%), slightly high in CD4+ (~ 40 %), but most abundant in the CD8+ T cells (~ 80%). Importantly, this tendency was similarly reflected within the IFNγ- and IL4- producing memory T cell subtypes.

Conclusions
These data suggest that CD45RO is not ubiquitously expressed in cattle, and CD45RA/RO clustering is more related to their distinct T cell subtypes than memory assessment. Results indicate that CD45RA/RO may not serve as authentic markers for memory T cells in cattle.

Notes:

25 - IgM antibodies play a major role in the elimination of *Streptococcus suis*

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Session: Immunology 1, 1/22/2022, 09:30 - 09:45

Objective
No effective commercial vaccine is currently available to prevent infections by *Streptococcus suis*, an encapsulated bacterial pathogen causing severe diseases in swine. *S. suis* has been found to modulate some immune cells, however the interactions between *S. suis* and B cells along with the development of the antibody response have not been studied in detail. The aim of this study was to characterize the development of the adaptive immune response after *S. suis* infections.

Methods
Infections with *S. suis* serotype 2 were performed in C57BL/6 wild-type mice along with knock-out lines for factors involved in antibody class-switch recombination and affinity maturation (*Aidca-/-* and *Ung-/-*) and T cell communication (*Tcrb-/-*). Spleens and sera were collected at various timepoints post-infection. Splenic germinal center B cell population dynamics were assessed by flow cytometry. Pathogen-specific antibody titers in sera samples were detected by ELISA against whole bacteria or purified capsular polysaccharide (CPS). Antibody functionality of sera samples was evaluated by opsonophagocytosis killing assay.

Results
Mice displayed delayed germinal center formation following infection. Anti-*S. suis* IgM and IgG induced partial killing of the bacteria *in vitro* and reduced bacteremia *in vivo*. The functional affinity of antigen specific IgGs did not improve following infections. Anti-CPS antibodies were mainly IgMs and not dependent on class switch recombination and affinity maturation. Lower anti-CPS antibody production was observed in *Tcrb-/-* mice and was paired with hampered bacterial elimination. Depletion of total IgGs from the sera of mice had no influence on observed bacterial killing *in vitro*.

Conclusions
S. suis can affect germinal center formation, a property that could explain the observed impaired functionality of IgGs. In contrast, IgMs targeting the bacteria and its CPS were fully able to eliminate *S. suis* and did not require class switch recombination and affinity maturation. IgM production plays an important role in *S. suis* elimination, and it could be the objective of future effective vaccines.

Financial Support
Fonds de recherche du Québec; Swine and poultry infectious diseases research center

Notes:

27 - Antigenic map of the hemagglutinin of influenza A virus of the H9 subtype

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Session: Virology 1, 1/22/2022, 08:30 - 08:45

Objective
Influenza A viruses (FLUAVs) of the H9N2 subtype are enzootic in poultry in Asia, the Middle East, and Africa, causing significant economic damage to the poultry industry due to high morbidity and associated mortality. Due to their zoonotic potential, the World Health Organization (WHO) places H9N2 FLUAVs among those with pandemic concern. To determine molecular signatures of antibody recognition of the hemagglutinin (HA) of the H9 subtype FLUAVs, phylogenetics, and antigenic cartography were combined

Methods
Analyzing the HA1 portion of H9 FLUAVs, 11 consensus sequences were produced to capture the potential antigenic diversity of these viruses on a global scale. We created 11 chimeric HA sequences containing the HA1 of these consensus sequences on a constant HA2 portion from a prototypic H9 strain. Nine chimeric HAs were successfully rescued by reverse genetics, and the resulting viruses were used to generate antisera in quails

Results
Antigenic cartography maps were generated, plotting the cross-hemagglutination inhibition (HI) data from the panel of sera against the chimeric constructs. ACMACS k-cluster analysis implemented with the Ward hierarchical-clustering approach allowed the identification of 4 H9 HA antigenic clusters. Furthermore, few amino acid positions of putative antigenic relevance allowed two-way complete antigenic cluster transitions. Although mutations at amino acid positions 150, 180, and 217 (H9 HA numbering) had a relatively significant impact on HI activity, only the mutations E180A, R131K/E180A, and F150L/Q217I led to complete cluster transitions

Conclusions
These studies suggest that a combination of a few amino acid residues modulates HI activity in the HA of H9 FLUAVs. Our studies provide significant insights into the antigenic profile of H9 FLUAVs with essential implications for understanding antigenic drift and for improved vaccine development

Financial Support
U.S. National Institute of Allergy and Infectious Diseases under federal contracts HHSN272201400008C and 75N93021C00014

Notes:

28 - A panel of pig macrophage-derived cell clones that differs in ability to support various steps of PRRSV replication

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Session: Virology 1, 1/22/2022, 08:45 - 09:00

Objective
Porcine reproductive and respiratory syndrome virus (PRRSV) has a narrow cell tropism limited to pig macrophages (PMs). Despite its clinical importance, however, research on viral replication has to date been hampered by limited availability of the PM-derived cell lines that support viral replication.

Methods
Here, we isolated eight PM-derived cell clones, all of which were susceptible to PRRSV infection but differed in their ability to support PRRSV replication. Of the eight clones, we selected three (Cl2, Cl3, and Cl7) expressing a similarly high level of CD163, a known entry factor for PRRSV, but forming a wide range of plaque sizes upon infection with both type-1 and -2 PRRSV, to identify a step(s) of viral replication responsible for the difference in plaque formation.

Results
By performing a series of experiments using an infectious cDNA clone of a type-2 PRRSV, its replication-competent and -incompetent replicons with or without a luciferase gene under the translation control of a cardiovirus-derived internal ribosome entry site, we found that (*i*) the highest level of viral entry was detected in Cl2, followed by Cl3 (~28% lower relative to Cl2) and Cl7 (~67% lower); (*ii*) the highest efficiency of viral translation in the absence of viral RNA replication was observed in Cl3, followed equally by both Cl2 and Cl7 (~26% lower relative to Cl3); (*iii*) the highest level of viral RNA replication was monitored in Cl3, followed by Cl7 (~47% lower relative to Cl3) and Cl2 (~69% lower); and (*iv*) the highest level of virus production was marked in Cl7, followed by Cl2 (~42% lower relative to Cl7) and Cl3 (~64% lower). Furthermore, the three cell clones showed differences in antiviral responses to PRRSV replication, which correlates well with the difference in viral RNA replication efficiency.

Conclusions
Our results show that three PM-derived cell clones differ in their ability to support viral entry, translation/RNA replication, and virus particle production. These cell clones will provide a valuable tool for a better understanding of how PRRSV-host cell interactions occur during viral replication.

Financial Support
U.S. Department of Agriculture

Notes:

29 - Rapid detection of equine CXCL16 allelic variants associated with equine arteritis virus persistence

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Session: Virology 1, 1/22/2022, 09:00 - 09:15

Objective
Equine arteritis virus (EAV) can cause long-term persistent infection (LTPI) in the reproductive tract of stallions. Following natural infection, up to 70% of the infected stallions continue to shed EAV in semen for more than one year (LTPI). Thus, LTPI stallions play a pivotal role in maintaining EAV in the equine population. Two alleles of the equine chemokine C-X-C motif chemokine ligand 16 (*CXCL16*^{S} and *CXCL16*^{r}) were identified and correlated with the susceptibility or resistance of a subpopulation of CD3^{+} T cells to *in vitro* EAV infection and to the establishment of LTPI in stallions. Genotyping stallions based on *CXCL16*$^{S/r}$ would allow identification of those at the highest risk of establishing LTPI. Thus, we developed a TaqMan® allelic discrimination assay for genotypification of equine *CXCL16* gene targeting a single nucleotide polymorphism within exon 2.

Methods
Blood samples from 160 horses spanning four different breeds were collected and genotyped using the new TaqMan® allelic discrimination assay. These results were compared to CD3^{+} T cell susceptibility to EAV infection by flow cytometry and DNA sequencing by Sanger sequencing.

Results
Genotyping by Sanger sequencing determined that all horses with the resistant CD3^{+} T cell phenotype (n=56) were *CXCL16*$^{r/r}$ while horses with the susceptible CD3^{+} T cell phenotype (n=104) were either *CXCL16*$^{S/S}$ or *CXCL16*$^{S/r}$. Genotypification with the new TaqMan® allelic discrimination assay showed perfect agreement (100%) with Sanger sequencing and flow cytometric analysis.

Conclusions
In conclusion, this new TaqMan® allelic discrimination genotyping assay provides a new diagnostic method for medium to high-throughput genotyping of equine *CXCL16* with perfect agreement compared to Sanger sequencing allowing accurate identification of stallions at higher risk of becoming EAV carriers. Thus, it will assist with targeted vaccination practices and selective breeding with particular emphasis on stallions carrying the *CXCL16*^{S} allele to help prevent the occurrence of the carrier state.

Financial Support
U.S. National Institutes of Health; U.S. Department of Agriculture, National Institute for Food and Agriculture; Food Animal Residue Avoidance Databank

Notes:

30 - Diagnosis and characterization of a novel strain of EHDV-8 in Tunisia in 2021

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Session: Virology 1, 1/22/2022, 09:15 - 09:30

Objective
Epizootic haemorrhagic disease (EHD) is an infectious disease of wild and domestic ruminants sustained by Epizootic haemorrhagic disease virus (EHDV), an *Orbivirus* existing in multiple serotypes transmitted by *Culicoides* midges. In late September 2021, EHDV was reported in cattle farms in central/western Tunisia that rapidly spread all over the country with more than 200 confirmed outbreaks. Clinical signs included fever, conjunctivitis, lacrimation, drooling, erythema of nasal and oral mucosa, teat erosions. In this work, we describe the identification and characterization through a combination of classical and molecular techniques of a novel EHDV-8 strain. This is the first evidence of serotype 8-like virus circulation since 1982.

Methods
A total of 174 whole blood samples were collected from symptomatic cattle and tested for EHDV RNA by real time RT-PCR. Positive samples were used for virus isolation and NGS analysis by Illumina and MinION technologies. Isolates were tested by virus neutralization (VN) for serotype identification. A total of 415 serum samples of cattle were tested by c-ELISA and serum neutralization (SN).

Results
Complete consensus sequences of the ten genome segments were obtained from blood samples. We were able to identify a novel EHDV-8 strain otherwise not typeable with serotype specific molecular tests. Seg-2 of EHDV-8 TUN2021 clustered (77% nt identity) with the only available VP2 of EHDV-8 Australia 1982. Seg-6 (VP5) was close to the EHDV-2 Australia 1979 whereas the remaining segments clustered with African EHDV strains. Thus, the Tunisian strain is most likely a reassortant between different serotypes. Shotgun Metagenomic by MinION produced reads classified as EHDV but was unable to identify the serotype due to limitation of the reference database. VN clearly identify isolates as EHDV-8 whereas SN did not allow the identification with certainty of the serotype as for well-known cross-reactivity between EHDV-6 and 8.

Conclusions
This is the first evidence of EHDV-8 in the field. Certainly, more surveillance is needed to assess the origin of this strain.

Notes:

31 - Characterizing the effect of coinfection ratios on bluetongue virus reassortment in *Culicoides sonorensis*

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Session: Virology 1, 1/22/2022, 09:30 - 09:45

Objective
Bluetongue virus (BTV) is a segmented, double-stranded RNA virus transmitted by *Culicoides* biting midges that can result in devastating disease in susceptible ruminants. Reassortment between BTV strains may enhance its ability to spread to new regions. In prior in vitro BTV coinfection studies, progeny genotypes were dominated by the parental strain with the higher initial dose. Our study evaluated in vivo reassortment of progeny virus in *Culicoides sonorensis* midges coinfected with different ratios of BTV-10 and BTV-17.

Methods
Midges were fed blood containing BTV-10, BTV-17, or a combination of BTV-10: BTV-17 at 90:10, 75:25, 50:50, 25:75, or 10:90 ratios. Midges were collected every other day for pan BTV and COX (housekeeping gene) qRT-PCR. A curve was fit to the ΔCt values (pan BTV Ct - COX Ct) for each ratio group and linear portions evaluated by pairwise comparisons with *P* values adjusted with Tukey's method. On day 10, midges were processed for BTV plaque-isolation. Genotypes of plaques were determined by next generation sequencing.

Results
Comparison of linear portions of ΔCt curves demonstrated no differences between ratio treatment groups. Plaque genotyping indicated that most plaques fully aligned with one of the parental strains. However, there was reassortment evident in a pool of midges coinfected with BTV-10: BTV-17 ratio of 75:25. Of the 10 plaques analyzed from that pool, one plaque's segments all aligned completely to BTV-10. The remaining 9 plaques had segment contributions from both parental strains.

Conclusions
Different coinfection ratios of BTV-10 and BTV-17 demonstrated similar virogenesis dynamics and most pools of coinfected midges contained plaque genotypes that completely aligned with a parental strain. However, one pool of coinfected midges resulted in mostly reassorted plaques. Thus, reassortment within the midge may be an infrequent event, but reassortants may have an advantage over the parental strains and overtake parental strain populations. BTV reassortment patterns and its consequences are important to understanding BTV expansion.

Financial Support
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Notes:

33 - Spatial epidemiology: pushing the boundaries

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Session: AVEPM - Schwabe Symposium, 01/22/2023, 10:30 - 11:15

The spatial distribution of disease is a foundation of the epidemiological approach which strives to understand how diseases affect populations, and the options available for disease control and prevention. Embedded in the celebrated works of John Snow on cholera in London in the 1850s was a dot map. The clustered distribution of cases provided a valuable clue to the source of infection. For much of the following century, spatial epidemiology was analogous to disease mapping, with subjective interpretation of spatial patterns being a major barrier to maximizing the epidemiological value of spatial disease data.

More recently, data and statistical approaches have led to a renaissance in spatial epidemiology. Whether this has been driven by an explosion in the collection and availability of spatial data, or the development of increasingly sophisticated tools for spatial data analysis is moot; combining data and tools, spatial health data is now mined for clusters and hotspots, autocorrelation and trends, and disease causation. Within the veterinary context, advances have been made in each of these areas, but particularly with a focus on surveillance data analysis and the better design of surveillance programs. The outputs of spatial analysis have become increasingly available and routinely used. Indeed, GPS and GIS have become integrated to the extent that they are no longer considered special topics. But despite the massive leap forward in technology, acquiring and assessing high quality data in veterinary medicine remains a challenge, and the interpretation of spatial analytical outputs remains under-developed.

And so, the science (and art) of disease mapping and spatial analysis is still as critical as when John Snow produced his dot maps in the 1850s. Whilst we continue to push the boundaries, we must be mindful of how we can, and should, use this information to improve animal and human health.

Notes:

34 - Veterinary epidemiology: A vision of the future

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Session: AVEPM - Schwabe Symposium, 01/22/2023, 11:15 - 12:00

There have been numerous advances in the design and conduct of studies that fall under the broad heading of veterinary epidemiological research over the past twenty years. Easier access to instructional material and the peer reviewed literature (through, for example, open access journals), better means for collecting and validating data, widespread availability of high specification computer hardware, advances in statistical methodologies and well designed, powerful (often open source) statistical software have removed many of the barriers to the conduct of high-quality research. Given the discipline has advanced so far, what are the likely opportunities and threats in the short to medium term future? We address this question using the broad categories of information acquisition, methodological advances and data inference.

Open-access journals and the widespread availability of instructional material are likely to continue to increase in the coming years. While these resources provide those with sufficient time and interest a means by which to learn the basics of epidemiology, increases in the number of journals and a corresponding increase in the number of published papers (i.e., those printed and those appearing on-line) has led to a net decrease in the quality of peer review. Now, more than ever, practitioners need to critically appraise most, if not all, of what they read both on-line and in print. Veterinary degree programs need to be modified to ensure that critical appraisal is a skill taught and applied across the entire curriculum, not just a topic addressed in a single subject such as veterinary epidemiology.

In terms of methodologies, there is a need for the discipline to embrace advances in the field of causal inference and to develop approaches to allow causal inference to be applied to hierarchical (i.e., clustered) data sets that are common in veterinary epidemiological research. Infectious disease simulation models will continue to be used. Future challenges relate to development of methods to: (1) allow appropriate model parameters to be selected, particularly in the early stages of an infectious disease outbreak response; and (2) ensure decision makers make full use of modelling outputs to inform appropriate decision making.

Current undergraduate and postgraduate epidemiology training programs provide instruction on bias, confounding and chance as threats to study validity. Although awareness of the negative impacts of bias on appropriate inference is important, there is a tendency for data sets (often collected at considerable expense) to be discarded due to concerns relating to bias. Moving to the future there is a need for veterinary epidemiology education to provide practitioners with tools to assess the magnitude and direction of bias in a given data set, allowing them to make more informed decisions with respect to appropriate inference.

Notes:

35 - Comparing the variation in measures of IgG and serum total protein in neonatal calf serum

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Session: General Health & Physiology 2, 1/22/2022, 10:30 - 10:45

Objective
Immunoglobulin G (IgG) and serum total protein (STP) are measured in neonatal calves to monitor passive absorption of maternal antibodies. Variability in test results may affect accurate assessment of disease risk. Our objective was to compare the variability of STP determined by optical refractometery and IgG from a commercial bovine IgG radial immunodiffusion (RID) assay.

Methods
Sera from 6 calves was tested 13 times for STP using an optical refractometer and IgG was measured 28 times with RID. Levene's test was used to evaluate the homogeneity of variance within the tests (α=0.10). Unrestricted random sampling bootstrapping (5000 repetitions) was used to calculate coefficient of variation (CV) for each serum and test. Homogeneity of variance between test CV by serum was evaluated using Levene's test (α=0.10). Difference between test CV by serum was assessed with Kruskal-Wallis test (α=0.05).

Results
No difference was observed in the variance for STP between sera (p=0.39, 0.0529 g^2/dL^2). The average CV for STP was 4.2%. Homogeneity of variance was not observed in serum IgG (p<0.0001). Serum requiring dilution for IgG had more variance. Bootstrapped CV for STP and IgG had homogeneity of variance for only one serum (p=0.31). Serum total protein had a significantly smaller CV compared to IgG for every serum (p<0.0001).

Conclusions
STP produced less variance than IgG concentration. IgG concentrations measured using a radial immunodiffusion should be considered an imperfect test and agreement with other testing methods to assess passive absorption of maternal antibodies in neonatal calves should be interpreted with caution.

Financial Support
Mikell and Mary Cheek Hall Davis Endowment for Beef Cattle Health and Reproduction; Mississippi State University House Officer Grant

Notes:

36 - Effect of hemolysis on goat serum antibody testing by ELISA

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Session: General Health & Physiology 2, 1/22/2022, 10:45 - 11:00

Objective
Obtaining high quality serum for large surveillance studies is challenging and often blood samples are hemolyzed. We used both clean and hemolyzed goat serum samples to study the effects of hemolysis on the outcome of a ELISA test for the detection of antibodies against *Coxiella Brunetti*

Methods
We tested 7943 goat sera from 2019 Q-fever surveillance repository at NVSL. A computer algorithm was developed for rapid scoring of serum hemolysis based on RGB (redness) and HSV (darkness) pixel values of serum images. These values were used to generate serum quality scores ranging from 1 to 5. The quality scores 1, 2, 3, 4, and 5 represent transparent, pink, red, dark red and black serum, respectively. Blocking antibody percentages were determined in Q-fever ELISA (IDEXX) and classified into 6 classes. The effect of hemolysis on the detection of antibody were determined by 1) regression analysis of quality scores and blocking percentages and 2) analysis of variance (ANOVA) for each blocking antibody class.

Results
Regression analysis revealed very weak negative relation between quality score and blocking percentages (R^2 = 0.0018). ANOVA showed no significant effect of serum quality on blocking percentages for all samples (P> 0.7) except for blocking class1 (P<0.01). This suggests that serum hemolysis does not affect ELISA antibody detection in samples with >20% background blocking. However, serum samples in blocking class 1 had significantly low background blocking compared to all other serum quality classes (P<0.01). This indicates that serum with low hemolysis may contain less background blocking for Q-fever negative samples. However, such variation in blocking does not alter the test results.

Conclusions
This study provided evidence that hemolysis does not significantly influence results of Q-fever antibody detection. Therefore, hemolyzed sera can be used for surveillance studies without compromising test results. The computer algorithm developed in this study can be used in future studies to determine serum quality of large number of samples with low sample volume and avoids bias during rating.

Notes:

37 - Veterinary student proficiency and time efficiency in canine and feline ovariohysterectomies

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Session: General Health & Physiology 2, 1/22/2022, 11:00 - 11:15

Objective
Canine and feline ovariohysterectomies (OVH), or spays, are some of the primary means of surgical instruction in many veterinary medicine teaching curriculums. These procedures teach tissue handling, ligation, and suture techniques that are common in many other soft tissue surgeries. The goal for many programs is to provide veterinary students with large amounts of canine and feline spays to increase their efficiency and confidence when performing soft tissue surgery. However, the rate of improvement in the students' efficiency and confidence is poorly understood. The objective of this study was to determine the number of OHEs veterinary students need to complete to maximize their learning experience and their surgical efficiency.

Methods
Surgical duration (from the initial incision until the placement of the last suture), complications, incision length, and assistance from supervising veterinarian were recorded. Linear regression was used to test for a difference in time efficiency (surgical duration) associated with the number of cat and dog spays performed, adjusting for animal species, age, and body condition score.

Results
Student surgical times decreased significantly on a logarithmic scale with the number of surgeries performed. However, there was no significant difference between students that performed 51-59 surgeries and more than 60 surgeries. A decrease in incision length was not associated with the number of surgeries performed.

Conclusions
This study indicated that students achieve their maximum surgical efficiency after performing approximately 50 surgeries.

Notes:

38 - A survey of Southeast beef veterinarians regarding methods of bull breeding soundness evaluation

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Session: General Health & Physiology 2, 1/22/2022, 11:15 - 11:30

Objective
The purpose of this survey was to evaluate veterinarians' methods for performing bull breeding soundness evaluations (BSEs).

Methods
Veterinarians in Mississippi, Louisiana, and Arkansas were emailed an internet survey link. Logistic regression was used to test respondent characteristics for associations with BSE methods. Outcomes of interest included whether respondents evaluate semen morphology as part of every BSE, and how often they detect nuclear vacuoles.

Results
Of 204 responding veterinarians, 83 qualified for analysis. Of these, 10/73 (14%) indicated they do not always evaluate morphology during BSEs. When asked whether they thought semen motility or morphology were most predictive of bull fertility; 36/73(49%) chose morphology, 31/73 (42%) chose motility, and 6/73 (8%) were unsure. When shown an image of a spermatozoa with nuclear vacuoles, 1 (1%) indicated they see this defect often, 17 (23%) indicated sometimes, 34 (47%) indicated rarely, and 21 (29%) indicated never. The only factor associated with evaluating morphology as part of every BSE, was if the veterinarian indicated morphology was most predictive of bull fertility (OR=11.2, 95%C.I.=1.3-94.1). There was an interaction between the use of bright field microscopy at 1000X and 400X magnification to evaluate sperm morphology on how frequently respondents indicated they observed nuclear vacuoles. Those that used both objectives had greater odds of observing nuclear vacuoles sometimes or often compared to using 400X alone (OR=15.7, 95%C.I.=2.8-87.3).

Conclusions
Veterinarians' perceptions of which aspects of the BSE are most predictive of fertility influence their methods for performing BSEs, and these methods influence the frequency of detecting nuclear vacuoles.

Financial Support
Mississippi State University; Mikell and Mary Cheek Hall Davis Endowment for Beef Cattle Health and Reproduction

Notes:

39 - Colombian Creole Horse: frequency of oral and motor stereotypies

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Session: General Health & Physiology 1, 1/22/2022, 11:30 - 11:45

Objective
This study aimed to report the frequency of locomotor and oral stereotypies in Colombian Creole Horses in Girardota (Antioquia, Colombia) and associated risk factors.

Methods
A prospective cross-sectional study was conducted from 2019 to 2020, in which 102 stabled horses aged 28 months and older participated. A questionnaire was developed to collect information on the horses' daily barn routines. The horses were observed twice a day for 2 h for 3 consecutive days to record information related to stereotypy behaviors. The Fisher's exact test and the Mann–Whitney U test were utilized for data analyses. Associations were considered statistically significant at $p<0.05$

Results
Among the horses evaluated, 32.35% presented at least one stereotyped behavior. The most common was crib-biting (i.e., cribbing), with 17.65% exhibiting this behavior. Age, weight, gender, type of feeding, visual contact between horses, and natural lighting were all associated with oral stereotypies. Crib-biting was most common in young horses ($U=1.36$, $p\leq0.05$), wind-sucking was more common in lighter weight animals ($U=1.45$, $p=0.01$), and lip-smacking was more common in stallions ($\chi^2=9.10$, $p\leq0.01$). It is noteworthy that their feeding diet included bran, molasses, and gopher. Horses that did not have visual contact with other horses and those that did not have natural lighting were associated with pica ($\chi^2=9.52$, $p\leq0.02$; $\chi^2=3.72$, $p\leq0.05$; and $\chi^2=3.72$, $p\leq0.05$, respectively). Of locomotor stereotypies, kicking the wall was significant in young animals ($U=1.54$, $p=0.03$) and walking in circles in lactating mares ($\chi^2=13.20$, $p\leq0.02$)

Conclusions
Housing conditions in this study were found to have several risk factors affecting horses that exhibit stereotypic behaviors, and all these factors resulted in a higher frequency of stereotypies. Establishing risk factors for the presentation of abnormal behaviors allows for the implementation of better management practices in the production systems of the Creole Colombian Horse and will help improve their overall welfare

Notes:

40 - The effect supplementation with *Saccharomyces cerevisiae* fermentation product on mammary immune cell populations

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Session: General Health & Physiology 2, 1/22/2022, 11:45 - 12:00

Objective
Evaluate the impact of supplementation of dairy cows with a postbiotic product from *Saccharomyces cerevisiae* fermentation (SCFP) on the cell subsets present in milk collected before and after intramammary challenge.

Methods
Clinically healthy mid-lactation Holstein cattle (n=37, parity 1-5, ≥120 DIM) were randomized to receive daily supplementation with SCFP (NutriTek, Diamond V) or to a negative control group which received the basal diet. After 45-d of treatment, 500 mL of milk was collected from a rear mammary gland for a pre-challenge baseline analysis of immune cell populations. Following initial milk collection, the same gland was challenged with 2,000 CFU of *S. uberis 0140J*. After 3 days of challenge, milk samples were collected and cells from the milk samples were isolated and cell staining was performed using fluorescently labeled antibodies targeted against CD45, CD172α, CD14, CD16, CD3, CD8, NKp46, and a viability dye, Zombie NIR. Flow cytometric analysis was performed using the Cytek Aurora spectral cytometer and data were analyzed using FCS Express (v7; De Novo Software). The percentage of cell expression was then modeled using SAS v. 9.4.

Results
Prior to *S. uberis* challenge, SCFP supplemented animals had a greater percentage of $CD172\alpha^+$ cells with 23.0% (control) versus 28.7% (SCFP; $P<0.01$) on Day 0. The co-expression of $CD14^+$ among the $CD172\alpha^+$ population was evaluated and co-expression was estimated at 41.9% (SCFP; $P<0.04$) and control animals 36.4%. Three days post challenge, the immune cell populations were evaluated again and supplementation with SCFP did not affect the expression of CD16 ($P=0.11$), CD3 ($P=0.49$), CD8 ($P=0.06$), or NKp46 ($P=0.30$).

Conclusions
Before challenge with *S. uberis*, supplementation with SCFP increased the mammary immune cell population of $CD172\alpha^+$ cells possibly driven by the associated increase in $CD14^+$ population but did not affect the expression of other markers. Three days after challenge, SCFP did not influence mammary immune cells possibly a reflection of the severity of the immune response during the course of mastitis infections.

Financial Support
Michigan State University; Diamond V Mills Inc.

Notes:

41 - Novel BoHV-1-vectored Rift Valley fever virus-Sub Vaccine Induces RVFV-specific neutralizing antibodies in calves

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Session: Vaccinology 2, 1/22/2022, 10:30 - 10:45

Objective
Rift Valley fever virus (RVFV) is an emerging pathogen that maintains high biodefense priority based on its threat to cattle and sheep, its ability to cause human hemorrhagic fever and its potential for aerosol spread. Currently available RVFV vaccines have safety and efficacy limitations. Therefore, this research aims to develop a safe and protective bovine herpesvirus type 1 (BoHV-1) vectored RVFV subunit vaccines against RVF.

Methods
We have constructed a BoHV-1 quadruple gene-deleted (UL49.5, glycoprotein G [gG], gE cytoplasmic tail and US9; BoHV-1qmv) vaccine vector expressing chimeric RVFV envelope glycoproteins, Gn ectodomain (eGn) fused with granulocyte-macrophage colony-stimulating factor [GM-CSF] and Gc with self-cleavable peptide 2A (P2A) (designated as BoHV-1qmv RVFV-Sub). Group of eight calves were immunized with BoHV-1qmv RVFV-Sub vaccine, both intranasally and subcutaneously. Safety and efficacy against RVFV were determined. Immunogenicity against RVFV was evaluated by BoHV-1- and RVFV-specific neutralizing antibody titer in serum.

Results
We have characterized the BoHV-1qmv RVFV-Sub vaccine virus for growth kinetics and confirmed chimeric protein expression. The live BoHV-1qmv RVFV-Sub subunit vaccine is safe and highly attenuated in calves. The vaccine virus replicated well in the upper respiratory tract of immunized calves. A single dose of BoHV-1qmv RVFV-sub vaccine immunization resulted in robust BoHV-1- and RVFV-specific neutralizing antibody titer in serum. Specifically, all immunized calves developed very high neutralizing antibodies against BoHV-1.

Conclusions
Taken together, our vaccine efficacy study revealed that calves immunized with a single dose of BoHV-1qmv vectored RVFV-sub prototype subunit vaccine is highly immunogenic, safe and efficacious against RVFV in calves. Assay for BoHV-1qmv RVFV-sub vaccine-induced cell-mediated immune response against RVFV is under progress.

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

Notes:

42 - Prevention of bovine pinkeye by intranasal vaccination with *Moraxella* spp. antigens

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Session: Vaccinology 2, 1/22/2022, 10:45 - 11:00

Objective
Infectious bovine keratoconjunctivitis (IBK; pinkeye) can be difficult to control by parenteral vaccination with commercially available *Moraxella* spp. bacterins. To evaluate if an intranasal vaccine containing *M. bovis* and *M. bovoculi* antigens adjuvanted with a mucoadhesive polymer could prevent IBK and reduce disease severity, a randomized controlled field trial was conducted during summer 2022 in 172 crossbred beef steers in northern California.

Methods
Crossbred beef steers (n=172) without evidence of active IBK were enrolled in this study. Steers were randomly assigned to receive a 2-cc dose of polyacrylic acid plus *M. bovis* and *M. bovoculi* antigens (experimental vaccine group) or polyacrylic acid plus water (control group). Eye exams were conducted once weekly for 16 weeks following primary vaccination to identify corneal ulcers associated with IBK. Steers exhibiting ocular pain from IBK were treated with flunixin meglumine. Oxytetracycline was administered to animals with corneal ulcers >0.5 cm in widest diameter. Nonparametric statistical methods were used to evaluate differences between groups in proportions of animals that developed corneal ulcers associated with IBK on or after Day 21, drug treatments, and ulcer severity. Cattle that developed IBK following corneal trauma associated with plant awns were excluded from the analysis.

Results
Less than half as many steers in the experimental vaccine group developed corneal ulcers associated with IBK versus the control group. Numerically lower rates of treatment with oxytetracycline or flunixin meglumine were observed in the experimental group compared with the control group. These differences were not statistically significant, however, the consistently lower metrics associated with ulcer occurrence and severity support further investigation of this intranasal vaccine against IBK.

Conclusions
The results from this pilot study suggested that intranasal vaccination with *M. bovis* and *M. bovoculi* antigens adjuvanted with polyacrylic acid reduced the occurrence of corneal ulcers associated with IBK and disease severity.

Financial Support
University of California at Davis; School of Veterinary Medicine Dean's Office Discretionary Funds

Notes:

43 - Functional and structural characterization of potential immunogenic proteins of pathogenic *Leptospira*

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Session: Vaccinology 2, 1/22/2022, 11:00 - 11:15

Objective
Leptospirosis is a zoonotic infection considered one of the major causes of morbidity and mortality in humans and animals. Current animal vaccines do not induce long-term protection or prevent renal colonization. The goals of our study were to apply reverse vaccinology strategy using advanced bioinformatic, immunoinformatic and proteomic analyses to identify three immunogenic *Leptospira* proteins, predict their 3-D structures, functions, and immunogenic potential as vaccine candidates to prevent *Leptospira* infection.

Methods
Three-dimensional (3-D) structural modeling was predicted on I-TASSER and compared to solved 3D protein structures available on the Protein Data Bank. Plasmids containing the target genes were purchased commercially and inserted into competent cells of *E. coli* BL21 (DE3) by heat shock. Western blots were performed following an SDS-PAGE run and the reactions antigen-antibody were read using chemiluminescent substrate in a Blot Scanner.

Results
Protein 1 predictions suggest this protein presents an Ycel-like protein domain, which commonly contain 8-stranded β-barrel fold and can play an important function in the metabolism and/or transport of compounds. Protein 2 was predicted as possibly an integral transmembrane protein containing an α-helical region, which is common to chemoreceptors and histidine kinases. It is suggested this protein can be part of a family of bacterial receptors that mediate chemotaxis to diverse signals, presenting both methyl-accepting chemotaxis protein and HAMP domains. The protein 3 was predicted as a hypothetical protein containing a transmembrane α-helix and a non-cytoplasmatic domain/regions. The protein 2 and 3 were expressed in their insoluble forms, presenting the expected molecular masses of 22.3 kDa and 24.5 kDa, respectively.

Conclusions
Our results suggest that two proteins identified and expressed are integral membrane proteins that in theory are easily accessible to host immune system components, and can be potential candidates to develop a vaccine to prevent *Leptospira* infection.

Financial Support
Morris Animal Foundation

Notes:

44 - Protective efficacy of an intramuscular subunit vaccine for *Lawsonia intracellularis* in pigs

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Session: Vaccinology 2, 1/22/2022, 11:15 - 11:30

Objective
Lawsonia intracellularis (L. intracellularis) is an economically important bacterium that is the causative agent of ileitis in pigs which manifests as chronic disease (subclinical but reduced growth kinetics) or acute infection with mortality in grow-finish. We have identified three immunogenic putative surface proteins for vaccine development and herein we use several adjuvants to select the best response to intramulscular immunization.

Methods
1) Five-week-old pigs (n=6) were vaccinated (week 5 and 8) with recombinant F, G, and Y *L. intracellularis* proteins (50 ug each), with Montanide® adjuvants (M1, M2, M3, and M4; Groups 1-4). Groups 5-8 were immunized with adjuvants alone. Sera was obtained for antibody analysis over time. Gut mucosa was collected for antibody quantification and white blood cells were collected on Week 12 to measure antigen-specific IFNy production.
2) Next, the best 3 groups (and control group) were repeated and challenged with pathogenic *L. intracellularis* (n=20 per group) at week 12 then euthanized 18 days later. Clinical analyses will be performed to measure changes in piglet weight, body condition and demeanor. Fecal shedding of bacteria will be quantified. Intestinal lesions were assessed by gross pathology as well as immunohistochemistry.

Results
Pigs in Groups 1-4 responded with significantly higher 'G'-specific serum and gut antibodies in serum relative to controls. However, only vaccines formulated with M1 and M4 adjuvant also led to G-specific IFNγ production. For antigens F and Y, only vaccines formulated with M1 and M4 adjuvants induced significant antibody production. F antigen triggered significant IFNγ production when formulated with M1 adjuvant. The challenge trial is currently underway with antigens F, G, and Y formulated with M1 and M4. Results will be reported in August.

Conclusions
Intramuscular subunit vaccines generated strong antibody and cell-mediated immunity when formulated with M1/M4 adjuvants. A large-scale challenge trial is underway wherein we tested whether these vaccines protect against pathogenic *L. intracellularis* in a barn setting.

Financial Support
Dechra Development LLC Collaborative Research Agreement; Innovation Saskatchewan and the Ministry of Agriculture, Canada Foundation for Innovation through the Major Science Initiatives; Governments of Saskatchewan and Canada under the Agricultural Partnership

Notes:

45 - Overcoming roadblocks for RNA-targeted antiviral therapy in shrimp through an orally-delivered shrimp viral vector

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Session: Vaccinology 2, 1/22/2022, 11:30 - 11:45

Objective
RNA-targeted therapy such as RNA interference (RNAi) was identified as a promising antiviral therapeutic tool for invertebrates. However, the effectivity of RNAi relies on the delivery of double-stranded RNA by injection, which is a limitation for large scale application especially for aquatic organisms. To overcome this, we propose using a replication-deficient virus as vector incorporated into shrimp diet to deliver therapeutic RNA. As proof of concept of our antiviral strategy, our goal is to demonstrate the production, oral delivery, and expression of a gene payload packaged into a viral vector.

Methods
As the viral vector, we used the capsid protein of *Machrobrachium rosenbergii* nodavirus (MrNVcp), a shrimp non-enveloped RNA virus with a genome comprised of RNA dependent RNA polymerase (RNA 1) and capsid protein (RNA 2). We expressed the RNA 2 and replaced RNA 1 with GFP gene as payload to generate vector-payload complex (MrNVcp-GFP) with bac-to-bac® baculovirus expression system in Sf9 cells. MrNVcp-GFP was then mixed into a commercial diet and fed to *Litopenaeus vannamei.* Successful delivery and expression of GFP RNA were assessed by imaging of the viral particles in shrimp cells and RNA detection and quantification of both MrNVcp and GFP in shrimp cells.

Results
The production efficiency of MrNVcp-GFP in Sf9 cells was confirmed with the high GFP expression, analzyed using fluorescence microscopy and flowcytometry. Evidence of assembled viral particles were seen in ultrathin sections of Sf9 cells and negatively-stained purified viral particles using transmission electron microscopy (TEM). Successful oral delivery of the viral vector was evident as MrNVcp and GFP RNA were detected in various shrimp tissues of treated shrimp. In hemocytes cells, viral particles were also observed by TEM; and expression of GFP protein was detected.

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

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture, Agriculture and Food Research Initiative - Foundational and Applied Science; Aquaculture Pathology Laboratory

Notes:

46 - Identification of potentially immunogenic peptide epitopes from Ehrlichia *canis* and *Anaplasma platys*

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Session: Vaccinology 2, 1/22/2022, 11:45 - 12:00

Objective
Ehrlichia canis and *Anaplasma platys* are canine bacterial pathogens transmitted by a common tick vector. We utilized a reverse vaccinology approach to identify unique and shared peptide epitopes encoded in the *E. canis* and *A. platys* genomes with the potential to be used as vaccine candidates

Methods
Initial epitope prediction used Bepipred. A peptide microarray prepared from selected epitopes were screened using sera from dogs positive for *E. canis* and *A. platys*.

Results
We identified 25,928 potential B-cell epitopes. The 4,999 peptides with the best Bepipred scores (2,118 conserved and 2,881 potentially species-specific) were selected to synthesize a peptide microarray. Highly reactive peptides included 406 conserved between both species, 334 potentially specific to *E. canis* and 235 potentially specific to *A. platys*. Although many of these best ranked peptides are derived from well-known surface proteins (surface antigen *mssp4*, type IV secretion system proteins, ankyrin repeat-containing proteins, etc.), several others derived from putative enzymes or DNA-processing proteins, not typically exposed on the cell surface. Additionally, more than 20% of the best-ranked peptides appear to derive from proteins of unknown function. As an additional criterium to prioritize candidates for future validation experiments, we used molecular docking simulation to explore the binding of all reactive peptides to canine MHC alleles DLA-88*001:01 and DLA-88*508:01. A set of thirty peptides with the highest reactivity in microarray experiments were predicted to bind with relatively high docking energy scores to both alleles.

Conclusions
These findings suggest that traditional approaches for the identification of B-cell epitopes, which commonly focused on proteins annotated as membrane-bound or surfaced-exposed, may be biased when dealing with intracellular bacteria such as *Anaplasma* or *Ehrlichia*. In addition, these peptides are attractive candidates for experimental validation, since they can potentially act as both B- and T-cell epitopes.

Financial Support
University of Tennessee; American Kennel Club Canine Health Foundation

Notes:

47 - Mast cell histamine mediates intestinal inflammation to early weaning in piglets via histamine 2 receptor (H2R)

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Session: Immunology 2, 1/22/2022, 10:30 - 10:45

Objective
Early weaning (EW), a necessary but stressful early life management practice induces gastrointestinal (GI) inflammation which is detrimental for short and long-term health. The mechanism by which GI inflammation is triggered is unknown and thus early targeted anti-inflammatory interventions are lacking. Mast cells are major orchestrators of stress-related disorders, especially that of the GI tract. Our previous studies showed that histamine, a major mediator in mast cell granules, is released shortly after EW and is followed by increased gene expression of histamine 2 receptor (H2R) in ileal, jejunal, and colonic mucosa. The precise contribution of histamine and histamine receptor subtypes to weaning stress-induced GI inflammation is unknown. Here we tested the hypothesis that EW-induced GI inflammation is mediated by H2R.

Methods
Fifteen-day-old Yorkshire female and male castrate piglets (n=6-10) were administered either saline vehicle or the H2R antagonist, famotidine (10 mg/kg; intramuscular), 30 minutes prior to weaning. At weaning, piglets were weaned from their dams and housed in nursery pens with ad libitum access to water. At 24 hours post-weaning, mid-jejunum was collected for gene and protein expression. Markers of immune activation including myeloperoxidase (MPO), cytokines, and adhesion molecules were measured by qPCR, Western blot or ELISA. Unweaned control piglets remained with the sow and were collected at the same time as weaned piglets.

Results
Compared with saline-treated controls, piglets administered famotidine had increased gene expression of jejunal adhesion molecules and reduced gene expression of IL6 and reduced protein expression of jejunal MPO and IL1β. Studies to assess the impact of histamine receptors on gut function during the immune response to weaning are currently in progress.

Conclusions
Together, these data demonstrate that histamine via H2R plays an important role in early weaning stress-induced GI inflammation. This provides a potential target for mitigation of the pro-inflammatory stress response to EW practices.

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

Notes:

48 - Application of mRNA to bovine preputial epithelium induces expression of antibodies against *Tritrichomonas foetus*

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Session: Immunology 2, 1/22/2022, 10:45 - 11:00

Objective
Aerosol application of synthetic mRNA to mucosal surfaces can be used to induce the expression of antibodies to prevent infectious diseases. In bulls the lack of a robust immune response against the urogenital parasite *Tritrichomonas foetus* (Tf) leads to chronic infection; currently there are no treatments for bovine trichomoniasis. Application of synthetic mRNA encoding antibodies against Tf to the preputial epithelium of bulls could offer a new therapeutic approach.

Methods
A synthetic mRNA for secreted bovine IgG against Tf surface antigen TF1.17 was applied to preputial epithelium via an atomization device (Teleflex). Two calves were each treated with 3 mg of mRNA in water (total volume of 0.6 mL) and one untreated calf served as a negative control. Preputial secretions were collected with guarded culture swab, and calves were sampled prior to treatment (day 0) and on days(s) 1, 3, 5, 7 and 11 following treatment. Swabs were transferred to 0.3 mL PBS, vortexed and placed in a homogenizer column (Qiagen). The resulting homogenate was applied to 4% paraformaldehyde-fixed Tf mounted on slides and processed for immunofluorescent antibody (IFA) detection of bovine IgG binding to Tf. Laser scanning confocal microscopy and image analysis (Volocity v 6.3.1) were used to determine the fluorescent intensity of thirty Tf in each sample. Statistical analyses were performed using Graphpad Prism v 9.4.1 and a 2-way ANOVA was used to test for treatment effects.

Results
In both of the mRNA treated calves, levels of anti-Tf IgG were significantly higher post-treatment as compared to day 0. From day 0 to day 1 there was a 3-fold increase in mean fluorescent intensity observed in one calf ($P = 0.0175$) and a 10-fold increase was observed in the other ($P < 0.0001$). While the magnitude of the increase varied between the 2 treated calves, no significant increase was observed in the untreated calf.

Conclusions
These preliminary data support the feasibility of utilizing aerosol application of synthetic mRNA to stimulate mucosal immunity and protect against parasites such as Tf at the site of infection.

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

Notes:

49 - Mucosal and systemic secretion of antileukoproteinase during equine inflammation

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Session: Immunology 2, 1/22/2022, 11:00 - 11:15

Objective
Antileukoproteinase (SLPI) is a pleiotropic protein produced at the mucosal surfaces, where it acts to maintain homeostasis. The four most explored properties of SLPI are modulation of inflammation, inhibition of serine proteases, antimicrobial action, and tissue repair. Together SLPI aids in maintenance of barrier integrity and regulating the immune response. Here we characterized SLPI in the horse by developing monoclonal antibodies (mAbs) and defining its role in inflammatory diseases.

Methods
To characterize the role of SLPI in the equine immune response, novel equine mAbs were developed using hybridoma technology. These clones were used for the development of a fluorescent bead-based assay for detection of SLPI secretion. One clone was also used for characterization of cell populations by flow cytometry (FACS) and immunofluorescent (IF).

Results
The newly validated bead-based assay allowed for quantification of SLPI at the mucosa and systemically, through vaginal (n=14), and nasal secretions (n=24), saliva (n=6), and serum (n=184). Healthy horses show significantly higher secretion at mucosal surfaces compared to circulation ($p<0.001$). Using healthy horses to define a preliminary normal range, we observed a significant increase in SLPI secretion during clinical EHV-1 infection, systemically ($p=0.023$) and nasally ($p=0.019$). This trend was also observed during a case of Potomac Hose Fever by measure of systemic secretion of SLPI. To characterize the cell types producing SLPI FACS and IF were used. The primary SLPI producers within circulation are $CD14^+$ monocytes, whereas at mucosal surfaces both epithelial cells and mucosal lymphoid tissues produce SLPI.

Conclusions
Here we developed mAbs specific to equine SLPI which have been tested in a bead-based assay, FACS, and IF. This allowed SLPI detection for the first time in horses, both at healthy and disease stages. Ongoing work is investigating if SLPI plays a role in additional inflammatory diseases induced by pathogens, allergy, or injury, as well as defining its mode of induction and action in the mucosal immune response to infection.

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

Notes:

50 - Impact of equine mesenchymal stromal cell secretome on innate immune response during wound healing

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Session: Immunology 2, 1/22/2022, 11:15 - 11:30

Objective
Despite recent advances and a better understanding of the pathogenesis of wound infections, chronic wound infections continue to be a significant burden in equine medicine. One of the pathogens isolated from infected wounds is methicillin-resistant *Staphylococcus aureus* (MRSA). MRSA infections primarily delay healing by impairing immune cell functions in the wound and are harder to treat due to resistance to several antibiotics. Therefore, new alternatives are urgently needed. This study aimed to investigate the capacity of the equine mesenchymal stromal cell (MSC) secretome to improve pro-healing equine immune responses.

Methods
We used *in vitro* assays to evaluate and compare the effects of the secretome, collected as conditioned medium (CM), of donor-matched equine MSCs isolated from bone marrow (BM), adipose tissue (AT), and peripheral blood (PB), on equine neutrophil and macrophage functions. For neutrophils, chemotaxis, phagocytosis, and production of reactive oxygen species (ROS) were evaluated in the presence or absence of MSC CM and for macrophages, polarization, phagocytosis, and ROS production.

Results
There was a significantly increased chemotaxis of equine neutrophils when treated with AT- and BM-, but not PB-, MSC CM when compared to controls. Their phagocytic ability was decreased when treated with MSC CM, irrespective of MSC source, and neutrophil ROS production was not significantly altered when treated with MSC CM. Macrophages polarized into both M1 & M2, and showed unaltered phagocytosis and ROS production when treated with MSC CM.

Conclusions
The findings generated thus far indicate that MSC CM alters neutrophil functions *in vitro* and can polarize macrophages into both M1 and M2 phenotypes, suggesting that the equine MSC secretome might have the potential to restore impaired immune cell functions in infected wounds. Next steps are to evaluate the effects of the MSC secretome *in vivo*, in the context of MRSA infection.

Financial Support
U.S. Department of Agriculture

Notes:

51 - Trained immunity in bovine innate cell populations followed by subcutaneous BCG administration

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Session: Immunology 2, 1/22/2022, 11:30 - 11:45

Objective
Trained immunity is characterized by an altered epigenetic status of innate immune cells after an initial challenge, resulting in an enhanced immune response to an unrelated second challenge. The Bacillus Calmette Guerin (BCG) vaccine, administered to prevent tuberculosis, is a well-studied inducer of trained immunity in human and mouse monocytes. In the current study, we characterized the trained immune phenotype exhibited by monocytes and gamma delta T cells after BCG administration to pre-weaned calves.

Methods
In two studies, pre-weaned Holstein calves were subcutaneously administered 10^6 or 10^7 CFU BCG Danish strain. Control calves received PBS. Gamma delta T cells and $CD14^+$ monocytes were isolated from peripheral blood at various times after treatment. Cells were stimulated in-vitro with Lipopolysaccharide (1µg/mL), Pam3CSK4 (10µg/mL), or Poly IC (50ug/ml) for 48 hours, and IL-1b, IL-6, and TNFa production were measured by ELISA. Gamma delta T cells from control and BCG-treated calves were also subjected to ATAC-seq to evaluate chromatin accessibility in trained and non-trained cells.

Results
The PBMCs and CD14+ monocytes from BCG calves showed increased IL-1b and IL-6 secretion compared to control calves, consistent with the trained immune phenotype observed in other species. Interestingly, Gamma delta T cells from BCG calves also showed enhanced IL-6 and TNFa production upon in-vitro stimulation with LPS, Pam3CSK4, and Poly IC. Analysis of the ATAC-seq data from gamma delta T cells is currently underway.

Conclusions
BCG vaccine administered subcutaneously can induce trained immunity in bovine innate cell populations. While there are previous reports of innate training being induced in monocytes, we report for the first time that gamma delta T cells, a nonconventional T cell population, have the potential to be trained in vivo following BCG administration. This memory-like phenotype is exhibited by increased cytokine production in response to TLR stimulation. ATAC sequencing will provide novel information regarding the altered epigenetic status of trained innate immune cells in cattle.

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

USDA

Notes:

52 - Soluble epoxide hydrolase activity from various cells in an *in-vitro* model of bovine coliform mastitis

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Session: Immunology 2, 1/22/2022, 11:45 - 12:00

Objective
Soluble epoxide hydrolase (sEH) activity was reported recently to increase during severe bovine coliform mastitis and may contribute to mammary tissue damage by degrading anti-inflammatory and anti-oxidative lipid metabolites. This study determined the activity and expression patterns of sEH in different cell types that contribute to the inflammatory responses in an in vitro bovine coliform mastitis model.

Methods
Raw264.7 cells were used as a proxy for bovine macrophages in initial model development. Bovine-derived hepatocytes (BH) as positive controls, mammary endothelial cells (BMECs), and peripheral bovine mononuclear cells (PBMCs) that basally express the sEH gene and secrete cytokines during acute inflammatory challenges were used in further model development. Inflammatory and prooxidant challenges were induced with lipopolysaccharide and a free radical generator, 2,2′-Azobis(2-methylpropionamidine) dihydrochloride, respectively. The mRNA for cytokines (tumor necrosis factor α, monocyte chemoattractant protein 1) and lipid oxidizing enzymes (cyclooxygenase 2 and sEH), and transcription factor, peroxisome proliferator-activated receptor α, were analyzed by RT-qPCR. The sEH activity was assessed ex vivo by the metabolism of a synthetic metabolite. Untreated cells were negative controls, whereas the PPARα agonist, clofibrate, was a positive control. Data were analyzed by one-way ANOVA and Dunnett's procedure for post hoc adjustment.

Results
Prooxidant challenge was associated with increased sEH mRNA expression in BH; however, there was no corresponding increase in protein activity. Gene expression changes and sEH activity were not detected in Raw264.7 and BMEC cells. In Raw264.7 cells, sEH mRNA expression values were above the maximum cycle-to-threshold value (>35). Although not significant, all treatments numerically increased sEH activity in PBMCs.

Conclusions
Raw 264.7 cells and BMECs are not ideal for assessing sEH dynamics in in-vitro models of coliform mastitis. PBMCs hold promise for future evaluation of the regulation of sEH activity during inflammation and oxidative stress.

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

Notes:

53 - *Lawsonia intracellularis* does not activate canonical Wnt signaling in cell lines *in vitro* or in swine intestine

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Session: Disease Pathogenesis 1, 1/22/2022, 10:30 - 10:45

Objective
Lawsonia intracellularis is an enteric pathogen causing thickening of the intestinal mucosa layer and diarrhea in swine and increases intestinal cell proliferation and undifferentiation. Pathogens, inflammation, and carcinogens activate canonical Wnt signaling causing intestinal hyperplasia and lesions resembling those caused by *L. intracellularis*. Therefore, the objective of this study was to evaluate evidence of canonical Wnt signaling activation caused by *L. intracellularis* infection.

Methods
We analyzed mammalian cell lines and swine enteroids infected with *L. intracellularis* and intestinal tissue of infected pigs. Cell lines Caco-2 (n=2), intestinal porcine enterocytes (IPEC-J2) (n=2), intestinal epithelial cells (IEC-18) (n=2), McCoy cells (n=3), NIH 3T3 reporter cells expressing firefly luciferase in response to Wnt activation (Leading Light Wnt Reporter Assay kit) (n=3), and ileal enteroids (n=3) were cultured and infected with pathogenic (passage<20) *L. intracellularis*. Cell line media and McCoy cell lysate were collected after 48 hours and tested for Wnt activation using a Wnt Reporter Assay. The reporter cell line was treated with Wnt3a as a positive control of Wnt activation. Differences between infected and non-infected cell samples were evaluated by paired T-test in GraphPad Prism.

Results
Compared to cells treated with Wnt ligands, no detectable Wnt activation from media of infected or non-infected cell lines or enteroids was observed, except in reporter cells exposed to McCoy cell media (P=0.03). Activation in all samples was equal or lesser than that of 25 ng/ml Wnt3a, and not different from the negative control (no Wnt3a). To identify evidence of Wnt signaling in infected intestines, archive samples from experimentally-infected pigs were used to evaluate presence of nuclear ß-catenin, the indicator of canonical Wnt signaling activation. Immunostaining showed membrane but no nuclear ß-catenin in lesions and surrounding normal tissue.

Conclusions
In summary, we found no evidence of canonical Wnt signaling activation induced by *L. intracellularis* infection in tissues or *in vitro*.

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

Notes:

54 - Gene editing of pigs to control swine influenza virus infections

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Session: Disease Pathogenesis 1, 1/22/2022, 10:45 - 11:00

Objective
This study aimed to determine the effects of a *TMPRSS2* gene knock-out on influenza virus infection of pigs.

Methods
To determine the effects of transmembrane protease serine 2 (*TMPRSS2*) on influenza virus replication, primary respiratory cells were established from *TMPRSS2* knock-out (KO) and wild-type (WT) pigs and infected with either pandemic H1N1 (pH1N1) or H3N2 subtypes (TX98) of influenza virus. In addition, *TMPRSS2* KO and WT pigs were infected with either pH1N1 or H3N2 subtypes of influenza virus. Clinical samples were collected during the course of infection, and tissue samples were obtained at necropsy.

Results
Infection of primary respiratory cells with either pH1N1 or H3N2 subtypes of influenza virus showed a reduced and delayed virus replication in primary KO cells versus primary WT cells. KO pigs infected with either pH1N1 or H3N2 subtypes of influenza virus shed significantly less infectious viruses in nasal swabs than WT pigs. All KO pigs were negative for virus isolation in nasal turbinates at 3 and 5 days post infection (dpi) with both subtypes, whereas the virus was isolated from several WT pigs at these time points. Virus titers in bronchioalveolar lavage fluids and macroscopic lung lesions of KO pigs were lower than in WT pigs at 3 and 5 dpi.

Conclusions
Previous findings demonstrated the essential role of *TMPRSS2* in influenza A virus replication of various subtypes in mice and human and mouse airway cells. The role of *TMPRRS2* in influenza infections of pigs is still not known. The present study illustrates an altered replication kinetic, significantly decreased virus shedding and improved pathogenicity of influenza viruses in genetically modified *TMPRSS2* KO pigs as compared with WT pigs. The effect of the *TMPRSS2* deletion on influenza replication and shedding in pigs could support the commercial use of such genetically modified swine in order to minimize (i) the economic losses caused by swine influenza virus infection in pigs, and (ii) the emergence of the novel influenza virus with pandemic potential through genetic reassortment in the “mixing vessel”, the pig.

Financial Support
U.S. National Institutes of Health; Genus PLC

Notes:

55 - Experimental study comparing contemporary and historical PRCV isolates with and without subsequent IAV infection

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Session: Disease Pathogenesis 1, 1/22/2022, 11:00 - 11:15

Objective
The primary objective of this study was to compare the infection dynamics of porcine respiratory coronavirus (PRCV) strains isolated in 1991 and 2020. The impact of PRCV on a pig can be two-fold: induction of disease and/or potentiation of other respiratory pathogens. Hence the secondary objective was to investigate the impact of PRCV on subsequent influenza A virus (IAV) infection.

Methods
In brief, 35 4-week-old pigs were divided into six groups: PRCV-2020 (n=5), PRCV-1991 (n=5), IAV (n=5), PRCV-2020/IAV (n=5), PRCV-1991/IAV (n=5) and negative control pigs (n=10). On day 0 of the study, pigs were inoculated intranasally with 4ml of PRCV-1991 or PRCV-2020 strain. PRCV-2020, PRCV-1991, and five negative controls were necropsied at D3. Except for the negative controls, all remaining pigs were challenged at D5 with a contemporary IAV H1N1 isolate. Respiratory scores and rectal temperatures were recorded daily. Nasal swabs were taken daily until D10 and were tested by both PRCV and IAV PCR. Serum samples were collected at D3 and D10 and were tested by IAV NP ELISA and TGEV/PRCV differential ELISA. The second necropsy was done at D10, corresponding to 5 days post IAV challenge. Gross lung lesion scores were assessed and tissues were collected for histopathology and immunohistochemistry.

Results
Until D5, PRCV-2020 pigs had significantly higher RNA shedding than the PRCV-1991 pigs. Pyrexia was observed at D6 in all IAV infected groups, with no significant differences between IAV-infected groups. Higher ELISA antibody titers were observed in pigs infected with PRCV-2020, which correlates with clinical signs and PCR data. Necrosis & inflammation of nasal turbinate were detected in PRCV inoculated pigs. Necrotizing bronchitis and severe interstitial pneumonia were present in both co-infected groups.

Conclusions
Compared to the 1991 PRCV, the 2020 PRCV caused slightly more severe clinical respiratory disease and increased amount and length of shedding. Pre-infection of pigs with PRCV did not enhance disease or lesions caused by IAV infection five days later.

Notes:

56 - A polymerase mutation of genotype 3 hepatitis E virus enhances virus replication in a rabbit HEV infection model

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Session: Disease Pathogenesis 1, 1/22/2022, 11:15 - 11:30

Objective
Chronic hepatitis E virus (HEV) infection has become a significant clinical problem that requires treatment in immunocompromised individuals. In the absence of an HEV-specific antiviral, ribavirin (RBV) has been used off-label but treatment failure may occur due to mutations in the viral RNA-dependent RNA polymerase (RdRp), including Y1320H, K1383N, and G1634R. Chronic hepatitis E is mostly caused by zoonotic genotype 3 HEV (HEV-3), and HEV variants from rabbits (HEV-3ra) are closely related to human HEV-3. Here, we explored whether HEV-3ra, along with its cognate host, can serve as a model to study RBV treatment failure-associated mutations observed in human HEV-3-infected patients.

Methods
By utilizing HEV-3ra infectious clone and indicator replicon, we generated multiple single mutants (Y1320H, K1383N, K1634G, and K1634R) and a double mutant (Y1320/K1383N) and assessed the role of mutations on replication and antiviral activity of HEV-3ra in cell culture. Furthermore, we also compared the replication of the Y1320H mutant with the wild-type HEV-3ra in experimentally-infected rabbits.

Results
Our *in vitro* analyses revealed that the effects of these mutations on rabbit HEV-3ra are altogether highly consistent with those on human HEV-3. Importantly, we found that the Y1320H apparently enhances virus replication during the acute stage of HEV-3ra infection in rabbits, which corroborated with our *in vitro* results showing an enhanced viral replication of Y1320H.

Conclusions
Taken together, our data suggest that HEV-3ra and its cognate host is a useful and relevant naturally-occurring homologous animal model to study the clinical relevance of antiviral-resistant mutations observed in human HEV-3 chronically-infected patients.

Financial Support
U.S. National Institutes of Health

Notes:

57 - *Lactobacillus murinus* activated the aryl hydrocarbon receptor but failed to attenuate *Campylobacter*-induced colitis

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Session: Disease Pathogenesis 1, 1/22/2022, 11:30 - 11:45

Objective
C. jejuni infected IL-10-deficient mice provide a model of inflammatory bowel disease (IBD). Previously, C57BL/6 IL-$10^{-/-}$ mice unexpectedly failed to develop colitis upon infection with *C. jejuni* 11168. We isolated *L. murinus* from these mice, suggesting its role in suppressing *C. jejuni*-induced colitis. This was consistent with ability of this *L. murinus* isolate to attenuate inflammatory responses in an aryl hydrocarbon-dependent manner in an *in vitro* model of colitis. We hypothesized that *L. murinus* LM12 will attenuate *C. jejuni*-induced colitis in IL-$10^{-/-}$ mice.

Methods
4 groups of BALB/c IL-$10^{-/-}$ mice (10/group) were used to test the hypothesis. Mice were inoculated with Group A) $1X10^8$ cfu *L. murinus* LM12, Group B) $1X10^8$ cfu *C. jejuni* 11168, Group C) $1X10^8$ cfu *L. murinus* LM12 followed by $1X10^8$ cfu *C. jejuni* 11168 after 32 days, and Group D) sterile tryptic soy broth. Fecal samples were collected to assess colonization levels in the gut through culture and 16S rRNA gene sequencing. 30 days post-C. *jejuni* challenge mice were sacrificed and assessed for gut pathology.

Results
Group B (*C. jejuni*) and Group C (*C. jejuni/L. murinus)* treated mice developed severe colitis, while other groups had no lesions. *C. jejuni* colonized more abundantly in Group C (*C. jejuni/L. murinus)* than in Group B (*C. jejuni*) mice. Shannon alpha diversity for Group C (*C. jejuni/L. murinus*) mice was significantly less ($P<0.001$) than for Group A (*L. murinus*) and Group D (negative control) mice. Principle components analysis showed the gut microbiota in groups with *C. jejuni* were significantly different than in Groups A and D; both had dysbiosis with increased abundance of *Enterococcus* and *Lactobacillus* and decreased abundance of *Lachnospiraceae*. Colon histopathology scores showed that this *L. murinus* did not protect IL-10-deficient mice from IBD following *C. jejuni* infection.

Conclusions
Prophylactic inoculation of *L. murinus* was not effective in providing protection against colitis in IL-10-deficient mice even though presence of *C. jejuni* caused dysbiosis that significantly enhanced abundance of probiotic *L. murinus.*

Financial Support
Michigan State University; Albert C. and Lois E. Dehn Endowed Professor of Large Animal Clinical Sciences; Enteric Diseases of Food Animals: Enhanced Prevention, Control and Food Safety; University Distinguished Professor, Michigan State University; R21AI121748 National Institute of Allergy and Infectious Diseases, National Institute of Health; U19AI090872 National Institute of Allergy and Infectious Diseases, National Institute of Health

Notes:

58 - Host FBXO22 enhances uptake of *brucella* by macrophages & promotes secretion of pro-inflammatory cytokines

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Session: Disease Pathogenesis 1, 1/22/2022, 11:45 - 12:00

Objective
Brucella species are intracellular bacterial pathogens, causing the world-wide zoonotic disease, brucellosis. Brucella invade professional and non-professional phagocytic cells, followed by resisting intracellular killing and establishing a replication permissive niche. Brucella also modulate the innate and adaptive immune responses of the host for their chronic persistence. The complex intracellular cycle of Brucella majorly depends on multiple host factors but limited information is available on host and bacterial proteins that play essential role in the invasion, intracellular replication and modulation of host immune responses. Hence goal of our study was to identify and characterize the host proteins which play important role in invasion and intracellular survival of Brucella.

Methods
siRNA based screening was employed to identify the host proteins required for Brucella infection. One of the host protein FBXO22 was studied in detail where we have silenced/knocked down or over expressed FBXO22 to study its role in Brucella survival and entry by CFU and immunofluorescence. Further we have used various biochemical, molecular and cell biology techniques like immunoblotting, co-immunoprecipitation, luciferase reporter assay, qRTPCR and ELISA for understanding in detail about the role of FBXO22 during Brucella infection.

Results
Downregulation of FBXO22 by siRNA or CRISPR-dCas9 system, resulted diminished uptake of *Brucella* into macrophages, which was dependent on NF-κB-mediated regulation of phagocytic receptors. FBXO22 expression was upregulated in *Brucella*-infected macrophages that resulted induction of phagocytic receptors and enhanced production of pro-inflammatory cytokines through NF-κB. Furthermore, we found that FBXO22 recruits the effector proteins of *Brucella*, including the anti-inflammatory proteins, TcpB and OMP25 for degradation through the SCF complex.

Conclusions
Our findings unravel novel functions of FBXO22 in host-pathogen interaction and its contribution to pathogenesis of infectious diseases.

Financial Support
Department of Biotechnology - India

Notes:

59 - Adrift in the virosphere: animal health in the age of viral metagenomics

T. Goldberg
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Session: CRWAD Council Keynote and Special Symposium, 01/22/2023, 02:00 - 02:45

Viruses are the most abundant organisms on Earth, yet their diversity is the least explored. Advances in DNA sequencing technology have led to a dramatic recent expansion of the known "virosphere," meaning all viruses in all hosts and environments on our planet. Given this ever-expanding sea of viruses, how might we approach determining which particular viruses are most likely to affect animal and human health? The complexity of the problem is compounded by the fact that the vast majority of viruses identified using metagenomic methods cannot be cultured, and by the sheer volume of new viruses being reported. This talk discusses a range of current and proposed strategies for distinguishing those few viruses that are likely to emerge and cause animal and human disease from the many others that are not. These strategies range from laboratory experiments to predictive computational models, and from global surveys to narrowly targeted monitoring and surveillance efforts. The talk then presents recent examples of newly discovered viruses for which concrete predictions about cross-species transmission have been made. One central example is simian hemorrhagic fever virus and its relatives in the family *Arteriviridae*, which cause economically important animal diseases but have never been documented to infect humans. The talk emphasizes the value of incorporating methods from disparate fields of investigation in the biological and social sciences into an overarching epidemiological framework that takes advantage of careful field observational studies and fortuitous natural experiments. No single approach is adequate.

Notes:

60 - The impact of COVID on agricultural research

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Session: CRWAD Special Symposium, 01/22/2023, 03:00 - 03:45

Texas A&M AgriLife Research is the state's premier research agency in agriculture, natural resources and the life sciences. The agency conducts hundreds of projects spanning many scientific disciplines to deliver life-sustaining and industry-changing impacts to Texans and around the world. Our primary mission is to create, learn, and share knowledge about agriculture and the life sciences that nourishes health, strengthens communities, protects natural resources, and supports economies and our research focus is driven by a strategic plan that focuses on four primary priority areas: 1) leading-edge research and innovations; 2) sustainable production systems; 3) economic strength; and 4) healthy living. To accomplish our goals, the agency provides the research support to the Texas A&M University College of Agriculture and Life Sciences, School of Veterinary Medicine and Biomedical Sciences, and oversees >600 faculty across the state located at 13 Research and Extension Centers. Within this infrastructure, the agency is responsible for 4,465 cattle, 1,217 goats, 880 sheep, 220 swine, 81 horses, 45 guard dogs, and 28 deer. As a result, our agency had to adapt to the onset of COVID, manage research during the pandemic, and ensure the health and well-being of livestock and personnel throughout the pandemic.

Initially, agency mandates focused on eliminating face to face meetings, reducing the number of personnel who could travel in a vehicle, limiting the number of individuals who could be in an office and understanding the nature of human/livestock/wildlife spillover risks of coronavirus. As a result, new human transmission protocols were implemented, eliminating the opportunity for visitors to interact with animals and research personnel, and halting non-critical research. New standard operating procedures (SIOPs) were developed to offset caregiver illness and quarantine, along with halting international efforts. Research livestock and wildlife were impacted by a reduction in efficiency due to absenteeism as a result of employee infection. Social distancing also impacted daily operations, such as feeding and animal treatment. In addition, a significant reduction in workforce (i.e., limited student workers and temporary staff) ensured that only minimal maintenance and repairs were completed. Supply chain issues resulted in disrepair of critical research facilities, reduced operations of key research equipment and machinery and a reduction in efficiency.

While many outcomes of COVID were negative, some faculty had enhanced productivity in grant writing and publications. Online or virtual communications increased the efficiency of conducting meetings and teaching, and animal units were able to enhance their efforts in managing logistical efforts with fewer staff or student workers. As a result, efficiencies in operations have been made, and faculty and staff are more aware of the spread of disease and/or illness from pathogens, so are able to consider methods in reducing the spread. AgriLife Research has returned to full pre-COVID operations, while still dealing with delays in supply chains and a challenge in student labor who have become less reliable, who expect to work remotely and insist on higher pay. Our agency is better prepared for a future pandemic, while understanding the negative impacts of COVID on our research animals, personnel and facilities.

Notes:

61 - What happened? COVID-19 and the meat industry

K. McCullough
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Session: CRWAD Special Symposium, 01/22/2023, 04:15 - 05:00

The Coronavirus pandemic (COVID-19 or pandemic) was declared a national emergency on March 13. The meat and poultry industry, and the livestock sector more generally, was at the forefront of much of the discussion regarding the pandemic's adverse economic impact and worker safety. The North American meat industry implemented important protocols and procedures to protect its workforce from the threats posed by the pandemic. The learning curve dealing with this unprecedented disruption was steep at times and challenges were presented regarding testing and acquiring personal protective equipment. But from the pandemic's inception meat and poultry companies worked with federal, state, and local health officials to exchange information and implement practices and procedures to best protect workers.

While the news coverage highlighted some of the devastating impacts of COVID-19, it often did not accurately articulate what was going on inside meat and poultry plants or the efforts these plants were taking to protect employees. COVID-19 illness estimates show that after peaking in the spring of 2020, illness associated with meat and poultry plant employees took a steady decline and only saw increases following major holidays. The perceptions regarding worker safety based on spring 2020 conditions did not match the Fall of 2020 and beyond realities of worker safety in meat packing plants.

The pandemic's spread was unprecedented and placed the meat and livestock industry under great strain. Throughout the spring and summer of 2020, the North American meat industry implemented important protocols and procedures to protect its workforce from the threats posed by the pandemic. These efforts yielded considerable success, as evidenced by the data. The industry's efforts were guided by the urgency of protecting the health and safety of its workforce; and ensuring meat packing and processing plants, a vital, part of the nation's critical infrastructure, continue to operate to provide a safe and secure supply of high-quality, nutritious protein to the U.S. and the world.

Notes:

62 - Conducting research during a global pandemic: musings of an early career scientist

N.R. Noyes
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Session: CRWAD Special Symposium, 01/22/2023, 05:00 - 5:45

The COVID19 pandemic presented tremendous challenges for workers across all sectors, including academia. This talk will focus on challenges faced by academic researchers, with a special emphasis on early career faculty. The challenges will be briefly described and their potential short- and long-term impacts discussed. While the lived experience of the COVID19 pandemic is highly individualized, this talk will attempt to cover shared experiences. Special attention will be given to how these shared experiences might shape faculty perspectives and academic research amongst this particular cohort of early career scientists.

Notes:

63 - Heterologous prime-boost vaccination: from COVID-19 to HIV

S. Lu
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Session: Animal Vaccinology Research Network Symposium, 01/23/2023, 08:30 - 09:15

In the last 30 years, our team has established the concept of the heterologous prime-boost vaccination in which two different types of vaccines are used to delivery the same pathogen antigens to maximize the protective immune responses. This concept was further confirmed by recent finding that prime-boost with two different types of COVID-19 vaccines frequently can elicit better protective immune responses than using the same type of COVID-19 vaccine. Using the same concept, our group has developed the world first polyvalent DNA prime-protein boost HIV vaccines which demonstrated the robust antibody and T cell immune responses in recently completed multi-center, double blind human clinical studies managed by the HIV Vaccine Trial Network (HVTN). The immune responses showed broad cross reactivity against a wide range of HIV subtypes. My talk will share our vision on how the heterologous prime-boost vaccination may shape the future of vaccinology which should have impact to the development of both human and animal vaccines with improved immune responses and protection efficacies.

Notes:

64 - Time-release microneedle-based vaccine platforms

T. Nguyen
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Session: Animal Vaccinology Research Network Symposium, 01/23/2023, 09:15 - 10:00

Objective
The ability to transform medical polymers, commonly used for resorbable surgical sutures, into desired 3D forms/shapes/structures at nano and micro scales with "smart" functions, while sustaining the materials' excellent biocompatibility and biodegradability, provides significant applications in different biomedical fields, ranging from tissue engineering and controlled drug/vaccine-delivery to medical implanted devices. Here, I will present our recent research works to develop novel vaccine delivery systems which are made by a newly developed 3D additive manufacturing process. This vaccine system in the form of a skin patch relies on tiny microneedles which can be painlessly administered onto the skin at a single-time and pre-programmed to deliver stable vaccine antigens repeatedly over a long period, simulating the immunogenic effect of multiple bolus injections in the conventional vaccination process. Our lab also develops the microneedle patch with a sustained delivery for other medicines such as anti-HIV drugs or anti-viral antibodies.

Notes:

65 - Effect of veterinary feed directive regulation on violative antibiotic residues in tissues of food animals in the US

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Session: Food Safety, 1/23/2022, 08:30 - 08:45

Objective
To our knowledge, no study has quantified the effect of Veterinary Feed Directive (VFD) regulation on detecting violative levels of residues of penicillin, tetracycline, and sulfonamides in the tissue of food animals in the U.S. This study objective was to investigate the effect of VFD regulations on the detection of violative levels of residues of penicillin, tetracycline, and sulfonamides in the tissue of food animals that were slaughtered in slaughterhouses in the U.S.

Methods
A dataset of the United States National Residue Surveillance Program was analyzed, particularly an inspector-generated sampling dataset from 2014 through 2019, were analyzed using multivariable logistic regression models. Penicillin, tetracycline, and sulfonamides were selected as target antibiotics for the analysis because they are commonly used in food animals in the U.S. The final model's results were reported as odds ratio (OR) with a 95% confidence interval.

Results
With regards to penicillin, the type of animal and type of tissue sampled were significantly associated with residue violation, but VFD regulation was not. Regarding tetracycline, the type of animal and type of tissue sampled were significantly associated with residue violation, but VFD regulation was not. For sulfonamides, compared to before the VFD regulation period, the odds of detecting the violative level of sulfonamides residues in the tissue of food animals (irrespective of animal type) decreased by 36% after VFD regulation.

Conclusions
After the VFD regulation period, the odds of detecting the violative level of sulfonamide residues decreased significantly in the tissue of food animals. In contrast, there was no significant decrease in the odds of detecting the violative level of penicillin and tetracycline residues in the tissues of food animals. Further investigation of the factors that influence the violative level of penicillin and tetracycline residues in the tissues of food animals after VFD regulations would lend clarity to this critical issue.

Notes:

66 - Comparison of antimicrobial resistance among *Salmonella* serovars isolated from Canadian turkey flocks, 2013-2021

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Session: Food Safety, 01/23/2023, 08:45 - 09:00

Objective
The emergence of antimicrobial resistance (AMR) in *Salmonella enterica* isolates of turkeys is a significant issue. This study analyzed data collected between 2013 and 2021 by the Canadian Integrated Program for Antimicrobial Resistance Surveillance on-farm surveillance program to determine AMR and differences in AMR patterns among major *Salmonella* serovars recovered from pooled fecal samples of turkeys.

Methods
Salmonella isolates were tested for susceptibility to 14 antimicrobials using a broth microdilution method. Hierarchical clustering dendrograms (heatmaps) were constructed to compare the individual AMR status of *Salmonella* serovars. Differences in the probability of AMR among *Salmonella* serovars were determined using logistic regression models with the generalized estimating equation method to account for farm-level clustering.

Results
Of 1,367 *Salmonella* detected, 55.3% were resistant to at least one antimicrobial and 25.31% were multidrug-resistant (MDR). The *Salmonella* isolates were highly resistant to tetracycline (43.3%), streptomycin (47.18%), and sulfisoxazole (29.11%). The most common serovars identified were *S.* Uganda (22.9%). *S.* Hadar (13.53%), and *S.* Reading (12%). Streptomycin-sulfisoxazole-tetracycline (n=204) was the most frequent multidrug-resistant (MDR) pattern. Heatmaps showed *S.* Reading co-resistance to ciprofloxacin and nalidixic acid, *S.* Heidelberg to gentamicin and sulfisoxazole, and *S.* Agona to ampicillin and ceftriaxone. *Salmonella* Hadar isolates compared to all the other serovars had higher odds of resistance to tetracycline (OR:152.06, 95% CI:70.63-327.36) while the probability of being resistant to gentamicin and ampicillin was significantly higher in *S.* Senftenberg than in all the other serovars. Moreover, *S.* Uganda had the highest odds of being MDR (OR: 4.73, 95 % CI: 3.68-6.07).

Conclusions
The high resistance to some antimicrobials warrants re-assessment of antimicrobial stewardship strategies. Differences in AMR patterns highlight the need to implement serovar-specific mitigation strategies.

Notes:

67 - Natural and targeted bacteriophage treatments as a *Salmonella* pre-harvest mitigation technique in cattle feedlots

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Session: Food Safety, 1/23/2022, 09:00 - 09:15

Objective
This study assesses the effect of bacteriophage as a pre-harvest strategy to reduce *Salmonella* or shift to pan-susceptible *Salmonella* serovars in the feedlot environment to prevent dissemination into beef cattle and their products.

Methods
A 2 x 2 full-factorial unbalanced experimental field trial was conducted by applying treatments to the feedlot environment: 1) A naturally-occurring bacteriophage environmental slurry applied through dust abatement sprinklers on a weekly basis 2) A targeted bacteriophage cocktail applied via backpack sprayers on a bi-weekly basis. Samples were collected weekly from pen environment (n=18) and beef cattle (n=178; brisket swabs, rump swabs and fecal grabs). Subiliac lymph nodes were harvested at slaughter. Samples were selectively enriched and cultured for *Salmonella* and isolates (n=603) underwent whole genome sequencing.

Results
Salmonella prevalence was reduced in cattle samples from pens treated with natural phage (36.9%, 32.5%, 19.4%), phage cocktail (36.3%, 32.5%, 13.1%) and combination treatment (37.8%, 34.2%, 23.0%) compared to the control group (55.1%, 49.5%, 34.7%) for brisket, rump, fecal grabs respectively. *Salmonella* was detected in only one lymph node. A multilevel mixed effects logistic regression model was used to determine that the combination treatment was most effective at reducing *Salmonella*; however, rarely significantly. *Salmonella* serovars 61:l,v:1,5, Anatum, Cerro, Kentucky, Lille, Lubbock, Montevideo, Muenster, and Virginia were identified. A phylogenetic analysis determined that *Salmonella* serovar patterns were clustered by feedlot pen.

Conclusions
All bacteriophage treatment groups reduced *Salmonella* compared to the control group across the trial, albeit not significantly. Natural phage or combination treatment resulted in the largest reduction of *Salmonella,* especially within hide samples. We were unable to analyze the impact of treatments on *Salmonella* in cattle lymph nodes due to low prevalence. Overall, these results suggest that phage may be a promising treatment to reduce *Salmonella* in beef cattle and the feedlot environment.

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

Notes:

68 - Prevalence of shiga toxin genes in *E. coli* DNA extracted from finisher pig feces in Illinois, USA

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Session: Food Safety, 1/23/2022, 09:15 - 09:30

Objective
Shiga toxin-producing *E. coli* (STEC) is the leading cause of bloody diarrhea, hemorrhagic colitis, and hemolytic uremic syndrome (HUS), becoming a major concern to food safety and a threat to public health. Bovids are the key reservoir of the pathotype globally, however, recent studies show that STEC is also commonly detected in healthy pigs, suggesting contaminated pork products may be associated with HUS cases. Subsequently, the USDA National Animal Health Monitoring System (NAHMS) carried out a study to examine STEC prevalence in US swine farms but only 60 samples were included from Illinois. To fully survey the prevalence of STEC in Illinois, this study looks at the trends in pig fecal samples collected from different regions of the state over a year.

Methods
Samples were collected almost monthly from a local processing facility from October 2021 to October 2022. In total, 521 fecal samples were collected from healthy finisher pigs statewide then DNA was cultured and extracted using a commercial kit. DNA was tested through PCR by targeting the Shiga toxin-producing genes (*stx1, stx2*), the subtypes (*stx2a*, *stx2c*, *stx2d*, *stx2e*), as well as the *F18* fimbrial gene.

Results
Data showed that the *stx2* gene was more prominent (61%; 318/521) than *stx1* (0.38%; 2/521). Of these, 73% (233/318) had *stx2e*, 3% (10/318) *stx2d*, and 37% (119/318) had *F18*. A significant increase in the detection rate of the *stx2* gene was observed at the end of winter (March 19%; 19/100) compared to the end of summer (September 100%; 70/70). The detection rate in Oct 21, April, May, June, Oct 22 was 36, 67, 56, 85, 66, respectively. Moreover, the highest detection rate of the *F18* gene (64%; 45/70) in *stx2* positive samples was observed in September. Likewise, *stx2d*, which is a highly virulent clinical subtype associated with severe human illness, was highest in *stx2* positive samples in Oct 21 at 33% (6/18).

Conclusions
These data indicate a high prevalence of swine-adapted STEC in Illinois finisher pigs, particularly in the warm season, which is of food safety, porcine husbandry and welfare, and public health concern.

Financial Support
University of Illinois; AES Research Incentive Grant

Notes:

69 - *Ex vivo* evaluation of modified probiotic metabolites with prebiotic like components against *Campylobacter*

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Session: Food Safety, 1/23/2022, 09:30 - 09:45

Objective
Previously it was reported that cell free cultural supernatant (CFCS) of probiotic *Lactobacillus casei* with *mcra* (myosin-cross-reactive-antigen) over-expressed (LC^{+mcra}) with prebiotic like component, berry phenolic extracts (BPEs) can reduce growth of *Campylobacter* in *in vitro* condition but their effect in presence of normal bacterial flora in complex gut ecosystem is not known yet. The purpose of this study was to evaluate combined effect of CFCS-LC^{+mcra} with BPEs against *Campylobacter* in a simulated *ex vivo* poultry gut condition as well as their effect on normal microflora.

Methods
Freshly obtained cecal content from chicken (inoculated with kanamycin resistant strain of *Campylobacter jejuni*, CJ-KM) were incubated at standard condition with CFCS-LC^{+mcra} and/or BPEs. Effect on growth of CJ-KM and natural *Campylobacter* was observed by cultural methods and16S metagenomic analysis was used to determine the effect on microbiome. Significance in difference was determined by ANOVA.

Results
Combined effect of CFCS-LC^{+mcra} (10%) and BPEs (0.1 mg GAE/ml) on CJ-KM or *Campylobacter* growth reduction was more efficient than their individual effect at both 24h and 48h time points. After 24h of incubation, either CFCS-LC^{+mcra} or BPEs or their combination reduced growth of CJ-KM and *Campylobacter* by 1.65 log CFU/ml and 2.8 log CFU/ml, by 1.5 log CFU/ml and 2.67 log CFU/ml ($p<0.05$), and by 1.85 log CFU/ml and 3.3 log CFU/ml, respectively ($p<0.05$). The levels of Firmicutes decreased more in the control group (from 88.5% at 0h to 55.17%) than the treatments ($p<0.05$) and for Proteobacteria, treatments reduced the level of increment compared to control (cecal content without CFCS-LC^{+mcra} or BPEs) at 48h time point. There was also notable change in the percentage of abundance various genus at both 24h and 48h time points ($p<0.05$) but the change in alpha diversity at species level was not significant ($p<0.05$).

Conclusions
This *ex vivo* observation recommended that combination of CFCS-LC^{+mcra} and BPEs can effectively reduce colonization of *Campylobacter* without noticeable detrimental effect on gut microbiome.

Financial Support
University of Maryland; U.S. Department of Agriculture, National Institute for Food and Agriculture Exploratory Grant: 2018-67030-27426

Notes:

70 - Use of a postbiotic product to reduce *Salmonella* prevalence in the peripheral lymph nodes of cull dairy cattle

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Session: Food Safety, 1/23/2022, 09:45 - 10:00

Objective
The objective of this study was to evaluate whether the whole farm use of a postbiotic product derived from the fermentation of *Saccharomyces cerevisiae* (SCFP) was associated with a reduction in the prevalence of *Salmonella* in the subiliac lymph nodes (LN) of culled dairy cattle at slaughter in different regions and seasons.

Methods
In collaboration with two commercial processing plants in the Southwestern (SW) and Northeastern (NE) regions of United States, culled dairy cattle lots processed on the same week from dairy farms that fed SCFP or did not feed the product (no-SCFP) were identified and sampled. Up to 20 LN were collected from each farm. A total of 1,773 LN, collected between May 28, 2021, and November 30, 2022, were tested for *Salmonella* using culture and PCR methods.

Results
A total of 1,773 LN, collected between May 28, 2021, and November 30, 2022, were tested for *Salmonella* using culture and PCR methods. The overall crude *Salmonella* prevalence in LN (across regions and sampling months) was 9.8% (173/1,773). Although no significant difference in *Salmonella* prevalence was observed between SCFP and no-SCFP farms ($p = 0.73$), there was an interaction effect between season and region ($p = 0.04$). The prevalence of *Salmonella* in the SW region was 9.9% (7/71), 9.9% (14/142), 15.2% (23/152), and 22.1% (71/316), whereas in the NE region, the prevalence was 7.1% (5/70), 9.9% (20/202), 4.2% (21/487), and 4.0% (12/301) during the Winter, Spring, Summer, and Fall seasons, respectively. A subset (n = 123) of the positive isolates has been serotyped so far; 14 different serotypes were identified, with the dominant serotypes being Montevideo (47.2%; 58/123), Cerro (15.4%; 19/123), Muenster (9.8%; 12/123) and Mbandaka (8.9%; 11/123).

Conclusions
Our preliminary results indicate that *Salmonella* prevalence in culled dairy cows is not affected by the administration of the SCFP product, but instead, region and season affected the prevalence. Continued research to identify effective pre-harvest strategies to reduce the burden of *Salmonella* is necessary to ensure food safety and protect public health.

Financial Support
National Cattlemen's Beef Association - Beef Checkoff

Notes:

71 - Development of electrochemical sensors for on-farm detection of bovine infections

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Session: Diagnostic Testing, 1/23/2022, 08:30 - 08:45

Objective
The objective of this work is to demonstrate selective, sensitive, reliable, and repeatable electrochemical detection of antibody-binding to antigenic proteins (gE, gB and NS3) indicative of exposure to Bovine Herpes Virus-1 (BHV-1) and Bovine Viral Diarrhea (BVDV) respectively.

Methods
Capture antigen proteins were expressed within an *E. coli* expression system to sufficient levels to be applied to sensor platforms. A variety of chromatographic techniques were used to purify the expressed proteins. Western blot and ELISA assays were used to characterize the efficacy of recombinant protein antigen synthesis and to assess immune specificity. Several gold sensor surface linker molecules including carboxyl terminated thiol self-assembled monolayers and ortho-aminobenzoic acid (o-ABA) polymers were used for sensor functionalization. Surface Plasmon Resonance was used to independently demonstrate receptor functionalization and antigen capture. Multiplexed biosensor chips were designed and fabricated for use in electronic biosensing with chronoamperometry used to electrodeposit gold foam nanostructures and a biocompatible antifouling hydrogel on the surface of working electrodes. The biosensors were characterized using electrochemical impedance spectroscopy, cyclic voltammetry and differential pulse voltammetry (DPV).

Results
A two-step strategy comprising ammonium sulphate precipitation followed by anion exchange chromatography was demonstrated as the optimum approach to enrich/purify the viral antigen proteins expressed within *E. coli*. The o-ABA functionalized gold electrodes and microfabricated sensors demonstrated highly efficient electroanalytical performance with excellent reproducibility. Results for electrochemical sensing of NS3, gE and gB will be demonstrated.

Conclusions
Recombinant expression produced sufficient levels of viral protein antigens at required purity to allow for incorporation into developed electronic biosensor assay platforms. The fabricated sensor design increases the biosensing operational efficiency by enabling multiple immunologic assays to be run on single chips.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture; Department of Agriculture, Environment and Rural Affairs of Northern Ireland; Department of Agriculture, Food and the Marine of Ireland

USDA

Notes:

72 - Performance evaluation for the real-time detection of digital dermatitis in dairy cattle on edge devices

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Session: Diagnostic Testing, 1/23/2022, 08:45 - 09:00

Objective
Digital dermatitis (DD) is a bovine claw disease responsible for ulcerative lesions on the coronary band of the foot. DD is associated with massive herd outbreaks of lameness and influences cattle welfare and production. Early detection of DD can lead to prompt treatment and decrease lameness. Computer vision (CV) provides a unique opportunity to improve early detection. The study aims to train lightweight CV models for constrained environments and compare edge devices for the real-time detection of DD in dairy cows. CV models were trained for detection and scoring, compared using performance metrics and inference time, and automated for real-time detection using images and video on portable devices.

Methods
Images were collected from commercial dairy farms while facing the interdigital space on the plantar surface of the foot. Images were scored for M-stages of DD by a trained investigator using the M-stage DD classification system. The dataset contained 240 M0, 17 M2, 51 M2P, 114 M4H, and 108 M4P images. Models were trained to detect and score DD lesions, embedded on multiple edge devices, and compared for precision, recall, and mean average precision (mAP) in addition to inference time in frame per second (FPS).

Results
Tiny YOLOv4 achieved an mAP between 0.895 whereas Mobilenet SSDv2 yielded an mAP of 0.538. Both TensorFlow models were first converted to OpenVINO Intermediate Representation and then converted to blob files for use on DepthAI platform. Both models processed images at approximately 40 FPS on an OAK-1 or OAK-D-Lite connected to a Raspberry Pi or Jetson Xavier NX above the benchmark for real-time detection at 30 FPS. Tiny YOLOv4 was able to detect all five class labels on images, video files, and live video.

Conclusions
The CV models were able to detect DD lesions on an edge device with high performance and speed. The proposed CV tool can be used for early detection and prompt treatment of DD in dairy cows. This result is a step towards applying CV algorithms to veterinary medicine and implementing real-time DD detection on cattle farms.

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

Notes:

73 - Antigen cartography for the determination of historical SARS-CoV-2 variant exposure in animals using serology

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Session: Diagnostic Testing, 1/23/2022, 09:00 - 09:15

Objective
SARS-CoV-2 can infect many animals, and recent evidence suggests the continued circulation of ancient SARS-CoV-2 variants in white-tailed deer. Several variants of SARS-CoV-2 have since emerged, and it is critical to determine the specific SARS-CoV-2 infecting and/or circulating in a given animal species. As the window of virus detection by PCR is very narrow, serological assessment is an efficient and practical way of detecting spillover infection of animals. We developed a process to identify the exposure of animals to a particular SARS-CoV-2 variant by serology using pseudovirus neutralization assay coupled with antigen cartography.

Methods
Serum samples from cat (n=272) and white-tailed deer (n=170) were initially screened with a surrogate virus neutralization assay and the positive samples were further analyzed with pseudovirus neutralization assay using lentiviral backbone-based pseudovirus carrying spike from either B.1 lineage, Alpha, Beta, Gamma, Delta and Omicron variants. The reciprocal of serum dilution showing 50% inhibition of pseudovirus infection (NT_{50}) were used to create antigen cartography using ACMACS web-software.

Results
We tested 15 cat and 32 deer serum samples in pseudovirus neutralization assay and the antigen cartography positioned most of the cat and deer sera close to B.1 lineage (mean distances of cat and deer sera: 1.17±0.39, 1.13±0.63), Alpha (1.17±0.52, 1.09±0.63,) and Delta (0.75±0.55, 1.08±0.48,) variants compared to Beta (1.8±0.64, 2.64±1.18,), Gamma (1.59±0.58, 2.35±1.16) and far from Omicron (2.1±0.65, 3.67±1.07). It indicates that deer and cats were exposed to B.1 lineage, Alpha, Delta, or Beta, and cross-neutralize these antigenically related variants. Six out of 15 cat sera had significant NT_{50} to Omicron.

Conclusions
The pseudovirus neutralization coupled with antigen cartography is a valuable method to identify the SARS-CoV-2 variant that infected a given animal. This process help demonstrate the level of cross-protection of vaccines, re-infection, the emergence of novel variants, and circulation of ancient SARS-CoV-2 variants in animals.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture; Huck Institutes of the Life Sciences, Pennsylvania State University

Notes:

74 - MinION-based semi-targeted PCR-Seq of infectious bronchitis viruses: balancing inclusivity and sensitivity

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Session: Diagnostic Testing, 1/23/2022, 09:15 - 09:30

Objective
Infectious bronchitis is one of the most important and economically devastating diseases of poultry, causing a range of clinical signs. The etiology, infectious bronchitis virus (IBV; species: *Avian coronavirus*), is genetically diverse, with much of the diversity located within the S1 subunit of the spike gene, leading to the emergence of new serotypes and genotypes. Accurate IBV genotyping is an important step for IBV identification, vaccine planning, and tracking of this global virus. Genotypic classification is based on the complete S1 nucleotide sequence; however, its genetic diversity can hinder inclusivity of pan-IBV PCR assays. The objective of this study was to develop a semi-targeted PCR-based sequencing (ST-PCR-Seq) method to detect and characterize IBV from diagnostic samples.

Methods
An IBV-targeted hexamer was identified and used to design a reverse primer for cDNA synthesis, and an overlapping reverse primer for PCR. Total RNA was extracted from tissues using routine diagnostic methods. IBV RNA was reverse transcribed in a strand switching (SSW) reaction, followed by PCR amplification using the overlapping reverse primer and a forward primer targeting the SSW adapter. Samples were barcoded using the MinION PCR-based protocol. MinION libraries were multiplexed and synthesized using standard protocols. Raw reads were basecalled, demultiplexed, and taxonomically classified using a desktop PC.

Results
IBV ST-PCR-Seq was able to accurately identify varying IBV lineages in tissues, including multiple lineages per samples. ST-PCR-Seq provided more reads than random sequencing. Degenerate primers were required to improve inclusivity.

Conclusions
The results demonstrate the utility of using ST-PCR-Seq with the MinION for the identification and characterization of IBV. This method can be adapted for other viruses and other sequencing platforms.

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

Notes:

75 - Effect of swab retention in MTM on detection of ASF virus DNA in blood swab samples

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Session: Diagnostic Testing, 1/23/2022, 09:30 - 09:45

Objective
The preferred sample type for African Swine Fever Virus (ASFV) detection is whole blood. The use of Molecular Transport Medium (MTM) provides a safe, fast, and efficient method of sampling. The main objective was to compare cycle threshold (CT) for ASFV and internal control (IC) targets between blood in MTM with and without retention of blood swab, and to compare CTs for ASFV and IC targets between whole blood and blood mixed in MTM.

Methods
Three sample types (whole blood, blood in MTM with immediate removal of swab, and blood in MTM with retention of swab for 10 min) were generated from 48 ASFV positive and 12 ASFV negative blood samples. Xeno DNA was included as extraction internal control (IC) and DNA was extracted in triplicates. ASFV real time PCR were performed, and fluorescent data were analyzed with threshold of 0.1 ΔRN to determine CT values. ANOVAs of CT values was performed separately for each PCR target and CT values among three sample types were compared with two tailed t-test.

Results
No significant differences in CTs values were observed between blood in MTM in samples with or without retention of swab for ASFV and Xeno. ASFV CT for blood in MTM were about 2 CT higher than whole blood, possibly due to dilution of blood in MTM media. The Xeno CTs were similar between whole blood and blood in MTM with swab. No false negative results were recorded for all three blood sample types.

Conclusions
Results of the study shows that immediate removal of swab after transferring blood to MTM did not alter the PCR results and CT values of both PCR targets when compared to leaving the swab in the MTM tube. Our results support immediate removal of spun swab after swirling into MTM media does not compromise the specificity of the PCR assay.

Notes:

76 - Sensitivity of standing fecal flotation in hookworm identification in fecal aliquots from shelter canines

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Session: Diagnostic Testing, 1/23/2022, 09:45 - 10:00

Objective
Ancylostoma/*Uncinaria* spp (hookworms) are common canine intestinal parasites easily transmitted and in dogs and carry a zoonotic risk. Centrifugal fecal flotation is preferred for fecal detection but due to practicality, speed, and low economic cost, shelters and veterinary hospitals frequently use standing fecal flotation. Standing fecal flotation methods vary in flotation media and fecal mass. Fecal loop tools are often employed for fecal collection, and generally collect 0.5 or 0.25 grams(g). The objective of this study was to test the test sensitivity of standing fecal flotation to detect hookworm eggs when using varying masses of fecal samples compared to a 2-gm sample.

Methods
Thirty-one voided samples were collected from apparently healthy dogs in shelters. Eighteen dogs not yet treated for intestinal parasites, or those that had been treated within the previous three days or two weeks or longer, with at least one of 20 samples with hookworm eggs were included. For each sample, aliquots of 2, 1, 0.5, 0.25 g were evaluated for hookworm ova. Average EPG were calculated using the 2 g aliquot based on the presence of hookworms. Results were recorded with Excel and analyzed with SAS Studio 9.4.1.

Results
Hookworm eggs were detected from 331 of the 360 fecal floats from dogs with at least one sample with hookworm eggs. Compared to the 2 g sample standard, lower masses were had statistically lower test sensitivity (98, 96, 92, and 88 for 2, 1, 0.5, and 0.25-gram samples, respectively).

Conclusions
From a clinical standpoint however, each size aliquot provided a reasonable probability of identifying hookworm ova if the animal was shedding hookworm ova.

Notes:

77 - National surveillance of antimicrobial use in Canadian feedlots to date

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Session: Antimicrobial Use 1, 1/23/2022, 08:30 - 08:45

Objective
A pan Canadian feedlot beef cattle antimicrobial use (AMU) and antimicrobial resistance (AMR) surveillance network (CFBSN) was established in partnership between the Canadian government, feedlot stakeholders, veterinary practitioners, and producers. The surveillance network aims to (1) provide representative estimates of AMU within Canadian feedlots and (2) monitor AMU trends over time.

Methods
Sample size calculations were designed to select a representative sample of feedlot cattle in Alberta, Saskatchewan and Ontario. To be included in the project, feedlots must have a one-time capacity of >1,000 cattle, hold a valid veterinary-client-patient relationship, and participate in the finishing phase of cattle production. AMU data is collected from (1) sentinel feedlots enrolled in the project via the consulting veterinarian and (2) antimicrobial drug dispensing data directly from the supervising veterinary clinics. Sentinel feedlots retrospectively provide the following information specific to each participating production lot: active ingredients being prescribed, the concentration of the active ingredient, the route of administration, the indication for AMU, and the total number of exposures to the antimicrobial drug (AMD) throughout the production period. Additional data on farm demographics and animal health are part of the minimum data set required for sentinel feedlot surveillance. Data are collected in a standardized spreadsheet and uploaded to a project-developed relational database.

Results
Disease prevention was the primary reason for in-feed AMU. Most in-feed AMU regimens were for respiratory illness (41.62%), digestive illness (20.10%) and liver abscess (16.67%) prevention. Comparatively, disease treatment was the primary reason for injectable AMU, with most regimens for respiratory disease (40.81%) and lameness (26.21%). AMU trends are reported for the active ingredient, year, and cattle demographics.

Conclusions
The CFBSN provides robust data to support AMU transparency and antimicrobial stewardship discussions.

Financial Support
Government of Canada; Canadian Agricultural Partnership; Beef Cattle Research Council; Beef Farmers of Ontario; Public Health Agency of Canada

Notes:

78 - Surveillance of antimicrobial resistance in bovine respiratory disease pathogens on Canadian feedlots

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Session: Antimicrobial Use 1, 1/23/2022, 08:45 - 09:00

Objective
The Canadian Feedlot Beef Surveillance Network [RD1] is one of the few programs monitoring national trends in the antimicrobial resistance (AMR) of veterinary pathogens. The objective of this work was to provide representative estimates of and monitor temporal trends in AMR in select bovine respiratory disease (BRD) pathogens.

Methods
Deep nasopharyngeal samples were collected from a target 16 animals from 26 finishing feedlots representative of the fed cattle population in Canada from 2019-2021. Cattle from the same production lot were sampled at both arrival and rehandling, and the primary isolation of *M. haemolytica, P. multocida* and *H. somni* was conducted at Prairie Diagnostic Services, Inc. in Saskatoon, Saskatchewan. Recovered isolates were subject to susceptibility testing via the broth microdilution method and interpreted with reference to CLSI guidelines.

Results
P. multocida was recovered in greater proportions than *M. haemolytica* and *H. somni* at both arrival and rehandling across all years. The recovery of *M. haemolytica* was substantially lower at both time points than was estimated at the project outset; follow-up investigation revealed a negative correlation between time to processing after sample collection and *M. haemolytica* recovery. There were no statistically significant differences from 2019 to 2021 in the proportion of BRD isolates resistant to the tested antimicrobials at either time point. Significant increases in resistance to the macrolide and tetracycline class antimicrobials between arrival and rehandling were detected when the *P. multocida* isolates were combined across three years. A similar but non-significant increase in macrolide resistance between time points was observed for the combined *M. haemolytica* isolates.

Conclusions
Increasing resistance over the feeding period to antimicrobials used frequently in feedlot medicine is consistent with previous reports. The relatively small number of recovered BRD isolates per year may limit the statistical power to detect relevant changes in resistance prevalence between sampling times and/or across years.

Financial Support
Government of Canada; Canadian Agricultural Partnership; Beef Cattle Research Council; Beef Farmers of Ontario; Public Health Agency of Canada

Notes:

79 - Targeted metaphylaxis using rectal or infrared temperature in high-risk steers during the feedlot receiving period

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Session: Antimicrobial use 1, 1/23/2022, 09:00 - 09:15

Objective
The objective was to evaluate rectal temperature and infrared thermography as qualification for targeted metaphylaxis, and the impacts on clinical health, growth performance, hematology, and serum haptoglobin in high-risk, newly received beef steers during a 42-day feedlot receiving period.

Methods
Crossbred beef steers (n = 240, arrival BW = 258 ± 22.5 kg) were used where pen was the experimental unit. Experimental treatments included: injection with sterile saline (NCON); conventional metaphylaxis administered to all steers (CONV); targeted metaphylaxis administered to steers with rectal temperature ³ 39.7 °C (RECT); or targeted metaphylaxis administered to steers with ocular infrared temperature ³ 39.7 °C (EYE). Blood samples for quantification of complete blood cell counts and serum haptoglobin were collected on days 0, 14, and 42 relative to metaphylaxis.

Results
Metaphylaxis was administered to 0% of NCON, 100% of CONV, 48.9% of RECT, and 48.6% of EYE steers ($P < 0.01$). Therapeutic treatment rate for BRD did not differ ($P = 0.16$), but overall treatment rate was minimal. Both methods of targeted metaphylaxis decreased total mL of antimicrobials administered compared to CONV ($P < 0.01$). Metaphylaxis accounted for 83.3% of total antimicrobials administered to RECT and 58.7% of total antimicrobials administered to EYE. Body weight, DMI, DMI as % of BW, and G:F did not differ at any time point ($P > 0.07$). From days 0 to 42, ADG was greatest in CONV and RECT least in NCON and intermediate in EYE ($P < 0.01$). No treatment differences in complete blood count were noted ($P > 0.10$).

Conclusions
Herein, both methods of targeted metaphylaxis decreased antimicrobial administration relative to conventional metaphylaxis. Steers administered targeted metaphylaxis based on rectal temperature had comparable ADG to those administered conventional metaphylaxis. Metaphylaxis is a widely used disease management tool in feedlots, and utilization of targeted preventatives with measures of body temperature may decrease total antimicrobial use while maintaining optimal health and growth performance outcomes.

Financial Support
Foundation for Food and Agricultural Research

Notes:

80 - Correlation between cephem and *bla*$_{CMY-2}$ resistance in *S. enterica* and *E. coli* from human and food animal sources

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Session: Antimicrobial Use 1, 1/23/2022, 09:15 - 09:30

Objective
The objective of this study was to compare and correlate the annual prevalence of ceftriaxone, cefoxitin, ceftiofur, and *bla*$_{CMY-2}$ resistance in *Salmonella enterica* and *Escherichia coli* from the national surveillance programs in the United States.

Methods
Using datasets retrieved from the surveillance programs of the United States National Antimicrobial Resistance Monitoring System for Enteric Bacteria from 2002 to 2018, we used Spearman's correlation analysis and beta regression model to correlate and compare the annual prevalence data, respectively.

Results
We observed a near-perfect positive correlation in annual prevalence between cefoxitin (ρ=0.97, P<0.0001), ceftiofur (ρ=0.96, P<0.0001), ceftriaxone (ρ=0.95, P<0.0001) resistance and *bla*$_{CMY-2}$ resistance in *Salmonella enterica* recovered from chicken retail meat. Similarly, we observed a very high positive correlation in annual prevalence between cefoxitin (ρ=0.94, P<0.0001), ceftiofur (ρ=0.91, P<0.0001), ceftriaxone (ρ=0.82, P<0.0001) resistance and *bla*$_{CMY-2}$ resistance in *Salmonella enterica* recovered from turkey retail meat. There was no correlation (i) between the annual prevalence of cephem resistance and *bla*$_{CMY-2}$ resistance in Salmonella isolates from the human surveillance program (P>0.05), (ii) in cephem resistance prevalence between the chicken and turkey retail meats (P>0.05), (iii) in cephem resistance prevalence between the retail meats and humans (P>0.05), and (iv) in *bla*$_{CMY-2}$ resistance between the chicken and turkey retail meats (P>0.05). *bla*$_{CMY-2}$ producing *Salmonella enterica* was less likely recovered in pork (OR=0.57, 95%CI: 0.35-0.94) and turkey (OR=0.23, 95%CI: 0.15-0.36) compared to chicken meat. Comparison of annual prevalence of cephem-resistance between the food animal and human sources varies depending on the bacteria type (*Salmonella enterica* vs. *Escherichia coli*) and surveillance period.

Conclusions
Correlation between the annual prevalence of cephem and *bla*$_{CMY-2}$ resistance suggests either data can be used as a proxy for decision-making in retail meat surveillance programs.

Notes:

81 - β-lactamase genes in Enterobacteriaceae from the food animal and human surveillance programs in the United States

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Session: Antimicrobial Use 1, 1/23/2022, 09:30 - 09:45

Objective
The spread of beta-lactamase-producing Enterobacteriaceae is a major public health concern around the globe. The study aimed to identify and explore the distribution of beta-lactam resistance genes reported in three surveillance programs (cecal, retail meat, and human) in the United States.

Methods
We retrieved and analyzed data from the United States National Antimicrobial Resistance Monitoring System for Enteric Bacteria from 2002 to 2021.

Results
A total of 109 beta-lactamase genes were detected in Enterobacteriaceae; 3 genes in *E. coli* (*bla*$_{\text{CMY-2}}$, *bla*$_{\text{TEM-1A}}$, and *bla*$_{\text{TEM-1B}}$), 6 genes in *Salmonella enterica* (*bla*$_{\text{CARB-2}}$, *bla*$_{\text{CMY-2}}$, *bla*$_{\text{CTXM-65}}$, *bla*$_{\text{TEM-1A}}$, *bla*$_{\text{TEM-1B}}$, and *bla*$_{\text{HERA-3}}$), and 2 genes in *Campylobacter* spp. (*bla*$_{\text{OXA-61}}$ and *bla*$_{\text{OXA-449}}$) have been detected across food animals (cattle, chicken, swine, and turkey) and humans over the study period. *bla*$_{\text{CTXM-55}}$ has been detected in *E. coli* isolates from the four food animal sources while *bla*$_{\text{CTXM-15}}$ and *bla*$_{\text{CTXM-27}}$ found only in cattle and swine. In *Salmonella enterica*, *bla*$_{\text{CTXM-2}}$, *bla*$_{\text{CTXM-9}}$, *bla*$_{\text{CTXM-14}}$, *bla*$_{\text{CTXM-15}}$, *bla*$_{\text{CTXM-27}}$, *bla*$_{\text{CTXM-55}}$, and *bla*$_{\text{NDM-1}}$ were uniquely detected in human isolates and *bla*$_{\text{SHV-12}}$ in cattle, swine, and humans. *bla*$_{\text{OXAs}}$ were the only genes detected in *Campylobacter* spp. The proportions of beta-lactamase genes in *E. coli* includes: *bla*$_{\text{CTXM-1}}$; 0.1-3.5% (2017-21), *bla*$_{\text{CTXM-15}}$; 0.3% (2018), *bla*$_{\text{CTXM-55}}$; 0.3-5.0% (2017-18), *bla*$_{\text{CTXM-27}}$; 0.3-2.3% (2016-21), *bla*$_{\text{CMY-2}}$; 0.21-12.4% (2016-21). The proportions in *Salmonella enterica* includes: *bla*$_{\text{CTXM-1}}$; 0.01-1.9% (2013-21), *bla*$_{\text{CTXM-15}}$; 0.05-0.91% (2015-21), *bla*$_{\text{CTXM-55}}$; 0.03-6.3% (2013-21), *bla*$_{\text{CTXM-65}}$; 0.4-14.7% (2013-21), *bla*$_{\text{CMY-2}}$; 0.7-57.1% (2004-21), *bla*$_{\text{SHV-2}}$; 0.7-5.3% (2011-18), *bla*$_{\text{SHV-12}}$; 0.07-1.96% (2014-21), and *bla*$_{\text{NDM-1}}$; 33.3% (2011). While the proportion of *bla*$_{\text{OXA-61}}$ in *Campylobacter* spp. ranges from 7.2-96.3% (2013-21).

Conclusions
This study provided information on the beta-lactamase genes detected in Enterobacteriaceae in food animals and humans in the United States. This information is necessary for a better understanding of the epidemiology of these genes in the USA and globally.

Notes:

82 - Prevalence and relatedness of *Salmonella* recovered from Ohio and Wisconsin livestock markets

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Session: Antimicrobial Use 1, 1/23/2022, 09:45 - 10:00

Objective
Livestock markets are critical infrastructure used for animal sales and movement that influence pathogen dissemination on a regional and national scale. Indeed, a 2016 *Salmonella* serovar Heidelberg outbreak, linked to surplus dairy calves at livestock markets, sickened 63 people across 17 states. This repeated cross-sectional study aimed to assess the prevalence of multidrug-resistant *Salmonella* ser. Heidelberg and other serovars of public health relevance in Ohio and Wisconsin livestock markets.

Methods
Twenty-four livestock markets (14 from WI and 10 from OH) that frequently sold surplus calves were enrolled. Market environments were sampled twice three months apart. State inspectors used boot swabs to sample the following areas: loading dock, main livestock thruway, and two pens used to hold surplus calves. Swabs were shipped to the Wisconsin Veterinary Diagnostic Laboratory for *Salmonella* culture, serotyping, and antimicrobial susceptibility testing. Whole genome sequencing was conducted at the Ohio Department of Agriculture and the Applied Microbiology Services Laboratory at The Ohio State University.

Results
Sample-level *Salmonella* prevalence was 99.1% (111/112) in Wisconsin markets and 94.9% (75/79) in Ohio markets. Negative samples from Ohio were from the same market across both sampling dates. Serogroups B, C2, and D1 isolates were selected for susceptibility testing and whole genome sequencing. Multidrug resistance (resistant to 3 or more classes) was exhibited by 41% (12/29) of tested isolates. *Salmonella* ser. Heidelberg was not recovered from any samples; however, other serovars of public health relevance were identified, including Newport and Agona. Sequencing data revealed related strains across markets. For instance, a closely related *Salmonella* ser. Panama group was recovered from three Wisconsin markets. These isolates clustered closely with isolates derived from retail meat, other livestock species, and clinical human cases.

Conclusions
Results suggest livestock markets play an important and under-recognized role in pathogen dissemination between livestock populations.

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

Notes:

83 - Prevalence of Q fever antibodies in a large study of domestic goat does in the USA and associated risk factors

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Session: Epidemiology 1, 1/23/2022, 08:30 - 08:45

Objective
Q fever (coxiellosis) is caused by the zoonotic pathogen *Coxiella burnetii.* Infection can cause significant reproductive and financial losses. However, Q fever is an underreported disease; and seroprevalence studies in United States livestock are limited to small sample sizes within a defined geographical area. To address this and other knowledge gaps, the NAHMS completed a national cross-sectional goat industry study in 2019 across 24 States, representing 75.8% of goat operations with 5 or more goats and 80.4% of U.S. goats on operations with 5 or more goats.

Methods
Serum samples were collected from 7,736 goat does 15 months of age or older across 647 operations that completed questionnaires. The presence of *C. burnetii*-specific antibodies was determined by indirect ELISA (IDEXX). ELISA and questionnaire data were combined to identify associated risk factors.

Results
These results are significant as less than 1% of operations suspected Q fever presence on their operation in the previous 3 years. This study identified an overall *C. burnetii* seroprevalence of 14.5% (SE = 2.3) in domestic goat does, and an operation-level prevalence of 21.0% (SE = 2.4) in the U.S. We identified that among does on positive operations, there was a higher seroprevalence among dairy producing does compared to non-dairy producing does. Study results also show that operation-level seroprevalence increased with increasing herd size, but geographical seroprevalence rates were similar among West and East regions.

Conclusions
This highlights a potential lack of disease reporting and importance of conducting studies which report disease prevalence. This study identifies risk factors such as herd health management activities that may be used to update doe management practices to prevent infection of a herd and to limit infection spread within an operation on which Q fever is present.

Notes:

84 - Prevalence and seasonality of Bluetongue Virus on the Front Range of Colorado in domestic ruminants during 2021

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Session: Epidemiology 1, 1/23/2022, 08:45 - 09:00

Objective
Bluetongue virus (BTV) is an economically significant pathogen affecting both wild and domestic ruminants around the world. Although endemic in the United States, climate variability has resulted in increased outbreaks for previously naïve populations, likely due to the range expansion of the insect vector, the *Culicoides* midge. Additionally, only serotypes 2, 10, 11, 13, and 17 are considered endemic in the US, with serotypes 11 and 17 historically reported in Colorado. This work aims to provide current information regarding the seroprevalence, seasonality associated with active infections, and to identify which serotypes are present on the Front Range of Colorado.

Methods
Serum and whole blood samples were collected from five sheep and five cattle sites along the Front Range of Colorado from August through December 2021. Serial samples were collected from the same animals at four sheep sites and one cattle site with cross-sectional samples collected from the remaining sites. Pan-BTV and serotype-specific RT-qPCR, along with cELISA, were used to evaluate these samples.

Results
Site-level seroprevalence and viral RNA prevalence in sheep flocks ranged from 7-50% (mean=33%) and 0-67% (mean=20%), respectively, while cattle herds ranged from 27-93% (mean=51%) and 7-40% (mean=22%), respectively. Of samples for which serotypes have been identified, BTV-6 and BTV-11 compromise the majority of positive samples with BTV-6 accounting for 53% of positive sheep samples and 31% of positive cattle samples. For flocks and herds in which serial sampling was performed, most positive cases occurred during the months of September and October, with RNAemia continuing through December.

Conclusions
The preliminary data from this work highlight the presence of an exotic BTV serotype, BTV-6, in addition to expanding upon previous studies examining prevalence of BTV within sheep and cattle populations in the region. These findings support the continued circulation of BTV in domestic livestock populations, exemplify BTV-6 as a newly endemic serotype, and will provide data for predictive modeling efforts.

Financial Support
U.S. Department of Agriculture, Agriculture and Research Services Agriculture and Food Research Initiative (2018-04648) as part of the joint U.S. Department of Agriculture - National Science Foundation-National Institute of Health-Biotechnology and Biological Sciences Research Council- Binational Science Foundation Ecology and Evolution of Infectious Diseases Program

Notes:

85 - True prevalence and predictive value of FeLV and FIV virus test results among northern Mississippi shelter cats

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Session: Epidemiology 1, 1/23/2022, 09:00 - 09:15

Objective
The objective of this study was to determine the true prevalence (TP) of FeLV and FIV in northern Mississippi shelter cats and to estimate positive (PPV) and negative (NPV) predictive values.

Methods
Zoetis Witness test was performed on blood collected from 337 apparently healthy and 27 sick shelter cats (>6 months of age) from northern Mississippi shelters. The uncertainty of test performance and apparent prevalence were simulated 5000 times using an excel spreadsheet model to obtain an estimate of uncertainty about true prevalence and predictive value.

Results
Three healthy cats (0.89%; 95%CI 0.18%, 2.6%) tested FeLV-positive, and nine (2.7%; 95%CI 1.2%, 4.01%) tested FIV-positive. Two sick cats (7.4%; 95%CI 0.91%, 24.3%) tested FeLV-positive and two cats (7.4%, 95%CI 0.91%, 24.3%) tested FIV-positive. No cats tested positive for both viruses concurrently. Median and 95% credible interval (CI) for FeLV TP was 3.4% (CI 0.01%, 19.9%) and 0.06% (0.01%, 0.51%) for sick and healthy cats, respectively. For FIV, TP was 0.1% (CI 0.01%, 14.1%) and 0.06% (CI 0.01%, 1.5%) for sick cats and healthy cats, respectively. Median PPV for FeLV was 36.1% (CI 0.11%, 93.8%) and 1.1% (CI 0.1%, 33.0%) for sick and healthy cats, respectively. Median NPV for FeLV was 99.8% (CI 96.6%, 99.9%) and 99.9% (CI 99.9, 99.9%) for sick and healthy cats, respectively. Median PPV for FIV was 1.2% (CI 0.09%, 84.5%) and 0.65% (CI 0.08%, 39.8%) for sick and healthy cats, respectively. Median NPV for FIV was 99.9% (CI 98.4%, 100%) and 99.9% (CI 99.9%, 100%) for sick and healthy cats, respectively.

Conclusions
Further testing needs to be conducted on sick cats before a recommendation can be made, however, shelters should be comfortable with not testing healthy cats based on the low prevalence.

Financial Support
Mississippi State University; Mississippi State University House Officer Grant

Notes:

86 - Causative agents for fowl typhoid and pullorum disease in poultry and approach to control

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Session: Epidemiology 1, 1/23/2022, 09:15 - 09:30

Objective
Due to the rise in consumer demand for organic products, many conventional poultry farms are now growing poultry without antibiotics or synthetic chemicals. In addition to this, pasture/organic poultry farms have increased significantly in the U.S. and they are also antibiotic- and chemical-free. Both antibiotic-free conventional and pasture poultry farmers are facing the re-emergence of bacterial diseases, including fowl typhoid caused by *Salmonella enterica* subsp. *enterica* serovar Gallinarum biovars Gallinarum (*S.* Gallinarum) and pullorum disease caused by *Salmonella enterica* subsp. *enterica* serovar Gallinarum biovars Pullorum (*S.* Pullorum). These diseases cause higher mortality rate in the birds and lead to non-profitable poultry farming.

Methods
In this study, we have investigated *S.* Gallinarum and *S.* Pullorum prevalence in the environment and farms' products. We also determined antimicrobial resistance patterns of *S.* Gallinarum and *S.* Pullorum. To achieve this goal, we aim to investigate potential sources and modes of entry for these bacterial pathogens. We have collected 1,285 samples from farms and investigated the prevalence of *S.* Gallinarum and *S.* Pullorum within farm ecology/environment by using cultural and molecular techniques. We have also determined the antibiotic resistance pattern of the isolates following CLSI guidelines.

Results
A total of 218 *Salmonella* isolates were recovered, with 17.9% prevalence from farms poultry samples in the year 2012-2014 ($P<0.05$) and 14.9% from chicken products in 2019 ($P<0.05$). From the isolated *Salmonella*, 9.2% was confirmed for *S.* Gallinarum and 5.0% for *S.* Pullorum. The overall multi-antibiotic resistance in recovered *S.* Pullorum and *S.* Gallinarum was 27.6%.

Conclusions
Overall, the data shows that both *S.* Pullorum and *S.* Gallinarum are commonly present in poultry farm environment as well as the products sold in the markets, which justifies implementing the required control strategies to reduce *S.* Pullorum and *S.* Gallinarum transmission.

Financial Support
University of Maryland; Indonesia Endowment Fund for Education (LPDP)

Notes:

87 - Prevalence and contributing factors of enteric parasites in cats and dogs on intake to Mississippi shelters

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Session: Epidemiology 1, 1/23/2022, 09:30 - 09:45

Objective
Shelters frequently administer multiple anthelminthic therapies to animals on intake, but superfluous use of anthelminthics may contribute to resistance over time. The objective of this study was to evaluate the prevalence of common enteric parasites in shelter animals on intake and factors that may contribute to parasitism.

Methods
Fecal samples were obtained from cats and dogs prior to administration of anthelminthics and examined via passive flotation. Presence or absence of common parasite eggs was noted along with descriptive characteristics (age, altered status, intake source). Multivariable, multi-level logistic regression with shelter as a random effect (SAS9.4) was used to test for associations (alpha=0.05).

Results
Overall prevalence of any parasite was 66% (58.6%-73.7%) in dogs and 44% (29.0%-60.1%) in cats. Prevalence of concurrent infections in dogs (n=161) with 2, 3, and 4 parasites was 33.5% (26.3%-41.4%), 3.73% (1.4%-7.9%), and 0.6% (0.01%-3.4%), respectively. Prevalence of concurrent infections in cats (n=43) with 2 parasites was 16.3% (6.8%-30.7%); no cats had more than 2 parasites concurrently. Hookworms (81%, 72.6%-88.2%) were the most common parasite noted in dogs, though roundworms (63%, 38.4%-83.7%) were the most common parasite in cats. Altered dogs had significantly lesser odds of parasitism (p=0.0021).

Conclusions
Internal parasitism was common among dogs and cats upon intake to a shelter. Altered dogs had less risk for parasitism but the reason for this relationship was not clarified in this study.

Financial Support
Mississippi State University House Officer Grant

Notes:

89 - Understanding Ontario horse owners' attitudes and perceptions towards biosecurity during the COVID-19 pandemic

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Session: Biosecurity & Infection Control 1, 1/23/2022, 08:30 - 08:45

Objective
What motivates horse owners to implement biosecurity could bridge the gap in the horse industry's ability to prepare for disease outbreaks. The COVID-19 pandemic has provided a chance to investigate attitudes toward biosecurity and whether these attitudes are translating into equine care. This qualitative study aims to explore horse owner perceptions, attitudes, and experiences relating to on-farm biosecurity during the COVID-19 pandemic.

Methods
Semi-structured interviews were conducted virtually with 9 horse owners across Ontario, Canada (June to August 2022). Recruitment utilized social media snowball sampling where equine and veterinary organizations shared advertisements with horse owners. Interviews were recorded, transcribed, and analyzed using reflexive thematic analysis.

Results
The COVID-19 pandemic resulted in increased public health measures implemented at Ontario equine farms, which simultaneously protected horse owners against COVID-19 and acted as increased biosecurity for horses. However, horse owners viewed these measures as COVID-19 specific and did not perceive them as playing a part in reducing equine pathogen spread. This distinction may be signifying which biosecurity practices horse owners see as effective. Vaccinations against equine pathogens were regarded by horse owners as bulletproof biosecurity measures for their horses. Additional measures beyond vaccines were seen as overkill unless there was perceived risk of an outbreak. However, many horse owners found that equine disease outbreaks were not being effectively communicated to them, which resulted in fewer horse owners feeling a need to implement biosecurity.

Conclusions
This study has provided insight into horse owners' perceptions of biosecurity. Biosecurity concepts were not successfully being translated into the horse industry, as horse owners were not distinguishing their experiences with COVID-19 as applicable to horse care. The findings from this study can be used to improve horse management practices and improve how biosecurity is communicated to horse owners.

Notes:

90 - Illinois veterinarians' attitudes, knowledge, and practices toward biosecurity

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Session: Biosecurity & Infection Control 1, 1/23/2022, 08:45 - 09:00

Objective
Biosecurity practices are crucial to prevent the spread of infectious diseases among animals and humans. Veterinarians play a vital part in implementing effective biosecurity methods in their practice and sharing biosecurity knowledge with their clients. There is a lack of information on Illinois veterinarians' perception, knowledge, and practices related to biosecurity. This study assessed the biosecurity practices of Illinois veterinarians employed in clinical practice and compared their biosecurity awareness and knowledge to veterinarians working in non-clinical settings.

Methods
All veterinarians registered with the Illinois State Veterinary Medical Association (ISVMA) were surveyed online between October- November 2021 using the Qualtrics^{xm} software. Differences in Illinois veterinarians' biosecurity knowledge and practices were assessed using logistic regression models. A significant association was demonstrated using the Wald $\chi 2$ test with a P-value ≤ 0.05.

Results
In total, 104 veterinarians completed the questionnaire of which 88% were veterinarians in clinical practice, and 12% were in other work settings. Among clinical veterinarians, 88% worked with companion animals and 12% with farm animals (bovine and swine). Sixty-five percent of the respondents were females. Seventy percent of the clinical veterinarians had more than 15 years of experience. Among all veterinarians, the odds of having biosecurity training (OR=5.75; 95 % CI = 1.69-19.56) and knowledge of biosecurity guidelines (OR=4.81; 95% CI= 1.37-16.92) were significantly higher in non-clinical veterinarians. Within clinical veterinarians, farm animal veterinarians compared to companion animal veterinarians had significantly higher odds of having biosecurity training (OR=15.31; 95% CI=3.67-63.97) and knowledge of biosecurity guidelines (OR=7.49; 95% CI = 1.82-30.91).

Conclusions
Based on the study results, a gap in biosecurity knowledge was evident among companion animal veterinarians, suggesting a need for biosecurity training and educational program.

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

Notes:

91 - Enhancing food sustainability through reducing the risk of transboundary anaimal diseases.

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Session: Biosecurity & Infection Control 1, 1/23/2022, 09:00 - 09:15

Objective
Animal disease outbreaks are a major concern for stakeholders across the food supply chain. Due to their international impact, discussions have shifted to preventive measures aimed at protecting livestock while ensuring food security and safety. Structurally, emergency assistance has been a response option during pandemics. However, this may not be sustainable because of public pressure to cut down government expenditure. Our hypothesis is that an indemnity policy that is conditioned on showing biosecurity protocols would increase adoption and reduce government expenditure during outbreaks.

Methods
We developed and launched a survey from March to July 2022 targeted at swine producers across the US. We examined the socio-psychological factors that influence farmers' decisions to adopt biosecurity measures on their farms. We presented farmers with different scenarios, such as being compensated for demonstrating their biosecurity plans or investing in livestock insurance and asked whether they would be willing to adopt these measures.

Results
The results showed that there are different groups of farmers with different motivations and incentives for implementing biosecurity measures and this can be identified by different behavioral groups of farmers.

Conclusions
All in all, the data suggest that understanding the factors that influence behavior and decision-making in the livestock industry can help to design more effective interventions and policies for disease prevention and risk management. For instance, disease reporting helps to identify and track outbreaks, allowing for timely response and control measures. Indemnification programs provide financial compensation to farmers for losses, helping to protect their income and livelihoods. However, challenges such as underreporting, overreporting, monitoring and surveillance, knowledge, trust, incomplete information, and inadequate funding for indemnification programs must be addressed in order to improve the effectiveness of these systems.

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

Notes:

92 - Discovering leverage points for enhancing biosecurity in swine production networks using agent based models

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Session: Biosecurity & Infection Control 1, 1/23/2022, 09:15 - 09:30

Objective
Human behavior, organizational strategies and public policies play a significant role in the introduction, spread and containment of animal diseases. Detection and mitigation strategies against the introduction of disease are commonly termed "biosecurity". While there are generally accepted biosecurity best management practices (BMPs) to support herd health, an improved understanding of the leverage points and mediating factors of biosecurity BMP adoption at the producer level can inform the design of biosecurity risk management strategies at inter-organizational network and policy scales. Using novel Artificial Intelligence (AI) technologies such as Agent Based Models (ABMs), the goal of this study is to discover leverage points for enhancing biosecurity in swine production networks.

Methods
In this study, an ABM simulates the spread of socioeconomically important diseases, e.g., porcine epidemic diarrhea virus (PEDV) and African swine fever (ASF), through livestock production chain networks. The model uses a GIS-based spatial framework, with three important hog-producing U.S. states—North Carolina, Iowa, and Illinois—defining the study areas. Four types of agents exist in the model: hog producers, feed mills, slaughter plants, and auction houses. More than 10,000 Monte Carlo simulation runs are performed to discover leverage points for enhancing biosecurity in the three sampled states for PEDV and ASF.

Results
We discovered that the fraction of biosecurity investments, and the proportion of risk-averse, risk-neutral and risk-tolerant producers at initial and final states of the simulation runs are the most important leverage points. A random forest algorithm applied to Monte Carlo results provides ranking of conditional leverage points for PEDV and ASF.

Conclusions
AI and complex systems modeling tools can provide critical foresight about leverage points to reduce the risk of pathogen transmission among swine supply chains of the US; and inform the design of risk management strategies at inter-organizational and policy network scales.

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

Notes:

93 - Understanding swine producers' and veterinarians' biosecurity perception and practices: a scoping review

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Session: Biosecurity & Infection Control 1, 1/23/2022, 09:30 - 09:45

Objective
Swine infectious diseases continuously pose a threat to the health and productivity of swine and the economy of the swine industry worldwide. Swine farms can be protected against the introduction and spread of endemic and foreign animal diseases by adopting effective biosecurity practices. Implementation of biosecurity systems can be influenced by the disease risk perception of swine producers, veterinarians, and other swine industry stakeholders. However, despite significant advances made in swine biosecurity over the last few decades, some swine producers do not see biosecurity as an important component of disease prevention. This proposed study aims to comprehensively review the existing literature on the biosecurity perception, knowledge, and practices of swine producers and veterinarians. In addition, we propose a thorough evaluation of the research methodology adopted to conduct these studies worldwide.

Methods
We used the Preferred Reporting Items for Systematic Reviews and Meta-Analyses extension for scoping reviews (PRISMA-ScR) framework for this scoping review. Five bibliographic databases were used to conduct a comprehensive search. Reference screening of screened full texts articles was conducted to identify any missed articles. A multi-stage screening process was followed. Two authors created and pretested two independent forms for the abstract screening and review characterization. The abstract and full-text screening was performed by a single reviewer using those forms.

Results
A total of 435 articles were identified based on the relevance screening criteria of their title. Of these, 216 duplicates were excluded. The remaining 219 articles will undergo abstract screening and analysis.

Conclusions
The results from this study can be used to develop a more effective biosecurity plan and scoring system and can guide animal health policymakers to improve disease prevention.

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

Notes:

94 - I have gloves!: food animal veterinarians' perceptions of zoonotic disease risks and prevention

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Session: Biosecurity & Infection Control 1, 1/23/2022, 09:45 - 10:00

Objective
Zoonotic disease exposure is expected while practicing veterinary medicine. However, reports of occupational infections among US veterinarians may indicate a lack of adherence to appropriate infection prevention strategies. To identify key targets for infection control strategies among veterinary personnel, this pilot study's objectives were to, 1) investigate food animal practitioners' perceptions about zoonotic disease exposure and personal risk of infection, and 2) evaluate infection prevention decision-making.

Methods
Three food animal practitioners from Trinidad & Tobago participated in this study. Each practitioner completed a 40 to 60-minute semi-structured interview conducted and recorded via Zoom®. Participants were asked about their demographics, perceptions of zoonotic risk, and routine field infection control practices. Each participant also responded to a case scenario of a diarrheic calf suggestive of *Cryptosporidium parvum*. Transcripts were generated using software, checked for accuracy, and thematic analysis was conducted using NVivo® software.

Results
Respondents agreed unanimously about the importance of zoonotic disease exposure risk. However, responses to the case scenario highlighted differences in practitioners' perceived level of risk and infection control decision-making, particularly regarding the appropriate level of PPE required for field practice, including the diarrheic calf scenario. This suggests that in the absence of employing 'standard' infection control practices, individual perceptions may lead to varying levels of exposure and preventable zoonoses in practitioners.

Conclusions
These preliminary results suggest that while food animal practitioners have a general awareness of their risk of exposure to zoonotic disease, the application of infection control practices is inconsistent, highlighting the absence of a standard. This study presents an opportunity for expansion to food animal practitioners providing greater insight into evidence-based, best practices infection prevention strategies in the veterinary profession.

Notes:

95 - Emerging technology and potential applications in animal health

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Session: Animal Vaccinology Research Network Symposium, 01/23/2023, 10:30 - 11:00

Innovation and emerging technology in animal health is often driven by funding source priorities, industry unmet commercial needs, and secondary technology development from the human vaccine segment. At the present time vaccine efforts are driven by the following general priorities:

- Pandemic preparedness, emerging diseases, and zoonosis
- Antimicrobial resistance
- Artificial intelligence and machine learning
- One health
- Sustainability
- Food security
- Availability of labor
- Animal welfare

The animal health industry has a wide variety of vaccines that range from autogenous, killed bacterins, killed viral, attenuated live, subunit protein, vector-based products, and a variety of combination vaccine products. Advances in both technology and CVB policy is leading a general shift in technology development to address the above priorities and providing novel, innovative, and faster vaccine solutions.

The CVB has recognized the need for the ability to rapidly respond to new and emerging diseases and so have proactively implemented new policy that allows for non-replicating vector systems to be licensed and the used to swap antigens for rapid response. The production platform (VSM 800.213) and prescription platform allow for the use of recombinant, non-replicating, non-viable platform technology for a streamlined vaccine registration. This creates opportunity for cost-effective investment in a variety of technologies that can receive a fully licensed USDA license but also participate in the production platform and prescription process. This would include but not be limited to protein expression systems such as baculovirus, defective and non-replicating viral systems such as alphavirus, and RNA/DNA vaccines.

Advances in replicating platforms continue to evolve and a variety of vector systems are showing value in safety and efficacy as well as rapid response potential as well as advances in delivery options. There is significant effort being invested in advanced polymers and antigen delivery technology that works across vaccine types. Nanovaccines and nanoparticles with advanced chemistry can be applied with a single dose, by routes other than intramuscular needle, and may have sustained release potential. Many of these compounds also have advanced opportunity to stabilize difficult antigens such as RNA and DNA and so open a door for new applications in animal health. Finally, the use of whole genome sequencing and advanced machine learning offers new insights into vaccine development, especially in complex bacterial pathogen vaccine development.

Notes:

97 - A systematic review of impacts of cattle on human risk of exposure to vector-borne diseases

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Session: One Health / Public Health, 1/23/2022, 10:30 - 10:45

Objective
Vector-borne diseases (VBDs) in humans account for more than one billion cases, one million deaths, and one-sixth of worldwide disability and illnesses annually. Due to agricultural, environmental, climate and anthropological changes, VBDs are on the rise globally. Cattle are intrinsically related to agricultural, economic, and cultural practices worldwide and are a major contributor to the GDP of nations. Previous research indicates a range of outcomes for the role of cattle in impacting human risk of exposure to VBDs but there has been no synthesis of the available literature.

Methods
Therefore, we performed a systematic review of the scientific literature using PRISMA 2020 guidelines to determine the effect of cattle on human health with respect to VBDs. We screened 470 peer-reviewed journal articles published between 1999 – 2019 using the databases Web of Science, PubMed Central, CABI Global Health, and Google Scholar and utilized forward and backward search techniques. After removing duplicates, we identified 127 papers that met the inclusion criteria. The articles were independently reviewed by two authors and interrater agreement was strong (Cohen's Kappa = 0.835).

Results
More than 50% of the papers in our final pool of studies were from the African continent, followed by Asia and Europe. In our analysis, cattle primarily were associated with increasing risk of VBDs in humans, especially for diseases transmitted by tsetse flies, ticks, followed by sandflies and mosquitoes. Cattle act as reservoirs of pathogens, cattle movements can introduce VBDs in new areas, and cattle can also modify their environment making it both suitable and unsuitable for different vectors. Treatment of cattle with pesticides can be beneficial to reducing human exposure risk under certain conditions, depending on vector feeding preferences.

Conclusions
Future research should focus on determining the specific mechanisms by which cattle impact VBDs and opportunities to exploit cattle management for vector control.

Financial Support
University of Illinois; U.S. Department of Agriculture, National Institute of Food and Agriculture, Hatch project 1026333 (ILLU-875-984)

Notes:

98 - Invasive plants as risk factors for tickborne disease in humans and livestock

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Session: One Health / Public Health, 1/23/2022, 10:45 - 11:00

Objective
Recent research has reported that the risk of tickborne disease in humans and livestock is increased in areas with an abundance of some invasive plant species. These plants may alter microclimate conditions to favor tick survival. The objective of this project was to examine the relationship between different species of invasive plants and the prevalence and diversity of tick species and associated pathogens.

Methods
Plots located at Dixon Springs Agricultural Center, Simpson, Illinois were classified as uninvaded and invaded by *Alliaria petiolata*, *Microstegium vimineum*, or *Lonicera maackii*. Tick abundance was assessed with a standard dragging protocol from April through December 2021. Collected ticks were identified for developmental stage, species, and tested for pathogens. Dataloggers at each transect recorded temperature and relative humidity. Ticks were tested using next-generation sequencing with barcoded, universal 16S rRNA primers for the detection of pathogens including Anaplasma, Borrelia, Ehrlichia, Francisella, and Rickettsia.
The Kruskal-Wallis test was used to assess associations between risk factors and quantitative outcomes. The Chi^2 test was used to assess associations between plant species and binary outcomes. Mixed multivariable regression models were used to control for potential confounders.

Results
A total of 531 ticks were collected (*A. Americanum,* n=147; *D. variabilis,* n= 25; and *I. scapularis,* n=2). There were significant associations between plant species and relative humidity ($p<0.001$). Plant species was significantly associated with presence of ticks (Chi^2 $p=0.014$). Plots positive for ticks were twice as likely to be invaded by *A. petiolata* compared to the uninvaded plots (OR = 1.94, p=0.013); *L. maackii* invaded plots (OR=1.86, p=0.02); and *M. viminuem* invaded plots (OR = 2.34; p=0.002). Multivariable regression models and pathogen test results will be presented.

Conclusions
Relative humidity and tick presence were significantly associated with the presence of invasive plants. Plots with ticks present were twice as likely to be invaded with *A. petiolata*.

Financial Support
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Notes:

99 - Ecology of avian *Escherichia coli* across niches and in response to vaccination

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Session: One Health / Public Health, 1/23/2022, 11:00 - 11:15

Objective
Avian pathogenic *Escherichia coli* (APEC) cause a range of diseases across all types of poultry. Understanding of the specific, highly virulent clones responsible for these diseases have been well studied and described. However, less is understood about the overall ecology of avian *E. coli* across the animal and its environment. Furthermore, we know very little of the ecological impacts of mitigation strategies on avian *E. coli* populations. The goal of this work was to understand strain-level *E. coli* ecology in this context.

Methods
A matched control-treatment field trial was conducted in which 20 commercial turkey farms were sampled. For each farm, a control barn was compared to a vaccinated barn on the same premises. From each barn, samples were collected every other week and included tracheal swabs, cloacal swabs, litter grabs, and bootsock samples. *E. coli* colonies were collected from each sample and subjected to a typing workflow which included PCRs, whole genome sequencing, and subsequent phylogenetic analyses.

Results
Distinct clonal differences were identified by sample type, with more high-risk APEC identified in tracheal versus cloacal and litter/bootsock samples. Further analyses of matched isolates from individual birds indicated that distinct populations of *E. coli* dominate in different bird niches. Vaccination using a live, attenuated vaccine had an impact over time, shifting the distribution of strains in all locations away from the vaccine strain phylogeny.

Conclusions
This work demonstrates that strains of *E. coli* differ substantially across anatomical site. Vaccination against *E. coli* in poultry not only has the potential to impact the occurrence of disease, but also to influence the ecology of *E. coli* in the healthy bird. However, some high-risk APEC were not impacted by vaccination due to their genetic differences from the vaccine strain used here. This suggests that vaccination approaches with broader heterologous immunogenicity may be more effective at reducing the overall populations of high-risk APEC within a production setting.

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

Notes:

100 - Genetic relatedness and antimicrobial resistance carriage of *Escherichia coli* isolates in a One Health continuum

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Session: One Health & Public Health, 01/23/2023, 11:15 - 11:30

Objective
The study aims to infer the genetic relatedness of *Escherichia coli* isolates within a One Health continuum using short-read whole-genome sequencing. It further investigates the genotypic antimicrobial resistance (AMR) within the isolates and epidemiological associations influencing AMR carriage.

Methods
Escherichia coli isolates (288) from routine surveillance projects were selected according to a stratified random sampling method with strata defined by source (feedlot cattle and broiler chicken fecal samples, retail beef and chicken meat, wastewater and well water) and class level phenotypic resistance. All samples were collected in Alberta, Canada in 2018 or 2019.
From whole-genome assemblies of Illumina short-reads, AMR genes were identified using *in silico* methods and phylogenetic trees inferred from core genome multilocus sequence type alignments. Epidemiological associations between AMR carriage and factors such as source, phylogroup, or species will be explored through regression analysis with adjustment for clustering.

Results
Preliminary phylogenetic analysis of fecal and retail meat samples did not reveal clear clustering of isolates based on source, species of origin (cattle vs chickens), or class-level AMR. Relative frequencies of many AMR genes differed between sources, even within samples originating from the same species (cattle, chickens). Data from water samples will be integrated prior to presentation.

Conclusions
Although genetic diversity in isolates from different sources was anticipated, the absence of obvious clustering by source was surprising given other findings in the literature. The unique epidemiological approach of this study may contribute to this disparity but may also provide new insight into AMR transmission. Other phylogenetic methods (whole-genome MLST, single nucleotide polymorphisms) will be applied to seek support for the initial inference of genetic relationships.

Financial Support
Government of Alberta; Beef Cattle Research Council; Natural Sciences and Engineering Research Council of Canada; Agriculture and Agri-Food Canada; Alberta Agriculture and Forestry

Notes:

103 - Alternatives to antibiotics: neonatal immunomodulation to improve disease resistance in animals

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Session: Antimicrobial Alternatives, 1/23/2022, 10:30 - 10:45

Objective
A potential immunomodulation mechanism for improving disease resilience is innate training, which relies on epigenetic modifications of immune cells for a heightened (ie, trained) response upon repeated microbial stimulation. Prior experiences indicate bacillus Calmette-Guerin (BCG) exposure increases cytokine production upon secondary exposure to microbe-associated molecular patterns, and epidemiological data suggest improved disease resistance in humans administered neonatal BCG.

Methods
Neonatal piglets received BCG or mock inoculum via intravenous (IV) route and were subsequently challenged with influenza A virus (IAV). Calves received intramuscular BCG or mock inoculum and were subsequently challenged with respiratory syncytial Virus (RSV). Body temperature was recorded and nasal swabs collected through the acute phase of viral infection. At necropsy, gross lung pathology was assessed. In addition, prior to IAV or RSV challenge, peripheral mononuclear cells were isolated from mock and BCG inoculated animals and stimulated *in vitro* with LPS to evaluate *in vivo* induction of a trained phenotype via cytokine production and gene expression.

Results
Upon *in vitro* LPS exposure, monocytes isolated from IV BCG treated piglets produced more IL-1beta and TNF cytokine than monocytes from mock pigs, indicating a trained phenotype. However, no significant differences in clinical presentation or nasal and lung viral titers were detected. Upon *in vitro* LPS exposure, monocytes isolated from calves after IM BCG administration produced more IL-1beta and IL-6 cytokine than monocytes from mock calves. Yet, no significant differences were detected in RSV shedding or lung titers.
Transcriptomic response of porcine monocytes is under evaluation, as well as epigenetic evaluation of porcine and bovine monocytes and bovine gamma delta T cells.

Conclusions
BCG administration induced a trained phenotype in porcine and bovine peripheral cells. However, it did not translate into a significant change in respiratory viral infection. Trials are ongoing to assess protection again bacterial infection in pigs and cattle.

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

Notes:

104 - Inhibition of protease RseP sensitizes polymyxin resistant Gram-negative bacteria

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Session: Antimicrobial Alternatives, 1/23/2022, 10:45 - 11:00

Objective
Polymyxin resistance has been increasingly appearing in various significant pathogens, such as *Escherichia coli*, *Klebsiella pneumonia*, and *Salmonella* spp., posing a serious threat to food safety and public health. Development of innovative strategies to mitigate polymyxin resistance is highly warranted. Recently, we demonstrated that the sigma E (RpoE) stress response pathway is essential for polymyxin resistance in Gram-negative bacteria. Here we aimed to examine if inhibition of RseP, a key protease activating sigma E stress response, can potentiate polymyxin against polymyxin resistant bacteria.

Methods
The batimastat (also named BB-94), a recently characterized RseP inhibitor and also a substrate of efflux pump, was used for *in vitro* growth assay in conjunction with the efflux pump inhibitor Phenylalanine-Arginine Beta-Naphthylamide (PAβN). The optimal concentrations of BB-94 and PAβN was determined by checkerboard assay. Diverse polymyxin resistant Gram-negative bacteria were subjected to MIC and growth assay (real-time growth curve and quantitative CFU measurement) to determine the feasibility of using RseP inhibitor to sensitize polymyxin resistant strains for combination therapy.

Results
The effective synergistical concentrations of BB-94 (12 μg/mL) and PAβN (100 μg/mL) were determined and used in all growth assays. With the aid of PAβN, BB-94 significantly sensitizes diverse clinical strains (7 *E. coli*, 3 *S.* enterica, and 1 *K. pneumonia*) to colistin by reducing MIC for up to 64-fold. In the presence of sublethal concentration of colistin, BB-94 and PAβN completely suppressed the growth of two colistin resistant *E. coli* in the real-time bacterial growth assay, which is further confirmed in four colistin resistant human pathogens (*E. coli*, *S.* Oslo, and *K. pneumoniae*) that consistently displayed about 6 log units growth reduction after 6 hours of treatment.

Conclusions
Inhibition of RseP is a promising approach to sensitize different polymyxin resistant clinical bacterial pathogens for combination therapy.

Financial Support
U.S. National Institutes of Health; University of Tennessee AgResearch

Notes:

105 - Epigenetic regulation of host defense peptide synthesis

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Session: Antimicrobial Alternatives, 1/23/2022, 11:00 - 11:15

Objective
Host defense peptides (HDPs) are an integral part of the innate immune system as the first line of defense against infections. Modulation of HDP synthesis has emerged as a promising host-directed approach to fight against infections. We have identified several classes of epigenetic compounds capable of inducing HDP gene expression. The objective of this study is to explore a possible synergy in HDP induction among different classes of epigenetic compounds as an antibiotic-free approach to infectious disease control and prevention.

Methods
Chicken macrophage cell lines and peripheral blood mononuclear cells were treated with or wothout structurally distinct histone deacetylase inhibitors (HDACi), histone methyltransferase inhibitors (HMTi), or DNA methyltransferase inhibitors (DNMTi) individually or in combinations, followed by analysis of the expression levels of HDP and cytokine genes as well as the major genes invovled in barrier function.

Results
We found that a combination of HDACi and HMTi or HDACi and DNMTi showed a strong synergy to induce the expression of multiple HDP genes. Tight junction proteins such as claudin 1 were also synergistically induced by HDACi and HMTi, while LPS-induced IL-1beta gene expression was suppressed.

Conclusions
Overall, we conclude that HDP genes are regulated by epigenetic modifications. Strategies to increase histone acetylation while reducing DNA or histone methylation exert a synergistic effect on HDP induction and, therefore, have potential for the control and prevention of infectious diseases without relying on antibiotics.

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

Notes:

106 - Bovine myeloid antimicrobial peptide-28 (BMAP-28) MIC for *Mannheimia haemolytica* and combination with florfenicol

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Session: Antimicrobial Alternatives, 1/23/2022, 11:15 - 11:30

Objective
Resistance to antimicrobials historically used for BRD is becoming prevalent; co-administration of antimicrobial peptides could improve efficacy and slow the spread of antimicrobial resistance. We measured the minimum inhibitory concentration (MIC) of BMAP-28 and florfenicol alone or in combination on three *Mannheimia haemolytica* (Mh) field strains.

Methods
Mh field strains 35-248, 25-7R5, and 28-32R53 were used in this study. Strains were individually plated on brain heart infusion (BHI) agar and incubated for 24hs at 37°C, 5% CO_2. After 24 hs, 0.5 McFarland standards where prepared and two ten-fold dilutions were made in BHI broth to reach the desired $1x10^6$ CFU/ml inoculum concentration. BMAP-28 and florfenicol dilutions were prepared in BHI broth, with concentrations ranging from 256 to 0.25 µg/ml for the individual MIC or 32 to 0.007 µg/ml for the combination MIC in a serial two-fold dilution pattern. Using a 96 well plate, 50 µl of the Mh inoculum were tested against 50 µl of the peptide and florfenicol alone or a suitable combination in a 1:1 ratio based on MIC results to peptide or florfenicol alone. BHI broth or inoculum were used as the sterile and growth control. Plates were incubated at 35+/-2°C in a shaker incubator for 20-24 hs and MICs were determined.

Results
BMAP-28 MIC for Mh strains 35-248, 25-7R5, and 28-32R53 was 64, 16, and 32 µg/ml respectively. Florfenicol MIC for Mh strains 35-248, 25-7R5, and 28-32R53 was 4 (Intermediate), 32 (Resistant), and 32 µg/ml (R) respectively. BMAP-28 and florfenicol combination MIC was 32 + 0.5 µg/ml (Susceptible) and 8 + 2 µg/ml (S) for the Mh 35-248; 8 + 16 µg/ml (R) for the Mh 25-7R5; and 8 + 16 µg/ml (R) and 16 + 8 µg/ml (R) for the Mh 28-32R53.

Conclusions
BMAP-28 has a measurable effect on Mh field strains and can be used in combination with florfenicol to lower the MIC of both peptide and antibiotic, with more impact to reduce the MIC of florfenicol in an intermediate Mh strain. Combined treatment with BMAP-28 may help improve antibiotic efficacy in susceptible and intermediate Mh strains and potentially help delay the onset of resistance.

Notes:

107 - Impact of sublethal biocide exposure on antibiotic resistance gene transfer in *Salmonella*

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Session: Antimicrobial Alternatives, 1/23/2022, 11:30 - 11:45

Objective
Salmonella enterica is an economically significant pathogen in livestock and human health, known for resistance to medically important antibiotics and capacity for environmental dissemination. Biocides are used in large volumes for cleaning and disinfection of farm buildings and food processing facilities leading to their accumulation in low concentrations throughout the farm environment. When present at sublethal concentrations, biocides alter bacterial characteristics causing selective survival of resistant bacteria. However, little is known about the impact of biocide application on promoting antibiotic resistance in *Salmonella*. We hypothesized that sublethal exposure to disinfectants would promote horizontal gene transfer in *Salmonella.*

Methods
Salmonella Agona (containing bla_{TEM}, ampicillin-resistant), a livestock environment-derived multidrug-resistant pathogen, and a susceptible strain of *E. coli* (made nalidixic acid resistant) was used as the donor and recipient strains respectively. Sublethal concentrations (SC) of two widely used biocides, bleach (chlorine) and Tek-trol (phenolic disinfectant) were determined by agar plating methods in which biocides were added in increments in broth until no inhibition in growth of donor and recipient bacteria was observed. Conjugation experiments were conducted by mixing equal concentrations of donor and recipient (1:1) along with the SC of disinfectants and incubating for 24 hours at 37°C. Donor, recipient, and transconjugant colonies were enumerated separately on tryptic soy agar containing ampicillin, nalidixic acid, and ampicillin-nalidixic acid respectively.

Results
The results were analyzed using a Mann-Whitney test which demonstrated that biocides, namely bleach at 50 – 100 ppm concentrations, and tek-trol at 4 – 6 mg/ml significantly increased conjugation frequency when compared to the controls.

Conclusions
This study provides insights to the potential contribution of biocides on the spread of antibiotic resistance in the environment. However, additional research is needed to better understand the impact of biocides on antibiotic resistance.

Financial Support
Ohio State University

Notes:

109 - A single cell analysis of pulmonary leukocytes in pigs

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Session: Immunology 3, 1/23/2022, 10:30 - 10:45

Objective
Respiratory diseases are a major health concern for pigs since they affect large numbers of animals and may result in important economic losses. Moreover, some respiratory pathogens such as influenza A viruses (IAV) have zoonotic potential. Current understanding of the pulmonary immune system of pigs is limited which poses an obstacle to the development of vaccines and therapeutics to effectively address respiratory infections. In this work, we performed a single cell transcriptomic analysis of the cellular composition of the pig lung.

Methods
Single-cell RNA-sequencing (scRNA-seq) was performed on lung leukocytes from 2 healthy 6-week-old mixed breed pigs. We obtained 24,560 cells at 4,225 mean reads, which were subjected to cluster analysis for cell phenotyping. We integrated our dataset with published scRNA-seq data of lung leukocytes from humans and mice to assess species similarities and differences. We also integrated our data with scRNA-seq datasets of lung leukocytes collected from 7 age matched pigs that we infected with pandemic H1N1 IAV to investigate influenza-induced changes in pulmonary immune cells.

Results
We identified into 23 distinct leukocyte clusters in healthy pig lungs. These were comprised of 2 NK cell clusters, 4 αβ T cell clusters, 2 γδ T cell clusters, 2 B cell clusters and 11 as myeloid cell clusters that included mast cells, 4 macrophage clusters, classical and plasmacytoid dendritic cells. Our interspecies integration found a high degree of overlap among pig, mouse, and human NK and αβ T cell subsets. In contrast, there were significant species-specific differences in myeloid cell populations and γδ T cell subsets. In comparison to naïve pig lung leukocytes, we found that IAV-infected pigs had lower proportions of NK and γδ T cells and higher levels of blood-derived lymphocytes.

Conclusions
Our data provides an atlas of pig lung leukocytes that includes cell types and gene expression signatures that appear to be unique to swine. These data provide a resource to better understand pulmonary immune responses in pigs, including against important respiratory pathogens.

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

Notes:

110 - Annotating cell transcriptomes in four immune tissues to establish a porcine single-cell immune atlas

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Session: Immunology 3, 1/23/2022, 10:45 - 11:00

Objective
A single cell-level understanding of the porcine immune system will provide tools for improving both disease resistance and use of the pig in biomedical modeling.

Methods
We performed scRNA-seq on bone marrow, lymph node, spleen, and thymus from healthy adult pigs and sequenced a total of 65,782 cells. QC removed duplicate cells and cells with high mitochondrial content, 50,559 cells and 18,673 genes were used for downstream analysis. We created a public searchable visualization tool for exploring these data.

Results
Each tissue was individually mapped using non-linear dimensional reduction, and distinct clusters were found and analyzed using several tools: IKAP, ROGUE, pair-wise differential expression testing, and random forest models. Using a combination of model defined and canonical immune cell markers, we were able to annotate each cluster as part of a diverse set of immune cell types. We used our published porcine PBMC scRNA-seq dataset to compare peripheral blood against tissue specific cell types, and also confirm our cell type annotations across shared cell types. We further validated these annotations using publicly available human scRNA-seq datasets specific for each corresponding porcine immune tissue. We compared expression profiles between similarly annotated cell types between human and pig, and identified cells with expression profiles that were unique to pig immune tissues. Finally, we integrated all of the immune tissue data to compare all cell types across the four tissues, and were able to identify shared and tissue specific cell types, and create an on-line visualization tool.

Conclusions
Our single cell-level transcriptomic study of immune tissues will be an important resource for improved annotation of porcine immune genes and cell types, including providing information for directed development of immune reagents to distinguish these cell types. On-line exploratory tool is useful for visualizing expression patterns. These data can be compared to human single cell transcriptomes to inform human translational biomedical research using pigs as a biomedical model.

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

Notes:

111 - Nonstructural protein 1-beta of PRRSV suppresses PML protein expression and promotes viral replication

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Session: Immunology 3, 1/23/2022, 11:00 - 11:15

Objective
The promyelocytic leukemia (PML) protein, also known as TRIM19, is one of the interferon (IFN) stimulated genes and plays a critical role in antiviral activities. Seven different PML isoforms share the identical sequence in the N-terminal region, but the C-terminal regions are variable. Porcine reproductive and respiratory syndrome virus (PRRSV) inhibits the host IFN response, and the nonstructural protein (nsp) 1b of PRRSV has been determined as the main IFN antagonist.

Methods
We investigated the interaction of PRRSV and PML and showed using siRNA that PRRSV replication was increased in PML gene-knockdown cells. In contrast, overexpression of all six isoforms of PML in MARC-145 cells restricted the PRRSV replication. Among the seven isoforms, PML-II or PML-IV showed the most significant suppression of PRRSV growth.

Results
Furthermore, PRRSV infection downregulated the PML protein expressions by 24 hours postinfection. The nsp1b protein of PRRSV was determined as the viral component that mediated PML reduction. The nsp1b-mediated reduction of PML expression was not the post-transcriptional event but rather post-translational regulation. PML was found to directly bind to nsp1b, as demonstrated by GST pull-down, colocalization, and co-IP assays. The mutations at two of the four potential SUMO interacting motifs (SIMs) in nsp1b resulted in the reduced ability to bind to PML proteins. Subsequently, double mutations in the SIM motif of nsp1b did not bind to PML, and the PML expression was restored compared to that in wild-type nsp1b-expressing cells.

Conclusions
Our findings demonstrate that SIMs of nsp1b play a critical role in the binding to PML and PML degradation. This study reveals a novel strategy for PRRSV to promote viral replication during infection.

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

Notes:

112 - Immune outcome of IFN suppression-negative and NF-κB activation-negative PRRSV infection in vitro and in vivo

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Session: Immunology 3, 1/23/2022, 11:15 - 11:30

Objective
Porcine reproductive and respiratory syndrome virus (PRRSV) suppresses type I interferon (IFNs-α/β) response and activates NF-κB signaling, leading to the production of proinflammatory cytokines and inappropriate innate and adaptive immune responses. In swine farms, co-infections of PRRSV and other secondary bacterial pathogens are common and result in hyperexpression of proinflammatory cytokines to cause porcine respiratory disease complex (PRDC). PRRSV non-structure protein 1β (nsp1β) has been demonstrated as a viral IFN antagonist, and the nucleocapsid (N) protein is the NF-kB activator.

Methods
First, we identified leucine at position 126 (L126) of nsp1β as the catalytic residue for IFN suppression and the nuclear localization signal (NLS) of the nucleocapsid protein as the NF-κB activation domain. Next, we generated two mutant PRRSV individually by reverse genetics for nsp1β L126A mutation to eliminate IFN suppression function and N protein NLS mutation (DNLS)to remove NF-κB activation function.

Results
The nsp1β L126A mutant PRRSV did not suppress IFN-β compared to wild-type PRRSV in cells, and the DNLS mutant PRRSV induced only a lower level expression of NF-kB dependent proinflammatory cytokines in porcine alveolar macrophages compared to wild-type PRRSV. Based on these findings, double mutations were Introduced to PRRSV and generated the nsp1β-L126A and N-DNLS PRRSV, and the phenotype of the double mutant virus was IFN suppression-negative and NF-κB activation-negative. Pigs co-infected with the double-mutant PRRSV and Streptococcus suis exhibited milder clinical signs with lower proinflammatory cytokines expressions compared to those of wild-type PRRSV and Streptococcus suis co-infection.

Conclusions
Our study demonstrates that the double-mutant PRRSV attenuates over-expression of proinflammatory cytokines and relieves the clinical severity of PRDC in pigs when co-infected with a bacterial pathogen, paving a novel way to developing an ideal vaccine candidate for PRRS.

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

Notes:

113 - Dietary zinc and BRD effects on intracellular zinc concentration and transporter expressions in bovine immune cells

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Session: Immunology 3, 1/23/2022, 11:30 - 11:45

Objective
Zinc (Zn) is the second most abundant transition metal in the body and is critical for innate and adaptive immune functions. Zn transporters regulate intracellular Zn homeostasis. In humans and mice, Zn deficiency may lead to increased disease susceptibility. However, trace mineral requirements, evidence of Zn dysregulation and transporter profiles in immune cells are poorly defined in cattle. We aimed to evaluate intracellular Zn concentrations, relative expression and profiles of Zn transporters in circulating immune cells from healthy growing steers, calves, and animals experiencing a respiratory infection.

Methods
Immune cells were collected during two separate animal trials. In trial 1, 18 Angus crossbred steers were stratified by body weight and randomly assigned to two dietary groups: control, receiving no supplemental Zn or HiZn; supplemented with 150 mg Zn/kg DM. Circulating immune cell populations were sorted with FACS and MACS methods and analyzed for intracellular Zn concentration using FluoZin-3 and Zn transporters via RT-PCR on day 33. In trial 2, 10 Holstein calves were infected with BRSV, followed by $6.88x10^8$ CFU of *Pasteurella multocida*. Whole blood was collected and analyzed for Zn transporters pre-infection and 48 hours post-infection.

Results
In trial 1, CD8 and gd T cells showed a decrease in intracellular free Zn in the HiZn group relative to the control group. Relative expressions of ZIP 12 in CD8 T cells and ZnT 7 in B cells were upregulated in HiZn steers. ZIP 11 and ZnT 1 in CD4 T cells were downregulated in steers from the HiZn diet. In trial 2, ZIP 3, 7, 9, 13, 14, and ZnT 4 - 7 and 9 were downregulated, and ZIP 2 was upregulated post-infection in calves.

Conclusions
Dietary Zn intake can influence intracellular Zn concentration and transporters in certain immune populations. Respiratory disease can also affect the expression of Zn transporters. Using RT-PCR, we can profile the different Zn transporters in circulating bovine immune cells from healthy growing steers, calves, and animals experiencing respiratory disease.

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

Notes:

114 - Passive immunization against rhodococcal roal pneumonia using nebulized mRNA

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Session: Immunology 3, 1/23/2022, 11:45 - 12:00

Objective
Pneumonia caused by *Rhodococcus equi* is an important cause of disease and death in foals. We hypothesize that nebulizing foals with mRNA encoding a monoclonal antibody (mAb) targeting poly-*N*-acetyl glucosamine (PNAG) can provide safer and more effective prevention of *R. equi* pneumonia by producing mAbs at the site of infection than transfusing foals with 1 to 2 liters of hyperimmune plasma, the current standard of care.

Methods
The first objective for this project is to design a chimeric mRNA construct based on an equine IgG_1 backbone with variable domains from a human anti-PNAG mAb that can be expressed in equine cells and to demonstrate that this mAb mediates opsonophagocytic killing of *R. equi in vitro.* Detection of expression will be performed by cultured primary equine bronchial epithelial cells and bronchial fibroblasts will be achieved by western immunoblot and ELISA. Opsonophagocytic killing assays will be conducted using supernatants from transfected equine cells that contain the expressed mAb, equine complement, and equine neutrophils.

Results
To date, multiple constructs of the chimeric anti-PNAG mAb have not yielded detectable antibody. We attempted to sort memory B cells from donor horses hyperimmunized against PNAG but we were unsuccessful to isolate single memory B-cells and expand these cells for sequencing their mRNA. We have taken 2 approaches to address the problem. First, new chimeric constructs have been designed for which the equine sequences are based on mRNA sequences of an equine IgG_1 mAb targeting the *R. equi* virulence-associated protein A (VapA) that we have successfully developed in the laboratory. Second, if the newly designed constructs fail, we can pursue the remaining objectives of this project (optimizing the dose and formulation of nebulized mRNA encoding a mAb, and testing the ability of the optimal dose of mRNA to protect foals against intra-bronchial infection with *R. equi*.

Conclusions
We have encountered problems but believe we are attempting to address these problems and have at least 1 solution in hand that will enable us to achieve our goals.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture; Link Equine Research Endowment and Department of Large Animal Clinical Sciences

Notes:

115 - Prevalence and pathology of equine parvovirus-hepatitis in racehorses from New York racetracks

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Session: Epidemiology 2, 1/23/2022, 10:30 - 10:45

Objective
Theiler's disease, a.k.a. equine serum hepatitis, is a devastating, highly fatal disease of horses. Equine parvovirus-hepatitis (EqPV-H) has been identified as the likely cause of this disease. While the incidence of Theiler's disease is low, the prevalence of EqPV-H DNA in horses is high, with up to 37% in some regions, suggesting that subclinical or persistent infection is common.

Methods
To determine the prevalence and pathogenicity of EqPV-H infection at New York racetracks, DNA was extracted from archived formalin-fixed, paraffin-embedded liver tissues from racehorses submitted for necropsy to the Animal Health Diagnostic Center as part of the New York State Gaming Commission-Cornell University postmortem examination program. A total of 191 liver samples from horses between 2 to 13 years old were evaluated. Extracted DNA was tested for EqPV-H using PCR and gel electrophoresis. PCR-positive samples were further assessed for tissue morphology using histology and detection of viral nucleic acid using *in situ* hybridization.

Results
Forty-two samples were PCR positive (22%). Of those, 31 samples had positive viral nucleic acid hybridization in hepatocytes with 11 samples showing positive hybridization in necrotic hepatocytes associated with inflammatory cells, indicating active hepatitis. Both individual hepatocyte necrosis and hepatitis were positively associated with EqPV-H detection ($p < .0001$ and $p = .0005$, respectively).

Conclusions
This study indicates that presence of EqPV-H in the liver and parvoviral-associated hepatitis are prevalent in racehorses from New York racetracks, thus warranting additional studies examining potential associations between EqPV-H infection and racehorse performance.

Financial Support
U.S. National Institutes of Health; U.S. Department of Agriculture; Harry M. Zweig Memorial Fund for Equine Research

Notes:

116 - Chronic hepatitis in horses with equine hepacivirus infection

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Session: Epidemiology 2, 1/23/2022, 10:45 - 11:00

Objective
Equine hepacivirus (EqHV) is closely related to hepatitis C virus, which causes persistent infection and chronic hepatitis in people. EqHV causes subclinical hepatitis during acute resolving infection, however, there is limited information on hepatitis associated with chronic infection. This study describes clinical cases of chronic hepatitis in horses infected with EqHV.

Methods
Case series of horses with inclusion criteria 1) chronic hepatitis, defined as at least one month duration of elevated serum liver biomarkers without improvement and/or elevated serum liver biomarkers with findings of chronicity on liver histopathology, such as fibrosis; 2) serum or liver EqHV qRT-PCR positive; and 3) liver histopathology performed. Liver biopsies were independently reviewed and scored according to the Batts and Ludwig system.

Results
Six horses were included. Horses were median 12 (4 – 19) years old, with 5 geldings and 1 mare. Four were Thoroughbreds, one Anglo-Arabian, and one Warmblood-Paint cross. Median duration of documented hepatitis was 13.5 (1-39) months with median duration of documented EqHV viremia of 9.5 (1-30) months. Clinical signs included weight loss, photosensitization, and hepatic encephalopathy. Histopathologic scores were 2 (0-3) out of 4 for portal/periportal activity, 2 (1-2) out of 4 for lobular activity, and 3 (2-4) out of 4 for fibrosis. Fibrosis was observed in 6/6, lymphocytic infiltrate in 5/6, and individual hepatocyte necrosis in 5/6 cases. One horse cleared viremia after 9 months and hepatitis markedly improved.

Conclusions
The similarities between these cases and HCV suggest it is likely that EqHV can cause chronic hepatitis and liver failure in horses.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture; U.S. National Institute of Allergy and Infectious Diseases

Notes:

117 - A survey of gastrointestinal nematodes (GIN) in North American bison (*Bison bison bison*), a 2023 update.

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Session: Epidemiology 2, 1/23/2022, 11:00 - 11:15

Objective
A significant factor of economic loss in bison production are gastrointestinal nematodes (GIN). However, parasites of bison have been understudied in the US for the past few decades. The composition of GIN infecting bison herds in the U.S. is unknown. It is hypothesized that species composition of GIN varies from farm to farm and region to region. Classical parasitology techniques are time-consuming and rely on morphology to reach diagnosis to the species level. A next generation sequencing (NGS) technique using internal transcribed spacer region (ITS) has been recently described to molecularly identify GIN of bison to the species level.

Methods
For this study, fecal samples were collected from 10 bison herds starting in August 2021 through July 2022. The eggs per gram (EPG) were quantified Coprocultures were set up individually for samples with high EPG. L3 stage larvae were harvested after 21 days of incubation, DNA was extracted, and the ITS-2 region was amplified using PCR. Deep amplicon sequencing was carried out on 50 samples.The sequencing data was analysed using the DADA2 pipeline utilizing IdTaxa to assign the species level identification of GIN.

Results
We present the GIN species found in 50 individual bison, the difference of parasitic biomes found in different herds along with the associated alpha diversity for each sample. *Cooperia oncophora* and *Ostertagia ostertagi* were found to be the most prevalent species. When combined with high fecal egg counts these parasites can be responsible for production losses in bison.

Conclusions
This study demonstrates how deep amplicon sequencing can be used to shed light on GIN communities within a single animal and within different herds of animals. This knowledge can help in the creation of more effective therapeutics for parasitic infections of ruminants.

Financial Support
Kansas State University; Center of Excellence for Bison Studies at South Dakota State University

Notes:

118 - Survey on *Anaplasma marginale* on grazing beef operations in California

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Session: Epidemiology 2, 1/23/2022, 11:15 - 11:30

Objective
The aim of this study was to describe anaplasmosis control and prevention management, and association with perceived herd-level exposure to *Anaplasma marginale* infection or anaplasmosis cases on cow-calf operations in California through a survey tool. The results of this study will be used to address knowledge gaps and improve best management practices for anaplasmosis control in California cow-calf herds.

Methods
A questionnaire was designed for beef ranchers on *A. marginale* infection status and anaplasmosis incidence, herd demographics, and management practices related to disease control and distributed through the network of cooperative extension advisors in November 2020. Response estimates for ranchers were survey weighted by region to adjust for nonresponse bias. Weighted chi square tests were used to detect differences in types of control measures used depending on the perceived *A. marginale* infection status or disease incidence.

Results
A total of 466 questionnaires describing 749 herds were obtained and used in this study. The most common control measures for anaplasmosis from the responses of 311 ranchers were: keeping a closed herd (53.3%), letting calves be exposed to ticks (31.1%), vaccination (22.0%), antibiotic metaphylaxis (1.3%), and 33.0% of producers report no control measures for anaplasmosis. The perceived herd-level prevalence of *A. marginale* infection and incidence of anaplasmosis in the last 5 years were 23.6% (n=745; 95% CI: 22.0-25.3%) and 13% (n=746; 95% CI: 11.7-14.3%), respectively. Control measures, including tick control ($P < 0.001$), vaccination ($P < 0.001$), and antibiotic metaphylaxis ($P = 0.01$), were significantly different between herds with or without perceived *A. marginale* infection.

Conclusions
There are differences in control measures between herds with or without perceived *A. marginale* infection in California. Biosecurity measures such as testing herd additions or changing needles between adult cattle are underutilized and offer opportunities for producer education.

Financial Support
Russel L. Rustici Rangeland and Cattle Research Endowment at University of California Davis

Notes:

119 - Apparent prevalence of hemotrophic mycoplasma infection in Michigan dairy calves

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Session: Epidemiology 2, 01/23/2023, 11:30 - 11:45

Objective
A high prevalence of infection with hemotrophic mycoplasma has been reported in mature cows but little is known about the prevalence of infection with *Mycoplasma wenyonii* (Mw) *or Candidatus Mycoplasma haemobos* (Mh) in calves and heifers. The aim of this study was to determine the apparent prevalence of hemotrophic mycoplasma in calves and replacement heifers on Michigan Dairy farms.

Methods
Blood samples were collected from 799 dairy calves and replacement heifers on 11 dairy farms in Michigan, between March and June 2022. Calves or heifers ranged in age from one day to 27 months old. Detection of Mw and Mh was determined using RT-PCR targeting the 16S rRNA region. Chi-square tests were used to compare the prevalence of organisms among farms. The association between age group and prevalence of hemoplasmas was evaluated using a logistic regression model.

Results
The herd-level apparent prevalence of infection of calves and heifers was 100% for both hemoplasmas and differed among farms ranging from 12 to 68% ($P < 0.001$). Apparent within-herd prevalence was 7% (58/799), and 10% (80/799) for Mh and Mw, respectively while 21.5% (172/799) were infected with both species. As compared to prevalence in the first 3 months of age, the likelihood of infection was similar (0.98; 95% CI:0.32-2.46) for animals 4-6 months of age, 4.5 (95% CI:2.2 - 9.5) times greater for calves aged 7-9 months, 32.7 (95% CI:17.3-64.3) times greater for calves aged 10 – 12 months, 68.2 (95% CI:35.9- 137.1) times greater for calves aged 13 -15 months, and 77 (95% CI:34.6-192.3) times greater for calves aged 16-18 months. All heifers older than 21 months were positive for infection with 1 or more hemoplasmas.

Conclusions
These preliminary findings indicate that infection with hemoplasmas increases greatly as heifers age. The widespread infection may indicate a newly emerging threat to dairy animal health.

Financial Support
Michigan Alliance for Animal Agriculture

Notes:

120 - Pregnancy success among eastern Kansas beef cows infected with *Anaplasma marginale* and/or bovine leukemia virus

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Session: Epidemiology 2, 1/23/2022, 11:45 - 12:00

Objective
Anaplasma marginale and bovine leukemia virus (BLV) are economically-significant, production-limiting cattle pathogens. In the U.S., both *A. marginale* and BLV are widely endemic in most states. Both *A. marginale* and BLV establish chronic infections often leading to cattle becoming life-long carriers and reservoirs. Maintenance of asymptomatic chronic infections may be immunologically taxing and could predispose cattle to secondary infections further exacerbating their disease state or causing poor production performance (e.g. calving success). The aim of this study was to evaluate the infection prevalence of *A. marginale*, BLV, and *A. marginale*/BLV co-infection in eastern Kansas cows; and, to investigate associations between infection state and pregnancy success.

Methods
Blood samples were collected from 2,859 clinically healthy cows undergoing routine pregnancy screening in eastern Kansas. Information captured for each cow included: age, breed, county of residence, pregnancy status and hematological parameters. Blood samples were screened for *A. marginale* and BLV using a combination of quantitative PCR and ELISA assays. Infection status, pregnancy status, and host variables were statistically analyzed using logistic regression and linear regession analyses.

Results
Among all sampled cows, 6.8%, 33.7%, and 21.4% were infected with *A. marginale* only, BLV only, and both, respectively. At farm-level, *A. marginale* prevalence ranged from 0% to 84.5%; the BLV prevalence ranged 0% to 96%; and, the prevalence of co-infection ranged 0% to 79.3%. Hereford had the greatest *A. marginale* prevalence of 37.7 % (132/350). Simmental-Angus Cross had the greatest BLV prevalence of 77.6% (455/586). Cows >6 years had the greatest *A. marginale* and BLV prevalence of 50.0% (350/700) and 85.0% (595/700), respectively. Of the 242 open cows, 42.6%, 30.6%, 43.4% and 16.5% were uninfected, *A. marginale-infected*, BLV-infected, or co-infected, respectively.

Conclusions
Investigating the impact of chronic infections on pregnancy success is important for optimal cow-calf herd management.

Financial Support
U.S. Department of Agriculture, Agriculture and Research Services; Kansas State University, College of Veterinary Medicine (MCAT 2021); National Institute for Food and Agriculture (2018-68003-27463) and the Foundation for Food and Agriculture Research

Notes:

121 - The role of bile metabolism disorder and inflammation on chicken necrotic enteritis

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Session: Disease Pathogenesis 2, 1/23/2022, 10:30 - 10:45

Objective
Necrotic enteritis (NE) has reemerged as a prevalent chicken disease with huge economy loss every year after restricting antimicrobial growth promoters. *Clostridium perfringens* induces NE in the presence of predisposing factors, such as coccidiosis. NE birds show bile metabolism disorders of reducing total bile acid pool in the small intestine. Few effective antibiotic alternatives are available to prevent NE, partly because of lacking mechanism insights. Here we hypothesized that reducing bile metabolism disorder and inflammation prevented chicken NE.

Methods
To examine this hypothesis, broiler chicks were allotted to one basal diet and three intervention diets supplemented with 0.3 mg/kg rapamycin, 1.5 g/kg of deoxycholic acid (DCA), and 1.5 g/kg DCA plus 0.3 mg/kg rapamycin. Feed supplementation was started from d 17. At 18 d, birds were orally infected with *Eimeria maxima* (15,000 sporulated oocysts/ bird) to induce coccidiosis. The birds were subsequently infected with 10^9 CFU/bird of *C. perfringens* at d 23 and 24 to initiate NE. Growth performance of body weight gain (BWG) was measured at d 18, 23, and 25. The birds were euthanized at d 25. Tissue and digesta in small intestine were collected histopathology and inflammation assay.

Results
Notably, birds infected with *E. maxima* and *C. perfringens* developed clinical NE and suffered severe growth performance reduction. Interestingly, rapamycin intervention alone didn't significantly inhibit the BWG loss induced by NE during NE phase (d23-25). Notably, DCA attenuated the BWG loss compared to NE birds. DCA plus rapamycin reduced BWG loss better compared to DCA alone. At cellular level, rapamycin with or without DCA strongly reduced *C. perfringens*-induced intestinal histopathology. Molecular analysis of ileal tissue showed that inhibiting mTOR by rapamycin reduced proinflammatory mediators *Infγ*, *Mmp9*, and *Il23* mRNA accumulation compared to NE birds.

Conclusions
Together, these results suggest that NE could be controlled through reducing *C. perfringens* with DCA and decreasing inflammation with anti-inflammation agent, such as rapamycin.

Financial Support
U.S. Department of Agriculture, Animal and Plant Health Inspection Services; Arkansas Bioscience Institute; AES Research Incentive Grant

Notes:

122 - Oral administration of microplastic polystyrene increased incidence of woody breast myopathy in broilers

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Session: Disease Pathogenesis 2, 1/23/2022, 10:45 - 11:00

Objective
Woody breast (WB) myopathy, characterized by abnormal hardness and pale color of chicken breast meat, is a significant metabolic disease and meat quality defect in poultry industry. However, etiology of WB is still unknown, impeding us to develop effective strategies to control WB myopathy. Microplastics (MPs) are the ubiquitous emerging pollutants that pose negative impacts on the health and sustainability of our ecosystem. Given a panel of strikingly similar changes observed in both WB myopathy and the animals treated with MPs, we hypothesized that MPs serve as an important etiological agent for WB myopathy. In this study, we aimed to examine if exposure of broilers to polystyrene (PS), a widely used microplastic representative, affected WB myopathy development.

Methods
Thirty newly hatched Ross 708 × Ross YP male broilers were assigned to two groups (15 birds per group in single floor pen). The birds in the control group received PBS while the birds in the treatment group received PS nanoparticles *via* oral gavage once a day for 56 days (5 mg/kg body weight per day). Birds were weighted weekly with body weight and body weight gain calculated. WB in live birds was determined by hand palpation weekly from Day 21 with WB incidence calculated. Upon termination, breast filets from all birds were collected for meat color and pH analysis.

Results
There is no significant difference between two groups with respect to body weight and weekly body weight gain. The WB incidence increased with age with the first occurrence of WB observed in both groups on Day 28. From Day 42, WB myopathy in the PS-treated birds developed faster than the control birds. On Day 49 and 56, the PS treatment group showed WB incidence of 69% and 76%, respectively, while WB incidence in the control group was 40% and 53%, respectively. No significant difference was observed for meat color and muscle pH between two groups.

Conclusions
This pilot chicken trial suggested that microplastics are potentially an important etiological agent for WB myopathy in broiler production.

Notes:

123 - Cloning and expressing microbial bile acid metabolizing enzymes to reduce *Clostridium perfringens* infection

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Session: Disease Pathogenesis 2, 1/23/2022, 11:00 - 11:15

Objective
Clostridium perfringens is the main bacterial pathogen responsible for the prevalent chicken necrotic enteritis (NE). The gut microbiota biotransformed bile acids into potent metabolites against pathogens through enzymes, such as 3β hydroxysteroid dehydrogenase (3βHSDH) and 3αHSDH, 5α reductase (5AR), and 5β reductase (5BR). In this study, we investigated transformation of bile acids to reduce *C. perfringens* infection.

Methods
The *3βHSDH, 3αHSDH, 5AR,* and *5βR* genes were PCR-amplified from three bacteria of *Eggerthella lenta, Parabacteroides merdae,* and *Clostridium scindens.* Each of the PCR amplicons were individually cloned into plasmids pDR111 and they were named pDR-3βHSDH, pDR-3αHSDH, pDR-5AR, and pDR-5BR. The ligated pDR plasmids were individually transformed into *E. coli* DH5α for amplification. The plasmids were then transformed and integrated into *Bacillus subtilis* for expressing the secreted bile metabolizing enzymes and were called bile acid metabolizing bacteria (BAMB). The secreted proteins were evaluated using SDS-PAGE and will be assessed by *C. perfringens* inhibition assay.

Results
Using gel electrophoresis analysis, the sizes of the *3βHSDH, 3αHSDH, 5AR,* and *5βR* genes were shown at 784, 814, 759, and 1224 bp, respectively. The transformed *E. coli* DH5α colonies with the target genes were PCR gene-typed at 784, 814, 759, and 1224 bp, respectively. The integration the plasmids into *B. subtilis* genome was verified by PCR at 923, 1596, 1129, and 1314 bp, respectively. The proteins in the supernatants and cell pellets of BAMB were subjected to SDS-PAGE and Western Blot (WB) by anti-His-tag. WB showed bands of 34.,59, 42, and 49kDa were present in the supernatants from respective BAMB. Lithocholic acid (LCA), CDCA, deoxycholic acid (DCA), or CA was cultured with individual or combined BAMB for 24 h.

Conclusions
In conclusion, bile acid metabolizing enzyme genes have been cloned and expressed as secretory proteins in *B. subtilis*. The bile acid metabolizing enzymes could be used to biotransform bile acids into more potent bile metabolites to reduce *C. perfringens* infection in chickens.

Notes:

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

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Session: Disease Pathogenesis 2, 1/23/2022, 11:15 - 11:30

Objective
Transmission from host-to-host is an essential part of the herpesvirus' life cycle. Using our chicken model for alphaherpesvirus transmission, we identified a conserved viral gene – namely glycoprotein C (gC) – to be essential for interindividual spread of Marek's disease alphaherpesvirus (MDV). The main objective of this project funded through U.S. Department of Agriculture-**National Institute of Food and Agriculture**
-AFRI grant no. 2019-67015-29262, is to determine the importance of the gC protein in host-to-host.

Methods
We used *Gallid alphaherpesvirus* 2 (GaHV2) or MDV, *Gallid alphaherpesvirus* 3 (GaHV3) (chicken), and *Meleagrid alphaherpesvirus* 1 (MeHV1) or turkey herpesvirus (HVT) in our host-to-host transmission models to test the ability of recombinant viruses to spread from bird-to-bird when they lacked gC expression. We have established that MDV expresses secreted forms of gC due to alternative mRNA splicing. These secreted gC proteins are important for transmission of MDV in chickens, therefore, we examined alternative mRNA splicing of GaHV3 and HVT gC in skin cells using RT-PCR and western blot assays.

Results
Previously, we determined HVT does not transmit in our chicken-to-chicken experimental model. Here, we tested the ability of HVT to transmit in a turkey-to-turkey experimental and natural infection model and found HVT did transmit efficiently. Importantly, gC was also required for transmission as a mutant HVT lacking gC was unable to spread in turkeys. Analysis of mRNA splicing of GaHV3 and HVT showed that both viruses produce mRNA splice variants of gC in both cell culture and in chickens.

Conclusions
Our results confirmed the conserved requirement for gC proteins during natural infection (transmission) for members of the *Mardivirus* genus. Interestingly, we also determined that the MD vaccines 301B/1 (GaHV3) and HVT also produce mRNA splice variants in both cell culture and in chickens confirming mRNA splicing of gC and expression of secreted gC is not unique to MDV. In addition, the host specificity of HVT was confirmed as it did not spread between chickens but did transmit between turkeys.

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

Notes:

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

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Session: Disease Pathogenesis 2, 1/23/2022, 11:30 - 11:45

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

Methods
In Specific Aim 1, we will use recombinant MDV expressing fluorescent proteins and epitope tagged UL13 and US10 to study the importance of US10 in transmission using an experimental and natural infection chicken model. The interactions of US10 and LY6E using immunoprecipitation assays. In Specific Aim 2, we will sequence the LY6E gene from chickens genetically resistant or susceptible to MD and determine whether LY6E is incorporated into the MD virion using virus extraction and western blotting.

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

Conclusions
Our results showed that expression of both UL13 and US10 are dependent on each other, with US10 protein expression abrogated in the absence of UL13, while UL13 localization is dysregulated when US10 is absent during MDV replication. However, contrary to our original hypothesis, US10 is not required for horizontal transmission of MDV. Thus, the abrogated expression of US10 in the UL13-mutant virus does not link US10 to the requirement of UL13 in horizontal transmission. Further studies are required to address the functional importance of UL13 during horizontal transmission including the potential role LY6E may play in this interaction.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture, Agriculture and Food Research Initiative grant no. 2020-67015-21399.

Notes:

126 - Longitudinal viral progression and immunological responses to bluetongue virus in experimentally infected ruminants

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Session: Disease Pathogenesis 2, 1/23/2022, 11:45 - 12:00

Objective
Bluetongue virus (BTV) is an economically important arthropod-borne pathogen that infects ruminant species worldwide. The severity of BTV infections range from asymptomatic to lethal, with the most severe cases succumbing to disease within one week. Animals that survive the infection often require months to fully recover. The immune response to BTV infection is thought to contribute to both the propagation of disease as well as being critical in the ultimate resolution of infection. Although BTV has been recognized since the 1800s, much of the cellular and cytokine response remains poorly understood due to limited reagent availability for the natural host species. To gain a greater understanding of the role the immune response plays in BTV infection, we infected cohorts of sheep and muntjac with two different strains of BTV, BTV-10 and BTV-17.

Methods
Interestingly, the two serotypes showed highly similar progression between the inoculated cohorts. Viremia was monitored by RT-qPCR using BTV specific primers on intermittent blood draws. Immune cells and cytokines were evaluated by traditional flow cytometry, RNA flow cytometry, RT-qPCR, and/or fluorescent based antibody arrays.

Results
BTV-inoculated animals exhibited clinical signs characteristic of BTV disease with some potential species-specific differences, specifically in the timing of immune response. Circulating virus was observed as early as 3 days post inoculation (dpi) and remained detectable for the remainder of the study (28 dpi). A distinct pan-leukopenia was observed between 8-14 dpi that rebounded to mock-inoculated control levels at 17 dpi consistently in sheep, and less so in muntjac. Interestingly, we observed increased expression of pro-inflammatory cytokines after 8 dpi, notably the pro-inflammatory cytokine CXCL10.

Conclusions
Taken together, we have established a model of BTV infection in two separate ruminant species and successfully monitored the longitudinal immunological response and viral progression using a combination of traditional methods and cutting-edge technology.

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

Notes:

127 - Development of "intelligently-designed" vaccines based on understanding of bacterial pathogenesis and host response

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Session: ACVM - Featured Speakers, 01/23/2023, 02:00 - 02:45

Many vaccines designed to protect animals against bacterial pathogens consist of killed or inactivated cells (bacterins). These vaccines produce a relatively short-lived humoral antibody response to multiple bacterial antigens. In cases where antibodies to a specific bacterial surface component are protective, bacterins have been successful. However, for pathogens in which cellular immunity or toxin-neutralizing antibodies are required, bacterins are inadequate. Furthermore, antigenic components expressed by a particular bacterium in the host may be far different from those expressed by the agent grown in culture medium. As a result, critical protective antigens may be missing from a bacterin. In order to develop a vaccine that will induce optimum protective immunity, a thorough understanding is needed of the components expressed by the pathogen in the host, which bacterial components contribute to bacterial virulence or pathogenesis of the disease, and the host immune response required to prevent disease.

Successful bacterial pathogens, particularly opportunistic pathogens, normally express multiple virulence factors that enable them to evade host defenses and obtain nutrients from the host. These factors are highly regulated and the genes that encode for these proteins may be turned on and off randomly or selected for based on the host environment. Once those factors required for virulence and protective immunity that are expressed in the host are identified, an intelligently-designed vaccine can be developed to effectively prevent disease.

Two examples of bacterial pathogens for which conventional bacterins do not induce adequate protective immunity to the host are *Actinobacillus pleuropneumoniae* and *Histophilus somni*. We now know that *A. pleuropneumoniae*, the etiologic agent of swine pleuropneumonia, produces several hemolysins, which are responsible for the disease swine pleuropneumonia. The capsular polysaccharide and lipopolysaccharide protect the bacterium against host innate immune defenses, but antibodies to these components are not protective. In contrast, neutralizing antibodies to the labile polypeptide hemolysins are highly protective, but antibodies to the denatured hemolysins are much less effective. Therefore, a vaccine utilizing a highly attenuated, but still toxigenic, strain of *A. pleuropneumoniae* can induce protective, neutralizing antibodies to native toxins without causing respiratory disease. *Histophilus somni* is one of the primary etiologic agents of bovine respiratory disease, but is also responsible for a variety of systemic diseases. *H. somni* has a wide array of virulence factors, all of which are designed to resist host defenses regardless of the site of colonization, resulting in a persistent host inflammatory response that is largely responsible for the disease. These virulence factors include a phase variable lipooligosaccharide that can be decorated with phosphorylcholine and sialic acid, and structurally mimic host cell oligosaccharides. A large fibrillar surface protein that binds the IgG Fc region of host antibodies is also involved in adherence, and contains a Fic motif that is cytotoxic for bovine cells. The bacteria also survive intracellularly in professional phagocytes by inhibiting phagosome-lysosome fusion. Furthermore, *H. somni* forms a biofilm during static growth and is documented to form a biofilm in the lungs and myocardium of infected animals. Of particular significance is that 50% of the bacterial genome is differentially expressed when the bacteria are in a biofilm compared to planktonic cells. Hence, the antigens expressed by *H. somni* in the bovine host are vastly different from the antigens expressed by the bacteria growing planktonically in artificial medium. An effective vaccine should target those antigens that are expressed in the host, and are required for survival or virulence of the bacteria. Note: This research has largely been supported by multiple grants from USDA-NIFA.

Notes:

128 - Inflammation and innate immune tolerance in the pathogenesis of bovine respiratory disease and *Mycoplasma bovis* pneumonia

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Session: ACVM - Featured Speakers, 01/23/2023, 02:45 - 03:30

Beef cattle often develop respiratory disease (BRD) soon after arrival to feedlots. During this transition period, calves experience well-characterized BRD risk factors such as weaning, dietary change, transportation, co-mingling with animals from other sources, and viral infections.

These BRD risk factors were previously thought to cause immunosuppression, but there is emerging evidence that they augment inflammatory responses in the respiratory tract of calves. Soon after arrival to the feedlot, inflammation in the respiratory tract of apparently healthy calves increases the development of later bovine respiratory disease caused by Mannheimia haemolytica, Mycoplasma bovis and other pathogens. In vivo, inflammatory stimuli recruit monocyte-derived macrophages to the lung, and these cells respond differently than resident alveolar macrophages to subsequent stimuli. In vitro, inflammatory stimuli enhance inflammatory and other harmful responses of monocyte-derived macrophages to subsequent Mycoplasma bovis infection. These are potential mechanisms by which inflammatory outcomes of BRD risk factors may contribute to development of bacterial pneumonia.

Leukocyte cell death is a key aspect of Mycoplasma bovis pathogenesis, is thought to provide a refuge from antibiotics and immune response, and might favour bacterial growth. The process of leukocyte cell death induced by Mycoplasma bovis is also exacerbated by prior inflammatory stimuli.

Subclinical inflammation, loss of innate immune tolerance, and development of BRD?
Overall, pro-inflammatory effects of bovine respiratory disease risk factors appear to worsen the responses of cattle to Mannheimia haemolytica and Mycoplasma bovis. It is hypothesized that this represents a failure of innate immune tolerance and could involve recruitment of pro-inflammatory monocyte-derived macrophages and neutrophils, or altered regulation of resident lung macrophages. These concepts may be relevant to improved understanding and management of bovine respiratory disease.

Financial Support:
This research was sponsored by the Beef Farmers of Ontario, Beef Cattle Research Council (Canada), Ontario Ministry of Agriculture and Food through the Ontario Agri-Food Innovation Alliance, and Zoetis.

Notes:

129 - Microbial community structure of the gastrointestinal tract of beef cattle in relation to liver abscess occurrence

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Session: Omics 1, 1/23/2022, 02:00 - 02:15

Objective
There is limited research characterizing the microbial community structure in the hindgut of feedlot cattle. The objective of this study was to investigate microbial community structure throughout the GIT. A secondary objective was to relate the GIT community structure to liver abscess presence.

Methods
Eighteen Akaushi crossbred steers enrolled in an all-natural finishing program were sampled during harvest. Oral swabs, fecal samples, and epithelial swabs from the rumen, abomasum, duodenum, jejunum, ileum, cecum, spiral colon, and distal colon were collected. Purulent material was also collected from livers with active abscesses. Microbial communities of all swab and abscess samples were characterized using 16S rRNA gene sequencing using DADA2 within QIIME2, and phyloseq.

Results
Among the 18 cattle, 3 had active liver abscesses, 2 had hepatic scars, and 13 had normal livers. Microbial community structures were markedly different throughout the GIT. Communities were richer and more diverse within the rumen, distal colon, and feces than in the oral cavity, duodenum, and jejunum samples ($P \leq 0.05$). The composition of oral and liver abscess samples were different from all other samples ($P \leq 0.001$). Further, rumen communities were structured differently than all other GIT communities ($P \leq 0.003$), but heteroscedasticity of the abomasum suggested no difference from the rumen. NMDS illustrated systematic changes in microbial communities from one end of the GIT to the other. While microbial communities of fecal samples were similar to the distal hindgut, there was little similarity between fecal and rumen communities.

Conclusions
This study represents one of the largest available detailing microbial community structures throughout the entire GIT, particularly in cattle. Results suggest that fecal communities are representative of hindgut communities, but not of foregut (i.e., rumen, abomasum) communities. These data will be valuable as a reference for further investigations of the gut microbiome of cattle.

Notes:

130 - Fusobacteria or Bacteroidetes? Microbial communities in bovine liver abscesses arise from throughout the gut

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Session: Omics 1, 1/23/2022, 02:15 - 02:30

Objective
Liver abscesses (LAs) are a common and important problem in cattle because of significant economic losses associated with liver condemnation, decreased growth and production, and lower carcass quality. Traditionally attributed to epithelial barrier dysfunction in the rumen allowing bacterial translocation *Fusobacterium necrophorum* to the liver, emerging evidence suggests they may also be seeded from more distal portions of the gut. This study aimed to use the largest 16S rRNA-based analysis to date of over 400 LAs to elucidate the impact of tylosin and antibiotic alternatives on LA community structure, identify the dominant taxa within LAs, and provide evidence that LAs are seeded from the bovine foregut and hindgut.

Methods
16S rRNA gene sequencing was carried out from the purulent material collected from 426 LAs in four separate trials. Sequence data was processed using a combination of QIIME2 and phyloseq. Microbial community composition was compared based on generalized UniFrac values, and discriminant taxa were identified using linear discriminant analysis effect size.

Results
Tylosin and antibiotic alternatives had little impact on LA microbial community diversity and composition. Importantly, members of Bacteroidetes (largely *Bacteroides* and *Porphyromonas*) and not Fusobacteria were the predominant taxa in over half of all LAs collected. Multiple liver abscesses from the same animal displayed remarkably similar community composition, suggesting multiple LAs within an individual are seeding from one location in the gut. Microbial taxa discriminant of LA prevalence were identified in the rumen, small intestine, and large intestine.

Conclusions
The pathogenesis of bovine LAs likely includes gut barrier dysfunction in addition to ruminal acidosis. Fusobacteria has a high relative abundance in LAs but is predominant in only half of all LAs. Tylosin likely acts indirectly on LAs by influencing gut barrier integrity.

Notes:

131 - Simultaneous long-read sequencing of porcine viral pathogens from field samples through TELSVirus workflow

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Session: Omics 1, 1/23/2022, 02:30 - 02:45

Objective
Viral co-infections are frequent on swine farms contributing to aggravated disease outcomes. Although important, limitations in the current sequencing techniques hinder the generation of full-length sequences of multiple viruses. To advance our understanding of viral co-infections, we have developed a workflow called "TELSVirus", or "Target-Enriched Long-read Sequencing of Virus". The workflow combines a "capture & enrichment" method with long-read, real-time sequencing, followed by an ensemble bioinformatics pipeline for data analysis. The aim of this study was to apply the "TELSVirus" workflow to swine field samples to generate full-length genomes and variant discrimination of the target pathogens.

Methods
First, we bioinformatically designed a panel of "probes" that selectively targets 100% of all complete genomes for 52 swine viruses. Then, a pilot of five field nasal swabs from weaned pigs with known influenza A virus (IAV) status (n=3 IAV qPCR positive; n=2 IAV qPCR negative) were subjected to the TELSVirus workflow. IAV RNA was extracted, followed by complementary DNA synthesis. Probe hybridization and enrichment were performed and subsequently, library preparation was conducted prior to loading the samples in the minION.

Results
The designed probes increased the percentage of IAV on-target reads from 0.1% up to 66% with no increase in the on-target read proportion for IAV qPCR-negative samples or reads that aligned to the host genome. The average coverage depth for the IAV positive samples was from 6 to 48 depending on each segment. While highly variable, the coverage positively correlated with information content and reliability of the obtained data. The RVHaplo pipeline identified 5 circulating haplotypes of IAV within a single sample based on the hemagglutinin, and neuraminidase gene segments.

Conclusions
In conclusion, the TELSVirus workflow can effectively increase the proportion of sequenced reads for the target viruses; generate long-read sequences with moderate coverage, and discriminate among different IAV variants that are simultaneously present in a single sample.

Financial Support
National Pork Board; University of Minnesota; U.S. Department of Agriculture Hatch Capacity Grant Funding; Minnesota Agricultural Research

Notes:

132 - Pooled piglet fecal samples accurately reflect individual-level fecal microbial composition

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Session: Omics 1, 1/23/2022, 02:45 - 03:00

Objective
This study aimed to characterize and compare the piglet fecal microbiome obtained from samples processed using both individual and pooled workflows, as well as pen floor composites.

Methods
A total of 20 litters (N=10 contained piglets ~1 days of age, N=10 contained piglets ~20 days of age) reared in a single commercial swine facility were selected for the study. All piglets (N=258) from all litters were sampled individually by inserting a sterile cotton-tipped swab into the rectum. Additionally, 3 composite floor samples were collected from each litter by swabbing the pen floor with a cotton-tipped swab. Swabs were stored at -80°C and then processed using four different methods: 1) individual samples were processed for DNA extraction ("individual", N=258); 2) raw material from individual samples was pooled (4 pools/litter) and DNA was extracted ("fecal pools", N=80); 3) raw material from individual samples was processed for DNA extraction and then the DNA was pooled (4 pools/litter), ("DNA pools", N=80); and 4) composite floor samples (3/litter, N=60). All libraries (N=478) were sequenced for microbiome analysis using the 16S rRNA V4 region.

Results
As a set, the individual samples contained 865 ASVs that were not detected in any of the pooled samples constructed from individual samples, while the DNA and fecal pools contained 168 and 171 ASVs, respectively, that were not detected in any of the individual samples. However, these ASVs tended to be both low abundance and low prevalence. Alpha and beta diversity were not significantly different between fecal and DNA pools. Microbiome composition of composite pen floor samples was significantly different from individual samples, as well as fecal and DNA pools (all PERMANOVA, $P < 0.01$).

Conclusions
Analyzing piglet fecal samples at the individual level provided the most comprehensive profile of the piglet microbiome. However, pooling samples captured most of the variability in individual microbiome profiles, thus should be considered for population-level piglet microbiome studies.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture; Agriculture and Food Research Initiative grant no. 2021-68015-33499

Notes:

133 - Is sequencing of pooled nasal swab samples effective for characterizing DNA for microbial community structures?

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Session: Omics 1, 1/23/2022, 03:00 - 03:15

Objective
Sequencing composited samples created by pooling raw samples could be a more efficient method for characterizing group-level microbiome composition compared to sequencing large numbers of samples from individual animals. The purpose of this study was to compare microbiome results of individual nasal swab samples collected from feedlot cattle with those obtained through compositing of raw swab material.

Methods
Nasal swab samples were collected from 50 feedlot calves with high risk of respiratory disease that were housed in 5 pens (10 cattle per pen) on day 14 after arrival processing. DNA was extracted from all individual nasal swabs, and from swab material composited prior to extraction to create 10 DNA composite samples (5 samples per composite with 2 composites per pen). 16S rRNA gene sequencing was performed on DNA from all individual samples and DNA extracted from swab composites. Sequencing reads were classified using DADA2, and data were normalized using CSS. Microbial community structures were analyzed using the R platform and phyloseq and UpSetR.

Results
The microbial community composition for the composite samples were similar to the mean community structure of individuals. Upset plots showed that richness was significantly higher in the individual samples, and that Shannon's diversity was slightly higher in the individual samples than the DNA extracted from swab composited samples (P=0.05). The majority of features were represented in both types of samples, and microbial community structures were not significantly different between sample types at the phylum level.

Conclusions
Microbiome composition of composite samples could be useful for group-level sampling of large populations. Additionally, composite samples contained all major taxonomic phyla that were found in individual samples, suggesting that pooling would have a similar sensitivity for detection of important features.

Financial Support
Texas A&M University

Notes:

134 - Updates to the MEGARes v3.0 database and its associated AMR++ bioinformatic pipeline for resistome analysis

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Session: Omics 1, 1/23/2022, 03:15 - 03:30

Objective
Antimicrobial resistance (AMR) is considered a critical threat to public health and increasingly, epidemiologic investigations feature high-throughput sequencing. We previously introduced MEGARes, a hand-curated, comprehensive AMR database with an acyclic hierarchical annotation structure that facilitates high-throughput computational analysis. In conjunction, we released AMR++, a customized bioinformatic pipeline designed to facilitate the use of MEGARes in high-throughput analysis for characterizing AMR genes (ARGs) in metagenomic sequence data. A complication faced by MEGARes and other ARG sequence repositories is the inclusion of ARGs whose AMR properties are only conferred when specific single nucleotide polymorphisms (SNPs) are contained within the gene sequence. Here we present an update to MEGARes and AMR++ to significantly expand the handling of ARGs requiring SNP confirmation by both improved annotation of the SNP loci, as well as the inclusion of a novel algorithm for SNP verification.

Methods
All publicly available ARG nucleotide sequence databases were downloaded and compared to MEGARes to identify novel sequences for inclusion in the update to MEGARes. A custom program and SNP database were developed to perform SNP verification in metagenomic sequenced reads. This was integrated into the AMR++ pipeline to check for the presence of resistance-conferring SNPs following alignment to the MEGARes database.

Results
Following all updates, the MEGARes acyclic hierarchical annotation scheme now encompasses 4 antimicrobial compound types, 59 resistance classes, 233 mechanisms, and 1,448 gene groups that classify a total of 8,733 accessions. The SNP verification program and database characterize 337 ARGs, whose resistance-conferring SNPs could not previously be confirmed in such a manner.

Conclusions
With the updates to MEGARes 3.0 and AMR++ v3.0(available at MEGLab.org), we provide important usability improvements to facilitate the processing of short-read sequencing data to perform resistome analysis, including the identification of ARGs requiring SNP confirmation.

Financial Support
Texas A&M University; U.S. National Institutes of Health; Minnesota Agricultural Research, Education and Extension Technology Transfer Program

Notes:

135 - Predictive models for metritis cure using farm collected data and hematological variables measured at diagnosis

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Session: Modeling, Methods, & Study Design, 1/23/2022, 02:00 - 02:15

Objective
Our objective was to evaluate the accuracy of predictive models for metritis spontaneous cure (SC) and treatment failure (TF) using farm collected data only, and with the addition of hemogram variables and circulating concentration of metabolites, minerals, and biomarkers of inflammation measured at time of diagnosis.

Methods
Data related to parity, calving related issues, body condition score, rectal temperature, and days in milk at metritis diagnosis was collected from a randomized clinical trial that included 412 metritic cows from 4 herds in TX, CA, and FL. Metritis was defined as the presence of red-brownish, watery, and fetid vaginal discharge, while cure was defined as the absence of metritis 14 d after initial diagnosis. Cows were randomly allocated to receive systemic ceftiofur therapy (to determine TF) or to remain untreated (to determine SC). At enrollment, blood samples were collected and submitted to cell blood count (CBC) and measurement of several minerals and biomarkers of metabolism and inflammation (BM). Univariable analysis to evaluate the association of farm collected data and blood assessed variables with metritis cure were performed, and variables with $P \leq 0.20$ were offered to multivariable logistic regression models and retained if $P \leq 0.15$ or if they improved model accuracy.

Results
The area under the curve (AUC; 95% CI) for models predicting SC using farm data only, farm + CBC, farm + BM, and farm + CBC + BM was 0.71 (0.62 – 0.78), 0.71 (0.64 – 0.79), 0.75 (0.67 – 0.82), and 0.75 (0.68 – 0.83), respectively. For models predicting TF, the AUC (95% CI) was 0.75 (0.68 – 0.82), 0.76 (0.69 – 0.83), 0.80 (0.73 – 0.87), and 0.80 (0.73 – 0.86) for models using farm data only, farm + CBC, farm + BM, and farm + CBC + BM, respectively.

Conclusions
Predictive models of metritis cure had fair accuracy, with SC models being less accurate than TF models. Additionally, adding BM variables marginally improved the accuracy of models using farm collected data, while CBC data did not improve the accuracy of predictive models.

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

Notes:

136 - Application of behavior data to predict metritis self-cure and treatment failure in dairy cows

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Session: Modeling, Methods, & Study Design, 1/23/2022, 02:15 - 02:30

Objective
The objective was to evaluate the performance of logistic multivariable models containing routinely available on-farm data (FD), behavior data (BD), and the combination of both to predict metritis self-cure (SC) and treatment failure (TF).

Methods
Holstein cows (n = 1061) were fitted with a collar-mounted automated monitoring device from -21 to 60 d relative to calving to monitor rumination and activity. Data related to parity, calving season, calving-related disorders, body condition score, rectal temperature, and days in milk at metritis diagnosis were also collected. Cows diagnosed with metritis, characterized by watery, fetid, reddish/brownish vaginal discharge (VD) were randomly allocated to: remain untreated (to determine SC; n = 90); and subcutaneous injection of 6.6 mg/kg ceftiofur crystalline-free acid (to determine TF; n = 97). Self-cure (VD mucoid, not fetid, and no additional treatment) and TF (VD watery, fetid, reddish/brownish and/or additional treatment) were defined 12 d after diagnosis. Univariable analyses were performed using FD and BD to assess their association with metritis SC or TF. Variables with a P-value ≤ 0.20 were included in the multivariable logistic regression models.

Results
To predict SC, the area under the curve (AUC) for the model containing only FD was 0.76 [95% confidence interval (CI) = 0.63, 0.86]. Sensitivity (Se) was 82%. The final model to predict SC combining FD and BD increased the AUC to 0.87 (95% CI = 0.76, 0.94), and 89% Se. To predict TF, the AUC for the model containing FD was 0.76 (95% CI = 0.64, 0.85), 80% Se. The final model combining FD and BD increased the AUC to 0.95 (95% CI = 0.86, 0.99), and 86% Se.

Conclusions
The addition of BD contributed to increasing the prediction of both models. Applying the model for SC, assuming a SC rate of 65% with Se of 89%, would allow for a reduction in antimicrobial use of 58%. The model for TF would identify 86% of the cows that would have TF. These models may allow for a reduction of antimicrobial use and better management decisions.

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

Notes:

137 - Connecting disjoint trial networks with a new trial: optimizing the estimation of a comparative effect in a NMA

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Session: Modeling, Methods, & Study Design, 1/23/2022, 02:30 - 02:45

Objective
In network meta-analysis, estimation of a comparative effect can be performed for treatments that are connected either directly or indirectly. However, disconnected trial networks may arise, which poses a challenge to comparing all available treatments of interest. Several modeling approaches attempt to compare treatments from disconnected networks but not without strong assumptions and limitations. Conducting a new trial to connect trial networks can create a single network that enables calculation of all possible comparisons and help researchers maximize the value of the existing networks. Here, we develop an approach to finding the best connecting trial given a specific comparison of interest.

Methods
We present formulas to quantify the variation in the estimation of a particular comparative effect of interest for any possible connecting two-arm trial. We propose a procedure to identify the optimal connecting trial that minimizes this variation in effect estimation.

Results
We show that connecting two treatments indirectly might be preferred to direct connection through a new trial, by leveraging information from the existing disconnected networks. Using a real network of studies on the use of vaccines in the treatment of bovine respiratory disease (BRD), we illustrate a procedure to identify the best connecting trial and confirm our findings via simulation.

Conclusions
Researchers wishing to conduct a connecting two-arm study can use the procedure provided here to identify the best connecting trial. The choice of trial that minimizes the variance of a comparison of interest is network dependent and it is possible that connecting treatments indirectly may be preferred to direct connection.

Notes:

138 - Surveillance of *Mannheimia haemolytica* through novel group-based sampling and molecular detection

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Session: Modeling, Methods, & Study Design, 1/23/2022, 02:45 - 03:00

Objective
Detecting *Mannheimia haemolytica* (Mh) in beef cattle is important to producers as Mh is recognized as a causal factor for bovine respiratory disease (BRD). Current methods of Mh surveillance are technical, costly, and impractical in commercial production. The objective of this study was to compare efficacy of less invasive, group-based Mh surveillance methods that could be employed in production agriculture.

Methods
Newly enrolled cattle were housed in 18 pens located at four feedlots in West Texas. Rope were hung above feed bunks for sampling and collected the following morning, and pen waterbowls were swabbed at the same time. Ten randomly selected cattle from each pen were sampled with nasopharyngeal (NP) swabs. Swabs and rope samples were cultured on blood agar and inspected at 24 and 48 hours for identification of Mh. DNA was extracted from swabs and ropes and analyzed using qPCR, including swabs collected from individual animals, and composite DNA samples. 16S sequencing was conducted on extracted DNA to characterize taxonomic diversity.

Results
Mh was detected in 67% of all individual NP swabs using qPCR, including at least one sample from all pens. In contrast, 15% of all NP samples were culture positive, including at least one sample from 70% of pens. qPCR detected Mh in 70% of composited DNA samples, pens for pooled samples of 3-4 individuals and 70% of all samples. Mh was not detected in culture for any rope or waterbowl samples. However, qPCR detected Mh in 61% of rope samples and 86% of waterbowl samples.

Conclusions
Detection of Mh by qPCR had a higher relative sensitivity than culture and would be necessary for analysis of the less-invasive, group-level sampling techniques proposed. Further refinement of group-level sampling techniques is warranted for yield of detectable microbes. Individual NP swabs provided the most viable samples for detection, and pooling of these samples for efficiency in downstream workflow could provide a compromise in terms of efficacy and cost.

Notes:

141 - Efficacy determination of an attenuated vaccine against channel catfish virus

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Session: Vaccinology 3, 1/23/2022, 02:00 - 02:15

Objective
Catfish aquaculture is undoubtedly one of the sustainable food production sectors contributing significantly towards the total U. S. finfish production. ICatfish herpesviruses belonging to the family Alloherpesviridae cause significant mortalities in catfish fry and fingerlings. Channel catfish virus (CCV) causes acute hemorrhagic infection and mortalities in catfish during the hatchery and nursery phases of culture. Considering the key role catfish industry plays in the national finfish production, it is imperative to control disease-related fish losses. Vaccines are considered to be the best and proactive (approach) disease management strategy against viral infections. An attenuated CCV vaccine was developed by serial passage (P41) of the wildtype virus in catfish cell cultures and its efficacy in reducing CCV-associated mortalities was determined.

Methods
An attenuated channel catfish virus vaccine was developed by serial passage (P41) of the wildtype virus in catfish cell cultures. This attenuated vaccine was administered to channel and hybrid catfish fingerlings both in the presence and absence of a stressor (netting stress). Thirty days post immunization, the immunized and non-immunized fish were exposed to wildtype CCV via immersion.

Results
Relative percent survival (RPS) was significantly high in the fish immunized with the attenuated vaccine compared to the non-immunized group confirming the protective immunity conferred by the attenuated virus vaccine. In addition, the efficacy of the vaccine was not compromised in presence of stressor.

Conclusions
The attenuated vaccine developed through serial subculturing of virulent wildtype CCV in CCO/BB cells was effective in curbing CCV-associated mortalities in catfish fingerlings. The CCV vaccine developed in this study is found to be efficacious in presence of acute stressors and the immersion route is promising for vaccine administration.

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

Notes:

142 - Field trial experience with an intranasal nonpathogenic novel vaccine against PRRS.

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Session: Vaccinology 3, 1/23/2022, 02:15 - 02:30

Objective
The objective was to evaluate the efficacy of a novel intranasal (IN) vaccine containing a natural nonpathogenic PRRSV strain (GX16) in a multi-site commercial farm located in Mexico.

Methods
The farm had 1,400 sows with an average of 850 births per week. 7,700 piglets were vaccinated at the third day of age (DA) by the IN route with a dose of 2.0 mL each. Records of clinical signs, mortality and body weight were kept, and sera samples of 20 piglets per group were taken every 3 weeks to perform ELISA and RT-qPCR tests for PRRSV and sequencing of positive cases.

Results
Clinical inspection 5 hours post-vaccination, at nursery and at weaning stages revealed no adverse effects induced by vaccination. RT-qPCR of umbilical cords were negative in 100% of the samples, meaning no transplacental infection of PRRSV. RT-qPCR in serum samples at 3 weeks of age (WA) resulted 100% positive for PRRSV, indicating replication of the vaccinal strain, as confirmed by the genetic sequencing and RFLP (1-6-2). At 6 WA, RT-qPCR revealed 75% of the animals were positive to a field PRRSV, and at 12 WA, 100% of the samples were positive, which indicates field virus exposure; it was confirmed that the field PRRSV had an RFLP 1-37-2. The ELISA tests were positive and increasing S/P ratios at 3 and 6 WA, meaning immune response to vaccination. S/P ratios found at 9 and 12 WA were related to the natural exposure to field PRRSV. The productive parameters of the IN vaccinated piglets showed an average mortality of 1.88% during the weaning period vs 3.32% of those that received the MLV vaccine in previous groups, and an improvement in the ADG of 65 g/day, resulting in 447.5 g additional gain in body weight at weaning stage.

Conclusions
The IN naturally nonpathogenic vaccine strain G16X proved to be a safe and efficacious immunoprophylactic tool in the prevention of clinical manifestations and productive damage induced by heterologous field PRRSV challenge, which is indicative that the vaccine does not damage the innate nor the adaptative immune response of the pigs, preparing them to resist the field challenge with PRRSV.

Notes:

143 - A modified live vaccine protects cattle against *Anaplasma marginale* transmitted by *Dermacentor variabilis* ticks

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Session: Vaccinology 3, 1/23/2022, 02:30 - 02:45

Objective
Bovine anaplasmosis, caused by *Anaplasma marginale* (Am), is transmitted biologically by ticks and mechanically by transfer of infected bovine blood. Towards the goal of vaccine development, we recently reported generating a targeted mutation by disrupting an essential gene. Cattle infected with the mutant did not develop disease. Further, cattle vaccinated with it as a modified live vaccine (MLV) were protected against blood challenge with the wildtype *Am* (AmW). Herein, we tested whether the mutant as a MLV was protective against AmW transmitted by ticks.

Methods
Male *Dermacentor variabilis* ticks, acquisition fed on cattle infected with the AmW during peak bacteremia, were removed and held for 5-13 days prior to infection transmission feeding. Three cattle were vaccinated with the MLV, while three other naïve steers served as infection controls. Infected ticks were allowed to feed on all six animals for seven days for AmW transmission, after which they were monitored for clinical signs and infection. Blood samples were collected over a 2-month period to monitor CBCs and infection levels by qPCR.

Results
Tick transmission control animals developed severe clinical disease with high fever, lethargy, and inappetence consistent of anaplasmosis. A drop in PCV to ~23% from ~35% was observed in these cattle after day 32 post-challenge and remained anemic for several days. During this time, the bacteremia peaked at ~1.75x10^6/µl blood. Blood cell abnormalities were noted, including anisocytosis, reticulocytosis, activated lymphocytes, and band cells. In contrast, MLV vaccinated cattle remained asymptomatic after tick transmission challenge without decrease in PCV and had a 7-fold lower bacteremia. Antibody levels were similar in all cattle.

Conclusions
Collectively, the data demonstrated that immune protection was stimulated by the Am MLV against tick transmitted AmW and warrants continued development of an effective vaccine for bovine anaplasmosis.

Financial Support
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Notes:

144 - *Role of rel, regulator of the stringent response, in establishment of persistent infections by mycobacteria*

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Session: Vaccinology 3, 1/23/2022, 02:45 - 03:00

Objective
Studies were conducted with a *rel* deletion mutant in Bacillus Calmette Guérin *Mycobacterium bovis* (BCG) vaccine (BCG_{rel}) to demonstrate the inability of mycobacterial pathogens to establish a persistent infection is attributable to development of CD8 cytotoxic T cells.

Methods
Studies were conducted with cattle to: 1) compare the primary immune response to vaccination of PBMC ex vivo in tissue culture with BCG and BCG_{rel} and 2) compare the recall response with PBMC from cattle vaccinated with BCG and BCG_{rel}.

Results
Comparison of the ex vivo primary immune response with PBMC from unimmunized cattle revealed both BCG and BCG_{rel} elicit development CD8 CTL with ability to kill intracellular bacteria. No clear difference was observed in killing intracellular bacteria. Comparison of the recall response with PBMC from vaccinated cattle revealed vaccination with BCG_{rel} elicited a more vigorous immune response as detected in killing of intracellular bacteria by CD8 CTL from cattle vaccinated with BCG_{rel}.

Conclusions
The mechanisms used by mycobacterial pathogens to establish a persistent infection remain unknown, impeding progress in development of a vaccine. The inability of *rel* deletion mutants to establish a persistent infection indicate genes under regulation of *rel* are involved. The results of the present study show, in absence of *rel*, the CD8 CTL response is increased suggesting genes under control of *rel* are able to dysregulate the immune response allowing for establishment of a persistent infection.

Financial Support
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Notes:

145 - Self-destructing attenuated *Salmonella* strains: innate immunity activators to improve food safety and animal health

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Session: Vaccinology 3, 01/23/2023, 03:00 - 03:15

Objective
Adequate induction of early protective immunity is one of the biggest challenges faced by the modern poultry industry. We aim to solve this problem by delivering Self-Destructing Attenuated Salmonella (SDAAS) strains in ovo to act as innate immunity activators to induce an early protective immune response against Salmonella and *E. coli,* allowing similar or improved hatchability when compared with non-inoculated eggs.

Methods
Embryonated eggs were inoculated in the amniotic sac at 18 days of incubation with BSG, $1x10^5$ CFU of strain χ12547 (Δ*alr-3* ΔP_{dadB66}::TT araC P_{araBAD} *dadB* ΔP_{asdA55}::TT araC P_{araBAD} *asdA* Δ*fliC180* Δ(*hin-fljBA*)-*219*), $1x10^8$ CFU of strain χ12553(Δ*alr-3* Δ*dadB4* Δ*asdA33* Δ*fliC180* Δ(*hin-fljBA*)-*219*) and $1x10^2$ CFU of wild-type *Salmonella* Typhimurium strain χ3761. Chicks were inoculated in the yolk sac at day of hatch with $1x10^7$ CFU of APEC strain χ7122 or orally with $1x10^2$ CFU of wild-type *Salmonella* Typhimurium strain χ3761. HEK-Blue cell lines, expressing mTLR4 or mNOD1 were stimulated with χ9052 (Δ*alr-3* Δ*dadB4* Δ*asdA33*) and χ12499 (Δalr-3 ΔP_{dadB66}::TT araC P_{araBAD} *dadB* ΔPasdA55::TT araC P_{araBAD} *asdA*) TLR/NOD activation was measured by SEAP activity. HEK-Blue-mTLR4/mTLR5 cells were stimulated with χ12518 (Δ*alr-3* ΔP_{dadB66}::TT araC P_{araBAD} *dadB* ΔP_{asdA55}::TT araC ParaBAD *asdA* Δ*fliC180* Δ*pagP81*::Plpp *lpxE* Δ*pagL7* Δ*lpxR9*).

Results
We have demonstrated that our SDAAS strains can be safely administered to chicken embryos at 18 days of incubation without reduction in hatchability. We have also observed that SDAAS strains induce superior activation of NOD1, TLR4 and TLR5 in HEK-Blue cells when compared with wild-type *S.* Typhimurium, LPS (TLR4) and flagellin (TLR5). Lastly, we have demonstrated that day-of-hatch chicks derived from eggs inoculated with SDAAS strains have lower mortality when challenged with wild-type APEC strain χ7122 and reduced intestinal colonization by *Salmonella* Typhimurium strain χ3761.

Conclusions
In this work we have demonstrated that SDAAS strains can activate PRRs, are safe and can provide early protection to newly hatched chicks against wild-type *S.* Typhimurium and APEC.

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

Notes:

146 - Adenoviral-vectored epigraph vaccine induces robust and durable immunity against H3 Influenza A Virus in swine

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Session: Vaccinology 3, 1/23/2022, 03:15 - 03:30

Objective
Influenza A Virus (IAV) is a significant pathogen in swine and causes considerable financial burden to the pork industry. Current vaccination strategies induce short-lived, strain-specific responses against swine Influenza A Virus (IAV-S). The substantial genetic diversity of IAV-S necessitates consistent revaccination and updating of commercial vaccines. Here we improve on current vaccination strategies by using a computationally derived set of immunogens to induce robust and durable immune responses in swine.

Methods
Swine were vaccinated intramuscularly with Ad-swH3-Epi, the whole inactivated virus (WIV) commercial comparator, FluSure XP, or DPBS then boosted three weeks later with the homologous vaccine. The swine were sequentially bled weekly for one month, then every month for 5 months. Virus-specific immune responses were characterized by hemagglutination inhibition assay (HAI) and IFN-γ ELISPOT assay to determine the kinetics of immune responses after vaccination. Swine were challenged with a heterologous H3N2 isolate, A/swine/Wyoming/A01444562/2013, and evaluated for reduced disease severity by lower presence of virus in the lungs, decreased microscopic lung lesion development, and reactivated T cell responses by 5 days post infection.

Results
A comprehensive analysis revealed an increased breadth of antibody responses against a wide array of IAV-S H3 isolates in Ad-swH3-Epi immunized pigs. We further identified enhanced levels of circulating antigen-specific IFN-γ secreting cells that remained significantly higher compared to FluSure XP immunized pigs. Challenge with a divergent IAV-S isolate showed that Ad-swH3-Epi immunized pigs had significantly lower presence of virus in the lungs, lower microscopic lung lesion development, and robust reactivation of circulating T cells, demonstrating strong protective efficacy and recall responses 6 months after vaccination.

Conclusions
This longitudinal study details the endurance of immune responses induced by vaccinating with a WIV vaccine compared to an adenoviral vectored Epigraph vaccine.

Financial Support
U.S. Department of Agriculture

Notes:

147 - Novel approaches to define *Salmonella* Dublin transmission dynamics and improve disease control in dairy cattle

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Session: Epidemiology 3, 1/23/2022, 02:00 - 02:15

Objective
Salmonella enterica serotype Dublin is an emerging pathogen among dairy cattle in the northeastern United States. Disease outbreaks can be severe, with a typical herd presentation of dairy calf pneumonia and high mortality. However, there are critical gaps in our understanding of *Salmonella* Dublin epidemiology. Our overarching goal is to utilize novel diagnostic techniques and sample types to comprehensively define *Salmonella* Dublin shedding patterns and transmission dynamics in dairy cattle, thus facilitating the development of improved diagnostic protocols and successful disease control strategies.

Methods
Northeastern U.S. dairy herds with a history of endemic *Salmonella* Dublin infection are being enrolled and intensively sampled. Samples are processed using a validated molecular screening procedure that is highly sensitive for detecting *Salmonella* Dublin, and whole-genome sequencing of selected isolates will be performed.

Results
Thirteen northeastern dairy herds have been enrolled thus far, and four have experienced an outbreak of clinical disease caused by *Salmonella* Dublin. All calves with confirmed infection (confirmed via blood culture) have been found to be *Salmonella*-positive based on nasal swab, saliva, and/or fecal samples collected within 1–3 days of apparent disease onset. All three sample types have been positive. Numerous environmental reservoirs of *Salmonella* Dublin have been identified on farms, including calf-related equipment and locations throughout the calf housing and maternity areas. *Salmonella* Dublin has still been isolated from environmental swab samples at 4 weeks following an outbreak.

Conclusions
Our laboratory technique is successful in identifying *Salmonella* Dublin-positive calves. All three calf sample types (nasal swab, saliva, and fecal samples) have been positive, demonstrating the ability to detect *Salmonella* in these readily accessible clinical samples. Several dairy farm environmental locations can serve as reservoirs of *Salmonella* Dublin, and the organism may persist in these locations for an extended duration beyond an outbreak of clinical disease.

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

Notes:

148 - Serological and molecular survey of SARS-CoV-2 in deer in Alabama, USA

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Session: Epidemiology 3, 1/23/2022, 02:15 - 02:30

Objective
While serological and molecular evidence of SARS-CoV-2 infection has been reported in white-tailed deer (*Odocoileus virginianus*) from the USA, deer sera from the UK (n=1,748) were found to be negative by a serosurvey. To further understand the geographical distribution of SARS-CoV-2 infected deer, we carried out a serological and molecular survey of white-tailed deer in a captive facility in the state of Alabama.

Methods
Blood samples (N=64), and nasopharyngeal and fecal swabs (N=7) used in this study were collected from the Auburn University Captive Facility in Camp Hill, Alabama, between Oct 2019 and Jan 2022. The blood samples were subjected to the SARS-CoV-2 surrogate virus neutralization test, then further to the virus neutralization test for the confirmation of any positive. FRET RT-PCR targeting spike protein of SARS-CoV-2 was performed for the quantification of the viral RNA present in the swab samples.

Results
The surrogate SARS-CoV-2 virus neutralization test identified one positive sample, which was later determined to be negative by the virus neutralization testing performed at USDA National Veterinary Services Laboratories. In addition, rectal and nasopharyngeal swabs from deer collected in January and February 2022 were found to be negative by SARS-CoV-2 PCR. Of 72 people who had close contact with the deer over the study period, 29 completed a voluntary questionnaire that showed three had been infected with the SARS-CoV-2 during the study period.

Conclusions
Our finding that the deer we studied appeared not to have been exposed to SARS-CoV-2 despite the presence of human infections in the facility indicates that spill-over of infections from humans to deer might not be common.

Notes:

149 - Effects of feed withdrawal prior to transport on pathogen transmission and virulence evolution

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Session: Epidemiology 3, 1/23/2022, 02:30 - 02:45

Objective
Feed withdrawal prior to transport is standard practice in livestock systems. This practice can shape dynamic nutrient-dependent feedbacks within and among hosts, with important consequences for pathogen transmission and the evolution of virulence. Yet, few studies have examined the evolutionary and epidemiological consequences of this standard practice. Here, we show that feed withdrawal can enhance or diminish disease severity, depending on whether feed withdrawal bolsters or inhibits immune function, inflammation, and pathogen growth and shedding rates.

Methods
To examine links between feed withdrawal and evolutionary epidemiology under various dietary contexts, we developed a nutrient-explicit quantitative epidemiological model. This model examines the relative costs and benefits of feed withdrawal which ultimately, depend on tension between within-host nutrients fueling: (1) basic host physiology and defense mechanisms versus (2) pathogen development, growth, and transmission. This model, therefore, tracks the outcomes of multiple resource-dependent processes, which may act simultaneously and exert contrasting effects on host and pathogen fitness.

Results
Feedbacks driven by nutrition-mediated competition between host immune function and pathogen production can create a unimodal relationship between feed availability and pathogen fitness. Subsequently, depending on the host's nutritional state, feed withdrawal prior to transport could backfire, and inadvertently select for more virulent parasites and larger epidemics.

Conclusions
These findings carry implications for the development of integrated treatment programs that consider links between nutrition management, disease severity, and subsequent spillovers to humans and the environment.

Notes:

150 - Are we teaching too much? Epidemiology education in the veterinary curricula

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Session: Epidemiology 3, 1/23/2022, 02:45 - 03:00

Objective
Epidemiology, considered one of the basic sciences in clinical medicine, is integral to clinical practice being specifically listed as core content in the veterinary curricula by the American Veterinary Medical Association Council on Education. Unfortunately, students tend to underappreciate its relevance to future practice and, as a result, student engagement and content delivery can be a challenge for educators. The objective of this study was to characterize core epidemiologic content, delivery, and learning strategies employed by educators in the veterinary curricula.

Methods
An on-line survey was conducted in the Fall of 2015 and Summer/Fall of 2022 of epidemiology educators responsible for delivery of core epidemiological content in the professional veterinary medical curricula at colleges in North America and abroad. The Associate Dean for Academic Affairs, or their equivalent, at each institution was contacted to identify educators responsible for delivery of this content at their respective institutions, both past and present. Subsequently, each identified educator received a personal invitation to participate in the survey.

Results
Survey participants included educators from North America and abroad, with the majority being contemporary teachers with at least 5 years of experience. In general, educators delivered content in a stand-alone course and facilitated learning through lectures and activities. While the pandemic impacted method of delivery (i.e., on-line/virtual learning), it also proved fruitful with new ideas and activities for content delivery and assimilation.

Conclusions
This study provides a consensus on core content that we should strive to incorporate into the veterinary curricula, offers insights into the impact of the pandemic on delivery of this content, and a suggests a pathway for the future of veterinary epidemiology education.

Notes:

152 - Prevalence and sources of *Salmonella* lymph node infection in special-fed veal calves

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Session: Epidemiology 3, 01/23/2023, 03:15 - 3:30

Objective
Peripheral lymph nodes (LNs) have been implicated as potential contaminants of ground beef, yet the source and timing of *Salmonella* LN infection in cattle is still unclear, limiting targeted intervention. The aim of this study was to leverage the vertical integration of special-fed veal production to identify preharvest environmental exposures, specifically in livestock trailers and harvest facility holding pens where calves spend 30 min to 4 h, that result in *Salmonella* LN infection.

Methods
Ten cohorts of 80 to 82 veal calves were followed through the harvest process, and environmental samples were collected in barns, trailers, and holding pens. Mesenteric LNs from 35 calves were collected at harvest, and 25 prefemoral LNs per cohort were pooled. Within the same cohort, for 12 samples for which the serovar of the environmental and calf LN *Salmonella* isolates matched, the isolates were submitted for whole genome sequencing to determine whether environmental exposure resulted in LN infection.

Results
Cohort-level *Salmonella* mesenteric LN prevalence ranged from 0% (0 of 35 samples) to 80% (28 of 35 samples), and pooled prefemoral LNs were positive for *Salmonella* in 3 of the 10 cohorts. *Salmonella* prevalence in samples from barns, livestock trailers, and harvest facility holding pens was 22% (13 of 60 samples), 74% (59 of 80 samples), and 93% (74 of 80 samples), respectively. Some environmental and LN isolates were multidrug resistant. Four instances of *Salmonella* transmission from trailers and/or holding pens to calf LNs were supported by sequence data. *Salmonella* serovars Agona, Give, and Muenster were identified in transmission events. One instance of transmission from the livestock trailer, two instances from holding pens, and one instance from either trailer or holding pens were observed.

Conclusions
Further research is needed to evaluate the extent of environmental *Salmonella* transmission in cattle and to determine whether targeted interventions in trailers or holding pens could reduce novel *Salmonella* LN infection in veal calves before harvest.

Notes:

153 - Evaluation of mitigation strategies to reduce bioaerosols in cattle feedyards

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Session: Biosecurity & Infection Control 2, 1/23/2022, 02:00 - 02:15

Objective
Antimicrobial use in food animals selects for antimicrobial resistant (AMR) bacteria, which may reach humans via the food chain, runoff, crop fertilizer, and dust. Evaluation of effective mitigation strategies in cattle feedyards to reduce quantities of viable bacteria in bioaerosols, including resistant bacteria is required. We aimed to evaluate a mitigation strategy based on water application to reduce bioaerosols and AMR determinants in cattle feedyards.

Methods
Cattle were randomized into 18 pens (10 steers/pen) and the experimental unit was a block (3 pens per block). Treatment (water) and control (no water) was applied daily to the blocks via a sprinkler system for the duration of the study (28 days). Bioaerosol, environmental, fecal, and hide samples were collected. Samples were processed, spiral-plated, and colony counted. *Enterococcus* were spiral plated and counted on M-Enterococcus agar (plain, tetracycline (TET), and erythromycin (ERY)) and *E. coli* were spiral-plated and counted on MacConkey agar (plain, TET, and ceftriaxone (AXO)) at CLSI/NARMS breakpoints. Compact dry plates were also used to process bioaerosol samples. Bacterial identification was confirmed by MALDI-TOF.

Results
Aerobic bacteria, *E. coli*, and *Enterococcus* in bioaerosols from control and treatment increased on day 14. Aerobic bacteria, *E. coli* resistant to TET and AXO, and all *Enterococcus* from the control group had the highest counts on day 21 and decreased on day 28. There was no significant difference between treatment groups for aerobic bacteria, *E. coli* or *Enterococcus* on any of the days. However, within groups, there was significant difference in counts between days. There was a significant decrease in overall *E. coli* and TET-resistant *E. coli* from hide samples from days 0 to 28 for both groups. Further analysis will include additional metadata including meteorological data to determine if trends may be related to climatic conditions.

Conclusions
Our results suggest that water application may have an effect on bioaerosols and AMR bacteria in cattle feedyards.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture Food Animal Residue Avoidance Databank;
U.S. Department of Agriculture, National Institute for Food and Agriculture Food Safety Challenge grant

Notes:

154 - Preventing particle bounce while preserving virus viability to improve the characterization of virus-laden aerosols

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Session: Biosecurity & Infection Control 2, 1/23/2022, 02:15 - 02:30

Objective
Airborne pathogens travel in association with particles. The behavior of these particles is largely driven by their size. To better understand airborne transmission of viral diseases, size characterization of virus-laden particles is essential. The Andersen cascade impactor (ACI) is an 8-stage impactor that separates aerosol particles in up to 9 size fractions. A common issue is that particles may bounce upon impact with a substrate, leading to eventual deposition on a further downstream stage than their target stage. Coating collection plates of ACI with adhesive materials may help decrease particle bounce. In this study, we aimed to evaluate different materials on preventing particle bounce while preserving virus viability when using ACI to characterize viral aerosols.

Methods
We evaluated nine materials: Tween 80, silicone oil, Span 85, Brij 35, glycerol, mineral oil, gelatin, bovine serum albumin and virus growth media, on viability of H1N1 influenza virus and bovine coronavirus (a surrogate of SARS-CoV-2). Coated plates were incubated with viruses for an hour, and viruses were eluted with media. Viable viruses were titrated in specific cells. Materials showing limited reduction of viability were selected to characterize viral aerosols artificially generated in a wind tunnel. The ACI was operated at 28.3 lpm for 30 min. Viral RNA was quantified by RT-qPCR, and viability by virus titration. Physical collection was assessed by fluorimetry, and total particle distribution was measured by an optical particle counter (OPC).

Results
Gelatin, silicone oil and mineral oil resulted in limited reduction of viability for both viruses. These materials were used to collect aerosols in the wind tunnel. Results of physical collection, viral load and viability of particles from various ACI stages revealed that there was no significant difference in size distribution between coated and uncoated plates, and the size distribution was similar to that of OPC.

Conclusions
Our results suggested lack of particle bounce when using the ACI to characterize viral aerosols generated under the conditions of this study.

Financial Support
University of Minnesota; College of Veterinary Medicine, Signature Programs

Notes:

155 - Evaluation of hand sanitation protocols on detection of swine influenza in hands of workers

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Session: Biosecurity & Infection Control 2, 1/23/2022, 02:30 - 02:45

Objective
Influenza A virus (IAV) is one of the most important respiratory pathogens being endemic in human and swine populations. Proper hand hygiene is important to prevent IAV transmission among pigs and between pigs and people. Although there are recommendations on hand washing procedures, these recommendations may not take into consideration hands contaminated with pig secretions, which may be protective to the virus. Furthermore, there is limited information on duration of IAV viability on hands of swine farm workers with contaminated secretions. We assessed four hand sanitation protocols on the effectiveness to inactivate IAV in hands of workers and IAV viability in hands after handling IAV-infected pigs.

Methods
Pigs were experimentally infected with an H1N1 IAV. Participants were recruited to model hand hygiene procedures. Hands were sampled before and after handling infected pigs, and after each of the procedures. Participants were allocated to one of the hand washing procedures: a) soap & water; b) water only; c) alcohol-based hand sanitizer, and d) wearing disposable gloves. Each procedure was evaluated in triplicate and compared to no washing. Samples were tested by IAV RT-PCR and virus isolation. A subset of participants also had their hands sampled to assess IAV viability for up to 120 min. All procedures were approved by the University of Minnesota IACUC, IBC and IRB committees.

Results
Upon handling infected pigs, hands of all participants became contaminated with IAV. All hand hygiene procedures resulted in a reduction of positive PCRs. However, reduction by the soap and water, and water only procedures was limited with water only procedure performing the worst. In contrast, alcohol-based sanitizer and change of gloves showed nearly no detection of IAV positivity. Participants that did not follow any procedure had detectable IAV up to 120 min.

Conclusions
There were differences among hand sanitation protocols. Results from this study provide guidelines on how to properly sanitize hands contaminated with pig secretions to minimize the risk of indirect transmission of influenza.

Notes:

156 - Factors influencing the avian influenza A/H5 environmental contamination at live bird markets in Dhaka, Bangladesh

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Session: Biosecurity & Infection Control 2, 1/23/2022, 02:45 - 03:00

Objective
Avian influenza virus (AIV) A/H5 persistence in the live bird markets (LBMs) environment is largely related to poor market-level biosecurity practices, which have been implicated as a source of human infection by AIV A/H5 in Bangladesh. This study aimed to characterize the differences in the proportion of AIV A/H5 environmental contamination in markets of two local government areas of Dhaka metropolitan, to quantify factors associated with the probability of market-level AIV A/H5 environmental contamination risk and work-zone specific (arrival, slaughtering and processing, and sales) contamination patterns.

Methods
We investigated 104 live bird markets within metropolitan Dhaka during January-March 2016, which are known for a higher level of circulation of AIV A/H5 in poultry in Bangladesh. Dhaka metropolitan was chosen because it has the highest population density (30,551 residents/km2) of any Bangladeshi metropolitan area. We performed univariable Fisher's exact test to identify the significant differences by location of Dhaka markets and ran Bernoulli generalized linear models and multinomial logistic regression models to quantify factors associated with the probability of market-level AIV A/H5 environmental contamination and work-zone specific environmental contamination patterns, respectively.

Results
Live bird markets located in Dhaka North City Corporation are qualitatively more vulnerable to AIV A/H5 environmental contamination compared to Dhaka South City Corporation markets. The probability of AIV A/H5 environmental contamination is equally likely in all market work zones investigated. Results showed higher environmental contamination in live bird markets that have both wholesalers and retailers compared with retailer-only markets and in March compared with January.

Conclusions
The findings provide policy-relevant insights into AIV A/H5 environmental contamination risk areas in the Dhaka metropolitan, which would be appropriate in the case of designing a market-level biosecurity intervention to minimize the human AIV A/H5 infection risk linked with live bird markets.

Financial Support
University of Queensland, Australia Higher Degree Research Scholarship Fund, with the LBMs data collected from the Department of Livestock Services, Bangladesh Government and Emergency Center for Transboundary Animal Diseases (ECTAD) of Food and Agriculture Organization (FAO) of the United Nations, Bangladesh; United States Agency for International Development (USAID) through the ECTAD of FAO, Bangladesh.

Notes:

157 - Optimizing Testing Frequency for Infectious Disease Control

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Session: Biosecurity & Infection Control 2, 1/23/2022, 03:00 - 03:15

Objective
Disease control programs are designed to find and remove (through isolation or culling) infectious cases before onward transmission. The frequency of testing required for this purpose differs depending on the dynamics of the test performance across the course of an infection. The objective of this study was to determine the appropriate testing frequency for different test modalities for SARS-CoV-2 control.

Methods
The COVID Detect study compared daily antigen and PCR results from 43 adults newly infected with SARS-CoV-2 to the presence of culturable virus in nasal turbinate swabs.

Results
A combinatorics approach determined that testing every 4 days with any testing method was sufficient to find cases with at least 95% sensitivity but was insufficient to find those cases while still infectious (as indicated by positive viral culture). These results will be compared to testing only after symptoms, and the potential impact of changing viral dynamics due to new variants and vaccination will be shown.

Conclusions
This approach can be applied to any disease control program based on repeated testing.

Financial Support
U.S. National Institutes of Health

Notes:

158 - Animal contact outbreaks of *Salmonella* and Shiga Toxin-producing *Escherichia coli* — United States, 2017–2021

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Session: Biosecurity & Infection Control 2, 1/23/2022, 3:15 - 3:30

Objective
Enteric diseases linked to contact with animals or their environment are estimated to cause 450,000 illnesses, 5,000 hospitalizations, and 76 deaths in the United States annually. Half of *Salmonella* outbreaks linked to animal contact are multistate; these outbreaks disproportionally affect young children, a population at increased risk for severe illness. We summarize multistate animal contact outbreaks of *Salmonella* and Shiga toxin-producing *Escherichia coli* (STEC) infections to characterize common sources.

Methods
We identified multistate outbreaks linked to animal contact using an internal CDC database of enteric disease outbreak investigations. We extracted patient demographic data from PulseNet, the national molecular subtyping network for enteric disease surveillance, and a secure data sharing platform used by public health partners during outbreak investigations. An outbreak was considered solved if an animal source was supported by epidemiologic, laboratory, or traceback evidence. A source was considered confirmed when two types of evidence were present. Etiology and type of animal were summarized.

Results
Eighty-five of 86 multistate outbreaks linked to animal contact were caused by *Salmonella* and 1 was caused by STEC, resulting in 6,388 illnesses, 1,386 hospitalizations, and 11 deaths. Among the 86 solved outbreaks, 66 (77%) had confirmed sources and 20 had suspected sources. Those <5 years accounted for 26% of animal contact outbreak-associated illnesses. Three quarters (62/86) of outbreaks, 5,546 (87%) illnesses, 1,144 (83%) hospitalizations, and 8 (73%) deaths were linked to live poultry. The remaining 24 outbreaks were linked to turtles (8), scaled reptiles (7), small mammals (5), ruminants, dogs, passerines, and cattle/horses.

Conclusions
Most multistate animal contact-associated outbreaks, illnesses, hospitalizations, and deaths were linked to live poultry. Most solved outbreaks had confirmed links to animal sources and disproportionally affected young children, demonstrating a continued need to investigate outbreaks using collaboration with partners through a One Health approach.

Notes:

159 - Challenges on developing a vaccine for protection against *Mycoplasma bovis* respiratory disease in cattle

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Session: ACVM - Featured Speakers, 01/23/2023, 04:15 - 05:00

Mycoplasma bovis is an important component of the bovine respiratory disease (BRD) complex. The economic impact of BRD in the USA feedlot industry has been estimated to be around $55,000,000 annually. *M. bovis* disease is often seen in feedlot animals previously infected by other bacterial or viral pathogens such as *Mannheimia haemolytica*, *Pasteurella multocida*, *Histophilus somni*, bovine respiratory syncytial virus (BRSV), parainfluenza type 3 virus (PI3V), bovine viral diarrhea virus (BVDV), and infectious bovine rhinotracheitis (IBR) caused by bovine herpesevirus-1 (BHV-1).

Control of the disease caused by *M. bovis* has been unsuccessful due to many factors including the capacity of *M. bovis* to evade and modulate the immune system of the host, the lack of known virulence factors, the absence of a cell wall which rendering antibiotics targeting cell-wall synthesis unusable, and the failure of vaccines to control disease on the field.

For the past 12 years, we have been involved in identifying *M. bovis* mechanisms of evasion of host-immune responses such as invasion of host cells, regulation of responses of immune cells such as T-cells, macrophages and monocytes. We also are focused on developing a vaccine for control of *M. bovis* disease in feedlot cattle. We first developed reproducible challenge models to test experimental vaccines in older cattle and later identified numerous *M. bovis* proteins that were used in proof-of-concept animal trials on animals vaccinated by the subcutaneous route. Our results suggest that for a vaccine to succeed, a different vaccination route may be needed to elicit a strong immune response in the lungs where the *M. bovis* infection is located.

Notes:

160 - Immunomodulation strategies to prevent and reduce bovine respiratory syncytial virus infection in preweaned calves

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Session: ACVM - Featured Speakers, 01/23/2023, 05:00 - 5:45

Bovine respiratory syncytial virus (BRSV) is the cause of enzootic pneumonia in young dairy calves and summer pneumonia in nursing beef calves. BRSV infection is associated with a high morbidity and mortality can reach up to 20% in some outbreaks. Moreover, BRSV is a major viral agent of the bovine respiratory disease complex (BRDC). Despite the widespread availability and use of vaccines for BRSV, the disease continues to be a leading cause of morbidity, mortality and economic losses to dairy and beef producers. There are challenges with immunizing cattle against BRSV. BRSV most severely impacts very young calves. These animals have an immature immune system which is inefficient at mounting potent and long-lived B and T cell responses. Furthermore, the presence of maternally-derived antibodies often prevent the induction of strong vaccine-induced immunity. BRSV infection often occurs in shipped, stress or comingled cattle. As is the case with very young calves, stressed animals are less able to mount a favorable protective response to vaccination. Thus, while vaccines are a necessary part of a disease prevention program, they are not ideal for using in very young or at-risk animals.

At Iowa State University, we are developing treatments to target the innate immune system in the calf, which may be useful to prevent, or to reduce the severity, of BRSV infection. The innate immune system is appealing as a target for novel therapeutic or preventative strategies. It is broadly specific and can therefore provide collateral protection against an array of invading bacteria and viruses. The innate immune system acts quickly, responding within minutes to hours, rather than the days required by the adaptive immune system. In very young calves, the adaptive immune system may not be fully matured; however, the innate immune system is active and primed for protection. My talk will discuss the role of the innate immune system in preventing BRSV infection, and how therapies that prime the innate immune system may improve the outcome of infection in young calves.

Notes:

161 - Showering as a biosecurity intervention to disrupt work-related shifts in swine worker skin microbiomes

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Session: Omics 2, 1/23/2022, 04:15 - 04:30

Objective
Swine workers can have frequent and varied contact with pigs during their workday and may harbor a higher prevalence of some bacteria (e.g., *Staphylococcus aureus*) compared to the general public. Close contact with swine is a risk factor for this prevalence. It is unknown if frequent exposure to swine results in detectable and sustained impacts to worker microbiomes or the antimicrobial resistance genes within (i.e., the "resistome"). We aimed to profile the microbiome-resistome of swine workers on a commercial farm, and to determine whether this profile changes throughout the workday.

Methods
Workers in a commercial Minnesota farrowing unit were enrolled into a cross-sectional study, involving taking composite skin-swab samples from four loci (hands, antecubital and popliteal fossae, and axillae), at three intervals during their workday (i.e., pre-showering in, end of work, and post-showering out). Dorsal swabs of sows or piglets were collected and matched to each worker directly contacting pigs in each pen. Resulting gDNA samples were subjected to enrichment for ARGs, mobile genetic elements, and virulence genes using custom probes and metagenomic sequencing. Microbiome profiling was performed via 16S rRNA V3-V4 sequencing. Bioinformatic pipelines were used to characterize taxanomic and gene composition; comparing between pigs and workers and between workday phases.

Results
We detected a shift in overall worker skin microbial composition at phylum, family, and genus levels when sampled prior to workday start (i.e. 'pre-showering-in') vs end of work but prior to showering out (PERMANOVA $P<0.001$). Post-showering out leads to a reversion from the microbiome at the end of work, to that of the composition observed at the start of the workday (PERMANOVA $P<0.001$). These results correlate with microbial networks showing dynamic shifts in community organization and dominance of keystone species following swine work and again after showering.

Conclusions
Our results demonstrate that workplace exposures significantly impact swine worker microbiomes, but existing interventions may dampen these impacts.

Financial Support
U.S. National Institutes of Health; University of Minnesota

Notes:

162 - Transcriptome analysis of beef cattle reveals influence of marketing on bovine respiratory disease risk

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Session: Omics 2, 1/23/2022, 04:30 - 04:45

Objective
Bovine Respiratory Disease (BRD) is the leading infectious disease in beef cattle. Our understanding of BRD development, treatment determination, and marketing system influences remains an important topic to cattle producers. The purpose of this study was to compare at-arrival whole blood transcriptomes from cattle that were shipped through two different marketing settings and determine differentially expressed genes (DEGs) between BRD and healthy cattle across both settings.

Methods
Eighty-one commercial steers (mean: 236 kg, s.d.: 35 kg) were raised in Mississippi, abruptly weaned, and shipped to Texas. Half were randomly placed into a livestock auction market, then an order buyer facility for 3 days (Auction), and half were directly transported three days after weaning (Direct); all cattle were shipped to Texas simultaneously. Blood samples were acquired from all cattle upon Texas arrival. Samples from cattle treated for clinical BRD during Texas backgrounding (n=12 Auction, n= 20 Direct) and twelve randomly-selected clinically healthy cattle (n=6 Auction; n=6 Direct) were utilized for RNA-Seq. Isolated mRNA from cattle was sequenced (NovaSeq 6000; ~35M reads/sample), and reads were processed through ARS-UCD1.2-guided assembly (HISAT2/Stringtie). Gene-wise testing for differential expression was performed via edgeR glmLRT (FDR<0.05). Functional enrichment analyses of DEGs were performed with WebGestalt (FDR<0.05).

Results
A distinct difference in DEGs (n=2,961) between the Auction and Direct groups was found; DEGs encoded for antiviral defense (increased in Auction), cell growth regulation (decreased in Auction), immune activation and complement (increased in Auction), inflammatory mediation (decreased in Auction), and skeletal system development (decreased in Auction). Evaluation of BRD and healthy cattle within the Auction group yielded nine DEGs, encoding for platelet adhesion and collagen formation (both decreased in BRD).

Conclusions
The identified expression patterns and differences in at-arrival blood betters our understanding of BRD development as influenced by marketing systems.

Notes:

163 - Characterization of the respiratory microbiome and virome associated with Bovine Respiratory Disease Complex

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Session: Omics 2, 1/23/2022, 04:45 - 05:00

Objective
Bovine respiratory disease (BRD) is one of the most significant health problems in cattle and the most expensive animal disease afflicting herds in the cattle industry. Effective immunization or antimicrobial therapies that substantially reduce the prevalence or severity of BRD have not been developed despite decades of research, due to the multifactorial etiopathogenesis of the disease that encompasses an array of infectious agents, as well as environmental and management potentiating factors. In this multidisciplinary project, we aim to 1) investigate the prevalence and distribution of the respiratory microbiome and virome associated with BRD in beef herds at the US Meat Animal Research Center (USMARC) and in beef and dairy herds in Ireland (Teagasc); 2) employ next-generation sequencing, third-generation sequencing, bioinformatic technologies, and high throughput sensitive and rapid diagnostics to identify respiratory viral and bacterial agents associated with BRD; and 3) elucidate the dynamics of secondary viral and bacterial infection by monitoring experimentally virus infected animals in longitudinal studies.

Methods
To date, nasal swabs have been collected from herds in the US and Ireland for year one and year two, and evaluation of the bacterial and viral populations through next-generation sequencing and qPCR has been completed.

Results
In the U.S. herd, bacterial communities were different between sampling time (initial vaccination, preconditioning, and weaning) as shown by beta diversity. In the Irish herd, relative abundance of *Mycoplasma*spp. was more elevated in the BRD diagnosed calves than the pen matched healthy calves as revealed by 16S rRNA gene sequencing. Assessing the viral communities in the Irish herd, we found Bovine coronavirus to be prevalent in all calves, regardless of health status; however, we identified a higher prevalence of Bovine rhinitis A virus in BRD diagnosed calves.

Conclusions
Analysis of these specific respiratory pathogens will present a clearer picture of the distribution of bacterial and viral populations in cattle prior to and after weaning.

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

Notes:

164 - Whole genome sequencing of *Moraxella bovis* strains reveals two genotypes with different genetic determinants

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Session: Omics 2, 1/23/2022, 05:00 - 05:15

Objective
Moraxella bovis and *Moraxella bovoculi* both associate with infectious bovine keratoconjunctivitis (IBK), an economically significant and painful ocular disease that affects cattle worldwide. *M. bovoculi* has not been shown to directly cause IBK and is comprised of two genotypes that differ in their gene content and potential virulence factors. *M. bovis* is a causative IBK agent, however, not all strains carry a complete assortment of known virulence factors. The goals of this study were to determine the population structure and depth of *M. bovis* genomic diversity, and to compare core and accessory genes and predicted outer membrane protein profiles both within and between *M. bovis* and *M. bovoculi*.

Methods
Thirty-six strains of *M. bovis* were selected for sequencing and whole genome assembly. These strains were isolated between 1978 and 2020 from 17 US states and one Canadian province. The genomes were sequenced on both Pacbio and Illumina platforms and assembled into high quality, circular chromosomes and plasmids. Gene content between *M. bovis* and *M. bovoculi* was compared.

Results
We identified two genotypes (1 and 2) of *M. bovis.* The two *M. bovis* genotypes share a core of 2,015 genes, with 121 and 186 genes specific to genotype 1 and 2, respectively. The two genotypes differ by over 11,000 single nucleotide polymorphisms, as well as their chromosome size and prophage content, encoded protein variants of the virulence factor hemolysin, and by their affiliation with different plasmids. A core of 1,403 genes was shared between the genotype 1 and 2 strains of *M. bovis* and previously sequenced chromosomes of genotype 1 and 2 *M. bovoculi*, which included a total of nine predicted outer membrane proteins.

Conclusions
There are two genotypes of *M. bovis* that differ in both chromosome content and plasmid profiles and thus may not equally associate with IBK. Immunological reagents specifically targeting select genotypes of *M. bovis*, or all genotypes of *M. bovis* and *M. bovoculi* together could be designed from the outer membrane proteins identified in this study.

Financial Support
U.S. Department of Agriculture, Agriculture and Research Services "Genomic Intervention Strategies to Prevent and/or Treat Respiratory Diseases of Ruminants" Project #3040-32000-034-00-D, University of Nebraska; Nebraska Experiment Station with funds from the Animal Health and Disease Research (section 1433); Hatch Capacity funding programs (#1002196, 1007070, and 1017646)

Notes:

165 - Stable flies harbor a low complexity microbiota dominated by mastitis-associated bacteria

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Session: Omics 2, 1/23/2022, 05:15 - 05:30

Objective
Hematophagous *Stomoxys* (stable) flies have long been implicated as potential vectors of bovine mastitis-causing bacteria due to their close association with raw manure and soiled bedding in barn settings, which both sustain large fly populations and serve as a major reservoir for opportunistic bacteria. Despite this, the overall composition and diversity of bacterial communities associated with *Stomoxys* flies, especially in relation to their associated habitats, remain to be investigated. Here, we present the first high-throughput culture-independent examination of *Stomoxys*-associated bacterial communities through longitudinal sampling of fly and manure samples collected from two connected dairy facilities in South Central Wisconsin.

Methods
Samples of *Stomoxys* flies and manure were collected on a weekly basis from July through September 2021. High-throughput 16S rRNA gene amplicon sequencing was then used to characterize and compare bacterial communities present on or within flies and in manure collected on the same sampling date.

Results
Bacterial alpha diversity was overall higher in manure samples as compared to fly samples, with manure-associated bacterial communities being dominated by members of the Bacteroidales and Eubacteriales. In contrast, flies harbored relatively low-complexity communities dominated by members of the Enterobacterales, Bacillales, and Lactobacillales. Many of the same bacterial strains were detected in both flies and manure collected from each facility, including clinically-relevant *Klebsiella* and *Staphylococcus* spp. Clinically-relevant taxa were also not only present in flies but exhibited dramatically elevated abundances in fly samples as compared to manure samples.

Conclusions
This research lays the foundation toward an improved understanding of the transmission of mastitis-causing bacteria in dairy barn settings.

Financial Support
University of Wisconsin; Dairy Innovation Hub

Notes:

166 - Complete genome sequence of a novel canine distemper virus strain, isolated from a fox in United States

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Session: Omics 2, 1/23/2022, 05:30 - 05:45

Objective
Canine morbillivirus (Canine Distemper Virus; CDV) is an RNA virus in the Morbillivirus genus of the Paramyxoviridae family. CDV is a highly contagious, systemic and fatal disease that affects dogs all over the world, despite extensive and widespread vaccination in developed countries such as the United States. CDV endangers a wide range of wild animal populations and can cross species barriers hence represents a significant challenge at the wildlife-domestic animals interface. Understanding the evolution of emerging strains and the transmission risk from wildlife to domestic animals is highly important to mitigate the effect of spillover events on household animals. In this study, we present the genomic and phylogenetic analysis of a complete genome of a CDV strain from a one-year-old female bat-eared fox (*Otocyon megalotis*) in Tennessee, United States.

Methods
RNA was isolated and cDNA synthesis was performed using NEB first and second strand synthesis kits. A library was prepared from cDNA using a Nextera™ DNA flex library preparation kit and sequencing was performed with an Illumina MiniSeq instrument, yielding greater than 980,000 150-base paired-end reads. SPAdes v3.14.0 was used to assemble the Illumina Miniseq short reads into an assembly graph using a variety of k-mer sizes.

Results
TaqMan-based real-time PCR for three genes including phosphoprotein (P), haemagglutinin (H) and nucleocapsid protein (N) confirmed the presence of CDV in the sample. Phylogenetic analysis of the complete genomic sequences and separately the hemagglutinin gene sequence revealed a unique lineage circulating in US wildlife.

Conclusions
More CDV strains from wild animals will be sequenced to gain a better understanding of the CDV genomic evolution as well as the likelihood of transmission from wildlife to domestic animals. Therefore, the early detection of CDV in wildlife species is of considerable importance, and the application of molecular analyses to examine the organs of wild animals allows the detection and elucidates the distribution of viruses in relation to the different and new circulating genotypes.

Financial Support
University of Tennessee

Notes:

167 - Does antimicrobial-free beef production reduce transmission of resistant bacteria to human consumers?

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Session: Antimicrobial Use 2, 1/23/2022, 04:15 - 04:30

Objective
Despite consumer concerns about animal production practices, research is not available about whether antimicrobial drugs (AMDs) used in cattle promote antimicrobial resistance (AMR) in bacteria that are transferred to humans via the food chain. A nutritional trial was performed in humans to investigate differences in the fecal resistome and microbiome when people ate beef from cattle raised conventionally, including veterinary supervised use of AMDs, vs beef produced in "raised without antibiotics" (RWA) production systems.

Methods
Twenty-six adult participants were enrolled in a blinded, randomized, cross-over dietary trial (clinicaltrials.gov: NCT04023604). Diets provided were identical in the two 3-wk treatment arms with the exception of the beef source (conventional or RWA production). Fecal samples were collected throughout the 14-week trial from all participants, as were samples of beef consumed throughout the study. Target-enriched shotgun sequencing was used to characterize AMR genes (ARGs) and 16S rRNA gene sequencing was used to characterize microbial community structures.

Results
ARGs were more abundant in feces as expected, as qPCR and sequencing confirmed that bacterial abundance was profoundly lower abundant in beef samples. Resistome and microbial community structures in human feces were richer and more diverse than those of beef products. Shifts in microbial populations were observed at different time-points during the trial as participants moved through controlled feeding and dietary washout periods. Major differences were not identified in the fecal resistome associated with consuming beef products with different labeling claims (i.e., no label claims regarding antimicrobial drug exposures vs. RWA labeling).

Conclusions
This study represents one of the first investigations of risks for AMR transfer to consumers via food, relative to beef product differences pertaining to antimicrobial use during production. This study suggests that conventional beef cattle rearing practices, including prudent use of AMDs, poses minimal risk to consumers relative to AMR.

Financial Support
National Cattlemen's Beef Association - Beef Checkoff

Notes:

168 - Ionophore use in food animal production and potential impacts on human and animal health: a scoping review

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Session: Antimicrobial Use 2, 1/23/2022, 04:30 - 04:45

Objective
Ionophores are polyether antibiotics that act by membrane depolarization in bacteria. They commonly are used as feed additives in North America to increase feed efficiency and weight gain of cattle and to control coccidiosis and clostridiosis in poultry. Because of a lack of identified resistance mechanisms specific to ionophores and toxicity on other host species, ionophores are typically classified as non-medically important drugs. To evaluate literature base supporting this classification, we conducted a scoping review of published literature regarding evidence of antimicrobial resistance related to use in animals.

Methods
Publication searches were conducted through PubMed and studies that pertained to four research questions were included in the review. If the reference was in English and published from 1990 and included ionophore use 1) in food animal production that was not a review article 2) in human clinical medicine 3) in food animal production and potential effects on bacteria susceptibility to either ionophores or medically important antibiotics (MIA), and potential effects on bacteria shedding or virulence 4) in food animal production and potential effects on coccidian susceptibility (using *in vivo* methods).

Results
Results from published research did not identify consistent evidence of antimicrobial resistance in bacteria to medically important antimicrobials associated with ionophore use in in vivo or in vitro studies, or increase of shedding or virulence in bacteria in livestock where ionophores were used. Development of resistance to ionophores in coccidia in poultry was reported.

Conclusions
There is a lack of published research indicating antimicrobial resistance in bacteria is an issue related to ionophore use in animals, suggesting that their classification as non-medically important antimicrobial drugs is appropriate. As such, it is appropriate to categorize ionophore use in separately from medically important antimicrobial drugs when summarizing surveillance data for antimicrobial use in animals.

Notes:

169 - Trends of human and veterinary antimicrobial consumption in Fiji from a "one health" perspective

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Session: Antimicrobial Use 2, 1/23/2022, 04:45 - 05:00

Objective
Globally, the demand for animal protein is increasing leading to increased antimicrobial use in food producing animals. Antimicrobials are frequently used in modern methods of animal production, which may put more pressure on evolution of antibiotics resistance bacteria. Despite the serious negative effects on animal and human health that could result from using antibiotics, there are no assessment of antibiotics consumption by the human and livestock sector in Fiji and other Pacific Island countries. The study objective was to evaluate patterns and class of antimicrobial used human and livestock sector from 2015 to 2019 in Fiji

Methods
The data was collected from the official antimicrobial import records held by Biosecurity Authority of Fiji (BAF) for animal use and Ministry of Health and Medical Services, Fiji for human consumption data.

Results
In 2017, 131.55kg of antibiotics were imported and used; 129.93kg in food animals while 1.62kg was used in companion animals. In the following year (i.e., 2018), 134.58kg of antibiotics were imported and used; 134.08kg in food animals while 0.50kg in companion animals. Lastly, in 2019, 156.90kg of antibiotics were imported and used; 153.56kg in food animals while 3.34kg in companion animals. This study has revealed that the tetracyclines, sulfonamides, beta-lactams and macrolides are the most commonly used drugs in food-producing animals in Fiji. Our study shows a considerable overlap between antibiotic classes sold for use in both human and veterinary medicine. Of the overlapping antibiotic classes, beta lactam/penicillin, tetracycline, sulfonamide, and macrolide antibiotic classes were found to be used in both human and animal health sectors. Overall, human drug stores had a broader range of antibiotics available for sale when compared to veterinary drugs.

Conclusions
The data is crucial for risk analysis and planning, and can be used to evaluate resistance surveillance data, assess the success of initiatives to promote prudent antimicrobial usage, and develop strategies to reduce antimicrobial resistance.

Notes:

170 - On-farm monitoring of antimicrobial use and resistance in U.S. broiler production: 2020-2021

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Session: Antimicrobial Use 2, 1/23/2022, 05:00 - 05:15

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

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

Results
Even with COVID-19 constraints, 346 and 356 farms were sampled in 2020 and 2021, respectively. Prevalence of farms raising animals without antimicrobials was 48.6 and 55.3% in 2020 and 2021, respectively. Farm level prevalence of *Salmonella* and *Campylobacter* was 258/702 (36.8%) and 243/702 (34.6%), respectively, with *Salmonella*-positive flocks being associated with *Campylobacter*-positive flocks (OR: 1.80, 95% CI: 1.2-2.7). *S.* Kentucky and *S.* Infantis were the most common serotypes identified. Most *S.* Kentucky isolates were pan-susceptible or resistant to tetracycline (TET) whereas most Infantis isolates were multidrug resistant. Most *Campylobacter* isolates were *C. jejuni,* and most were pan-susceptible or resistant to TET only; approximately one-third had resistance to ciprofloxacin.

Conclusions
Conclusions
This program was designed for sustainable monitoring of on-farm AMU and AMR in the U.S. broiler chicken industry. Based on industry feedback, the program does not require an excessive time commitment and provides value to company participants. To capture long-term associations between AMU and AMR, these datasets need to be collected in parallel at the farm level.

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

Notes:

173 - Altered cytokine and gene expression in vitamin A deficient mice after RSV infection and vitamin A supplementation

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Session: Immunology 4, 1/23/2022, 04:15 - 04:30

Objective
Bovine respiratory syncytial virus (BRSV) is a primary cause of lower respiratory tract disease in young cattle. Vitamin A (VA) deficiency (VAD) leads to increased susceptibility to severe respiratory infections. However, little is known regarding how VA impacts lung immune function. Our primary objectives were to investigate how VAD impacts innate immune function during viral infection and if local delivery of VA by polyanhydride-nanoparticles (NPs) restores the immune response in the lung.

Methods
Vitamin A sufficient (VAS) and VAD BALB/c mice were infected intranasally with 6 x 10^6 $TCID_{50}$ of RSV. Groups of VAD mice were treated intranasally with a dose of 600IU (0.18mg/kg) of NP encapsulated VA (all trans retinoic acid) 24 hours after infection. Lung tissues were collected on day 3 post-infection, and innate immune populations were analyzed by multicolor flow cytometry. Luminex bead-based multiplex assays and RT-qPCR were used to analyze cytokine production and gene expression in lung tissue lysates following RSV infection and NP supplementation.

Results
Cytokine and chemokine production was altered in the lungs of VAD animals after RSV infection, with reduced production of IL-1β, IL-6, IL-17, and KC (CXCL1). VAD mice had more neutrophils recruited to the lungs and changes in the accumulation of $CD27^+$ and $CD27^{neg}$ γδ T cell populations in the lung, suggestive of an altered balance by Th1 and Th17 type immunity. Intranasal VA supplementation after RSV infection induced greater production of GM-CSF, MCP-1, and IP-10 and upregulation in the expression of retinoic acid-producing enzymes and tight junction proteins.

Conclusions
VAD alters the inflammatory environment in the lung, which may contribute to disease susceptibility. Intranasal supplementation with VA encapsulated polyanhydride NPs alters chemokine expression in the lungs and may impact lung repair mechanisms through alteration in tight junction proteins.

Financial Support
Roy J. Carver Charitable Trust Grant #20-5306

Notes:

174 - First description of LPS-treated, pen-housed feeder cattle as a model of bacterial infection

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Session: Immunology 4, 1/23/2022, 04:30 - 04:45

Objective
Intravenous administration of 0.25µg/kg BW LPS in saline to catheterized cattle mimics infection with Gram negative bacteria as indicated by fever, malaise, and elevated haptoglobin. However, catheterization requires individual housing which does not mirror a feedlot environment. We report the results of a trial using this concentration of LPS to treat cattle without catheterization and housing them in a pen setting along with control/sham-treated animals as a prototype for collecting responses to LPS-treatment in a feed yard setting.

Methods
Thirty fall-born calves were used to mirror typical feedlot pen numbers. Animals were weighed, body temperature measured, and a blood sample obtained prior to administration of LPS. Fifteen feedlot steers were treated with LPS (0.25µg/kg; in 1-3ml of saline administered IV) and 15 feedlot steers served as controls and were treated with saline. After treatment, cattle were maintained on trial for four weeks. Blood samples were collected with a syringe via jugular venipuncture on Day 0 prior to treatment, then on Days 1, 7, 14, 21, and 28, after treatment. Response variables were modeled with fixed effects of pen and treatment using SAS.

Results
LPS-treated steers showed signs of malaise and had stopped eating within 2h. By 4h post-treatment, the animals had returned to normal behavior. On D1, haptoglobin levels were significantly higher (P=0.00159) in LPS-treated steers than in the controls. Neutrophil numbers were significantly higher (P=0.0007) in LPS-treated cattle on D1, however percent lymphocytes were significantly lower in those animals (P=0.0001). No animals required any post-treatment veterinary intervention and rectal temperatures, and weights were similar between both treatment groups for the next 28 days.

Conclusions
Our results show that LPS can be used to mimic a bacterial infection similar to that observed in calves suffering from BRDC. Because catheterization was not required, and no veterinary intervention was needed, this technique can be used in animals enrolled in studies where housing in typical feed yard pens is required.

Financial Support
U.S. Department of Agriculture, Agriculture and Research Services Project #3040-32000-036-00D, Strategies to Control Respiratory Diseases of Cattle

Notes:

175 - Impaired alveolar macrophage phagocytosis contributes to bacterial persistence in *M. ovipneumoniae* infected sheep

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Session: Immunology 4, 1/23/2022, 04:45 - 05:00

Objective
Mycoplasma ovipneumoniae (*M. ovi*) is a respiratory pathogen associated with mild atypical pneumonia in domestic sheep, or severe pneumonia in wild sheep. *M. ovi* is often transmitted by asymptomatic carriers with *M. ovi* colonization of the upper airways. How *M. ovi* evades the host immune system to achieve chronic colonization is unknown. We tested the hypothesis that *M. ovi* impairs the phagocytic capacity of alveolar macrophages (AMs), thereby preventing pathogen clearance.

Methods
We collected AMs from specific pathogen-free (SPF) domestic sheep using endoscopic bronchoalveolar lavage. Phagocytic function of these cells was assessed using an imaging cytometry assay with fluorescent beads or *M. ovi* bacteria. To assess impacts of *M. ovi* on phagocytic function with *in vitro* exposure, we first exposed the AMs to *M. ovi* for 24 h *in vitro* before performing the phagocytosis assay. We also collected AMs from 2-4 month-old SPF lambs infected with *M. ovi* alone, both *M. ovi* and *Mannheimia haemolytica*, and compared to AMs from the SPF lambs.

Results
Exposure of the AMs to *M. ovi in vitro* led to a 20.79% decrease in AMs with phagocytosed beads compared to untreated AMs (P=0.0161, Student's t-test). *In vitro* exposure did not show a significant difference between treatments for overall bead phagocytosis, but there was a 2.00% increase in AMs that had only phagocytosed a single bead rather than multiple beads (P=0.0319, Student's t-test) after accounting for individual sheep variation, sex, age, weight at infection, and twin status.

Conclusions
Overall, our results suggest that *M. ovi* inhibits the phagocytic capacity of AMs, as shown in our *in vitro* experiments and supported by the increase of AMs with single rather than multiple beads in the *in vivo* data. Additional factors such as individual variation between sheep also appear to impact phagocytosis, but the significant decrease both *in vitro* and *in vivo* points towards the significance of *M. ovi* infection on the ability of AMs to phagocytose.

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

Notes:

176 - Performance and immunometabolic responses to assess dietary anti-IL-10 on enteric disease outcomes in chickens

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Session: Immunology 4, 1/23/2022, 05:00 - 05:15

Objective
Pathogens use upregulation of host IL-10 to gain a competitive advantage. Performance and immunometabolic responses were evaluated in chickens fed anti-IL-10 during *Eimeria* and secondary *Clostridium perfringens* challenges.

Methods
Two replicate trials were conducted using 640 Ross 308 broilers housed in 32 raised wire floor cages (20 birds/cage) and assigned to a basal diet±0.03% anti-IL-10 for 24d. Upon arrival, chicks were gavaged with $1*10^7$ colony forming units (CFUs) of *Salmonella* Typhimurium (ST) or saline. On d14, a subset received saline or 15,000 sporulated *Eimeria maxima* (EM) M6 oocysts. On d18 and 19, half the *Eimeria*-challenged birds received culture media or $1*10^8$ CFUs *C. perfringens* (CP), resulting in 6 treatments. Body weight (BW) and feed intake were recorded weekly. Six birds/ treatment were euthanized for peripheral blood mononuclear cell isolation at baseline, 1, 3, 7, and 11d post-inoculation (pi) for analysis using Agilent real-time ATP and glycolytic rate assays (Santa Clara, CA). Data were analyzed using the MIXED procedure with diet, challenge, and diet*challenge fixed effects (SAS 9.4; $P \leq 0.05$).

Results
ST inoculation reduced baseline BW 4-7% vs. unchallenged birds ($P<0.007$). In replicate 1, baseline glycolysis increased 33% in unchallenged vs. ST-inoculated birds fed anti-IL-10, while the challenge main effect increased glycolysis 17% in replicate 2 ($P \leq 0.04$). Within 7dpi, EM±CP challenge reduced BW gain 49-55%, regardless of diet ($P<0.0001$). EM challenge in both replicates increased post-inhibition glycolytic activity 43% at 1dpi, regardless of diet ($P \leq 0.04$). In replicate 1, CP-challenged birds fed anti-IL-10 had 26% increased glycolytic ATP production vs. unchallenged counterparts at 1dpi while no differences were seen in control-fed birds ($P=0.03$). In contrast, the challenge main effect increased glycolytic ATP production 26.7% in replicate 2 at CP 1dpi ($P=0.005$).

Conclusions
Performance and glycolytic changes after EM challenge were consistent between replicates; however, variable diet-mediated immunometabolic shifts after secondary *CP* requires further study.

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

Notes:

177 - Multicolor flow cytometry panels for immunophenotyping pig leukocytes

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Session: Immunology 4, 1/23/2022, 05:15 - 05:30

Objective
Many aspects of the porcine immune system remain poorly characterized, which poses a challenge for improving swine health.

Methods
A useful tool to address this challenge is multicolor flow cytometry, which is capable of measuring a large number of parameters on the same sample. Herein, we describe three flow cytometry panels composed of monoclonal antibodies against CD3, TCR-δ, CD4, CD8α, CD8β, CD27, MHC II, CD172α, CD11b, CD11c, CD163, CD21, CD79α, IgM and immature B cells.

Results
These panels were used to phenotype leukocytes in blood, spleen, lung, thymus, and lymph node of conventional mixed-breed pigs at different ages. We describe subsets of helper T cells, cytotoxic T cells, γδ T cells, monocytes, macrophages, dendritic cells, mature B cells, and immature B cells in greater detail than most published pig flow cytometry panels, which usually consist of small number of antibodies.

Conclusions
Our results provide a resource to better understand the pig immune system as well as immune-related diseases that affect swine.

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

Notes:

178 - Identification of granulysin in the mucosal immune response to Equine herpesvirus type 1 (EHV-1) infection

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Session: Immunology 4, 01/23/2023, 05:30 - 05:45

Objective
EHV-1 is a widely spread pathogen of the horse, which infects the nasal mucosa leading to respiratory disease. Viral entry into the epithelium, followed by infection of lymphoid tissues allows the establishment of cell-associated viremia, which can lead to abortion or neurologic disease. An early mucosal immune response can be protective, preventing severe clinical manifestations. Transcriptomic profiling of nasopharyngeal samples was used to identify differences in gene expression in immune and non-immune horses during EHV-1 infection at the site of viral entry.

Methods
RNA sequencing was performed on nasopharyngeal swab samples from EHV-1 immune (n=4) or non-immune horses (n=4). Samples were taken before and during early (d1pi and d3pi), mid (d8pi and d10pi) and late (d18pi) infection, and differential expression was determined between groups. This screen highlighted the upregulation of genes with biological processes related to T cell function. To further characterize the identified genes, novel equine monoclonal antibodies (mAbs) were developed using hybridoma technology, following immunization of a mouse.

Results
We identified 30 genes that were significantly different between groups over the course of infection. For this work, we selected one protein, granulysin (GNLY), which was highly expressed only at the early time point in immune horses ($p=0.025$), while increasing in expression over time in susceptible horses ($p=0.0033$). To explore the role of GNLY in EHV-1 infection, mAbs were developed and characterized. One clone was validated for flow cytometry, with GNLY detected in CD8+ T cells following stimulation.

Conclusions
GNLY is a cytotoxic granule produced by T and NK cells during disease state. This protein is found in many mammalian species including the horse but is not encoded for in rodents. Thus, characterization of GNLY has implications both in equine health and as a unique natural model of function, unlike current mouse models. Here, we identified GNLY as a component of the mucosal immune response to EHV-1 and are characterizing mAbs to investigate its role in viral infection.

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

USDA

Notes:

179 - Inhibition of protein glycosylation blocks PRRSV and swine influenza virus replication

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Session: Virology 2, 1/23/2022, 04:15 - 04:30

Objective
Porcine respiratory and reproductive syndrome virus (PRRSV) causes reproductive failure in pregnant sows and respiratory disease in young pigs, and swine influenza virus (SIAV) causes respiratory disease outbreaks in pigs. Understanding the host factors critical for viral infection is a key step for devising a novel approach to prevent and control viral diseases. N-linked glycosylation is important for correct folding and trafficking of membrane-associated glycoproteins and mediated by a number of enzymes in the host cells including alpha-glucosidases I and II and protein disulfide isomerases (PDIs). As proper folding and glycosylation of envelope proteins of influenza A virus and membrane proteins of PRRSV are important for viral infectivity, we studied the roles of glycosylation and PDIs in the replication of PRRSV and SIAV in porcine-originated cells.

Methods
We used iminosugars that structurally mimic the substrates of ER-resident alpha-glucosidases I and II and siRNAs for PDI enzymes, which are molecular chaperone and folding enzymes, to determine their effects in the replication of PRRSV or SIAV. Porcine CD163-expressing cells, which were generated by transducing porcine-originated macrophage cells with a lentivirus vector carrying full-length CD163 gene, and LLC-PK cells were used for PRRSV and SIAV infection, respectively. Viral titers were measured by qRT-PCR or TCID50 method for the determination of EC50 for each compound. Reduction of virus replication was also measured by western blot analysis and fluorescence microscopy.

Results
Iminosugars including miglustat, MON-DNJ and NN-DNJ inhibited the replication of PRRSV and SIAV, and gene knockdown of PDIA3 and 4 using siRNA also led to significant reduction in the replication of these viruses in porcine-originated cells.

Conclusions
These findings indicate that N-linked glycosylation of PRRSV and SIAV envelope proteins has a crucial role in the infectivity of PRRSV and SIAV and may serve as a potential target for new intervention strategies for PRRSV infections.

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

Notes:

180 - Multiple transmissions of human influenza A virus in pigs leads to improved replication

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Session: Virology 2, 1/23/2022, 04:30 - 04:45

Objective
Influenza A virus is a prevalent respiratory disease of swine, leading to significant losses in pork production. Interspecies transmission of influenza A viruses (IAV) from humans to pigs is relatively common, and some human-origin influenza viruses become endemic in pigs. However, little is known about the evolutionary processes driving adaptation to pigs of IAV following interspecies transmission. Previous events appeared to be associated with mutations in the surface proteins and reassortment. This study aimed to determine whether there were selective bottlenecks during the transmission of human IAV in pigs.

Methods
To evaluate within and between host evolution of human IAV in pigs, we performed serial transmissions in pigs by placing naïve pigs in contact with pigs infected with a reassortant virus containing human HA and NA genes with a swine-origin backbone (HUsurface-SWinternal). A swine-adapted control virus with the same backbone was also included (SWcontrol). Nasal swabs were collected at 1, 3, 5, and 6 days post contact (dpc). Pigs were euthanized at 6 dpc, and BALF was collected. Viral titers were determined by RT-qPCR. Positive samples were sequenced using next generation sequencing (NGS) to further quantify diversity and variant analysis within and between hosts.

Results
Our results showed that all pigs in contact with HUsurface-SWinternal-infected pigs had a similar increase in nasal swab viral titers during the course of infection, leading to similar titers to directly infected pigs at 6 dpc. Pigs exposed to HUsurface-SWinternal showed an increase in the viral load in BALF samples with each contact, reaching titers similar to the SWcontrol virus in contact 4 pigs. Viral titers in BALF remained stable in SWcontrol-contact pigs.

Conclusions
Our study indicated that a reassortant virus carrying the HA and NA genes from a human seasonal IAV improved replication efficiency in pigs over time, particularly in the lower respiratory tract, suggesting that adaptive changes occurred.

Financial Support
U.S. Department of Agriculture

Notes:

181 - Entry of pseudoviruses carrying SARS-CoV and SARS-CoV-2 S protein into cells expressing human and animal ACE2

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Session: Virology 2, 1/23/2022, 04:45 - 05:00

Objective
Severe acute respiratory syndrome coronavirus (SARS-CoV) and severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) are zoonotic viruses that use angiotensin converting enzyme 2 (ACE2) for entry. Since 2019, several variants of SARS-CoV-2 has emerged, which led to increased concern over establishment of animal reservoirs. Viral entry into cells is the key step involved in host susceptibility, and we have previously shown varying entry efficiency of pseudotyped SARS-CoV-2 into cells expressing various animal ACE2. In this study, we extended our previous work and compared the entry efficiency of SARS-CoV and SARS-CoV-2 variants including Omicron to assess the host susceptibilities and zoonotic potentials.

Methods
Lentiviral-based pseudoviruses expressing the spike protein (S) from SARS-CoV (SA), SARS-CoV-2 parental strain (SA2-PA) and variants such as alpha (SA2-Alpha) and omicron (SA2-OMI) strains were generated. Cell lines stably expressing ACE2 from human, cat, dog, cow, rabbit, hamster, horse, white-tailed deer or camel were transduced with pseudoviruses, and virus entry was determined by luciferase reporter assay.

Results
We observed that entry efficiency of SA2-PA varied in the tested cells expressing animal and human ACE2, and SARS-CoV followed a similar pattern of entry to that of SA2-PA. Entry of SA2-Alpha was either comparable or increased compared to those with SA2-PA in each cell line. Similar phenomenon was observed with SA2-OMI in cells expressing human, cow, rabbit, hamster, white-tailed deer or camel. However, interestingly, entry of SA2-OMI significantly decreased in cells expressing cat, dog, or horse ACE2 with near abolishment of viral entry in horse-ACE2 expressing cells.

Conclusions
The results suggest that the interaction between S protein and ACE2 of different animals and humans leading to viral entry is similar between SARS-CoV and SARS-CoV-2. The observed increases and decreases in the entry efficiency of SA2-OMI with different animal ACE2 implies that Omicron variant, which increases viral entry in humans, may have varying effect in different animal species.

Financial Support
U.S. National Institute of Allergy and Infectious Diseases

Notes:

182 - BVDV compromises fetal immune organ development leading to post-natal health consequences: 2023

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Session: Virology 2, 1/23/2022, 05:00 - 05:15

Objective
To determine the effects of transient ncpBVDV fetal infections on the postnatal health and feedlot performance of calves.

Methods
Control (n=12) and TI heifer calves (n=11) were generated by inoculating seronegative heifers at day 175 of gestation with media or a ncp type 2 BVDV. Control and TI status were confirmed by serum neutralization titers to type 2 BVDV in calf serum immediately after birth and before colostrum intake. Calves were weighed and blood samples obtained at birth, 4, 5.5, 7, 7.5 months and 28-day intervals through the feedlot period. Plasma was assayed for markers of inflammation: oxidized glutathione synthase (GSH), glutathione disulfide reductase (GSSG), and ceruloplasmin. Total tract dry matter digestibility (DMD) was estimated by adding titanium dioxide (TiO2) to the diet of 3 control and 3 TI calves and fecal samples were analyzed for TiO2. DNA from PBMCs was obtained from 5 control and 5 postnatal TI calves at 4 months of age to identify differentially methylated regions (DMR).

Results
TI calves weighed less than controls from birth through 7 months of age ($p<0.05$). TI calves were 45 kg lighter at approximately 1 year of age and gained 0.10 kg less per day. TI calves had elevated levels of ceruloplasmin ($p<0.03$) and reduced glutathione (GSSG) ($p<0.01$) in plasma compared to controls; whereas, oxidized glutathione (GSH) was higher ($p<0.02$) in TIs compared to controls. When comparing PBMC DNA from control and TI calves at 4-Months of age, there were 3876 differentially methylated genes, with 1799 hyper and 2077 hypomethylated regions. Metabolic Processes, Regulation of Growth, Immune Response and T Cell Activation were primary GO and IPA pathways that were impacted.

Conclusions
It is concluded that fetal TI with BVDV may result in epigenetic changes that contribute to postnatal reduced body weights, decreased rate of gain, reduced feed efficiency, metabolic deficits and immune system competency. The 4-month PBMC Methyl-Seq data will be compared with Methyl-Seq and RNA-Seq data from tissues collected at harvest to confirm this hypothesis.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture 2019-67015-29866.

Notes:

183 - MicroRNAs as genetic markers for bovine coronavirus (BCoV) infection

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Session: Virology 2, 1/23/2022, 05:15 - 05:30

Objective
Bovine coronavirus (BCoV) is a Betacoronaviruses along with important emerging coronaviruses, SARS-CoV-2. BCoV is an ideal animal viral model for important emerging human coronaviruses. The roles of microRNAs in the pathogenesis, tropism and genetic markers for BCoV have not been thoroughly studied. We aim to identify some miRNAs as genetic markers and to explore the roles of these miRNAs in tropism, pathogenesis, and immune regulation of BCoV. Our preliminary data show that BCoV infection induces a marked differential display of miRNA expression profiles.

Methods
We used enteric and respiratory isolates of the BCoV to infect the bovine endothelial and bovine nasal turbinate cells. We extracted the total small RNA molecules from those two groups of the BCoV infected cells and a sham none infected group of cells. We obtained the miRNA expression profiles of the two BCoV infected and sham infected groups of cells.

Results
103 and 90 miRNAs are upregulated, while 329 and 324 are downregulated in bovine endothelial cells infected with the BCoV enteric and respiratory isolates. We selected eight top-ranked miRNAs (bta-miR-(497, 7, 454, 22-3P, 1, 374a, 9, and 130a) based on the prediction and the NGS data. Bta-miR-1 is highly induced in cells infected with BCoV respiratory isolates (27.49 folds) compared to 1.98 folds in the enteric strain. Bta-miR-130 and Bta-miR-130a are highly expressed in the case of respiratory pressures. Both Bta-miR-miR-340 and Bta-miR-miR-9-5p are highly induced in the enteric but not expressed in the case of the respiratory strains. Bta-miRNA-(474, 7, and 22-3P) are predicted to target BCoV-S and HE genes of both (enteric and respiratory) isolates, which may control BCoV tropism and pathogenesis.

Conclusions
We identified several miRNA candidates that may act as genetic markers for BCoV infection in general and distinguish between the enteric and respiratory isolates of the virus.

Notes:

184 - Expanding host range and cross species transmission potential of *Rocahepevirus ratti*

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Session: Virology 2, 1/23/2022, 05:30 - 05:45

Objective
Rocahepevirus ratti is an emerging Hepatitis E Virus (HEV) from the *Rocahepevirus* genus. It is commonly referred to as "rat HEV" as it was originally isolated from rats. Rat HEV was first found to be non-infectious to nonhuman primates, suggesting humans could not be a host. More recently, rat HEV cases have been identified in people. High seroprevalence for rat HEV in rats in the United States necessitates that we understand this emerging zoonotic strain. Lack of an infectious clone, cell culture and animal models have hindered this effort. In response to the increase in human infection by rat HEV, we sought to develop an infectious clone of the zoonotic rat HEV strain and identify permissive cells for virus replication, and to further study the cross-species transmission potential of rat HEV.

Methods
To establish a genetically tractable model for studying pathogenesis related to rat HEV, an infectious cDNA clone of genotype 1 LCK-3110 strain of rat HEV was constructed. Rat HEV infection was initiated in LMTK (mouse subcutaneous tissue), NSO-V (mouse myeloma), A549 (human lung), huh7 and HepG2C3A (human liver), BHK21 (baby hamster kidney) and LMH (leghorn male chicken liver) cell lines via transfection with *in vitro* transcribed viral RNA and replication was assessed. To test whether rat HEV transcripts have productively replicated in the target cells, we detected HEV ORF2 protein at the single-cell level using immunofluorescence and flow cytometry. Furthermore, cross species transmission ability in chicken was studied via intrahepatic inoculation. RT-qPCR for rat HEV was performed weekly in serum, fecal and tissue (liver, spleen, jejunum) and bile samples.

Results
LCK-3110 strain of rat HEV is capable of replicating in mouse subcutaneous tissue, human lung, human liver cells, and baby hamster kidney cells. However, the highest replication was seen in mouse subcutaneous, human lung and chicken liver cell cultures. A mild infection was seen in chicken.

Conclusions
Rat HEV is an emerging infectious virus expanding host range and has ability to spillover across species.

Notes:

185 - Applied research to enhance resistance to respiratory disease in cattle

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Session: AAVI - Featured Speakers, 01/24/2023, 08:30 - 09:15

Objective
The bovine respiratory disease (BRD) complex is a leading cause of morbidity and mortality in cattle. Historically, research focused on development of vaccines to prevent infection by individual viruses and bacteria that contribute to the complex. While numerous vaccines have demonstrated protective efficacy in experimental challenge studies, published field trials confirming efficacy of vaccines to prevent naturally occurring BRD are rare. Innate immune responses may be relevant to BRD resistance in high-risk cattle. To improve understanding of immunological pathways relevant to BRD resistance in beef stocker cattle at high risk for BRD, we defined gene expression profiles identified in transcriptomes from whole blood collected from high-risk cattle at arrival, and developed a disease prediction model from BRD occurrence within 28 days after arrival.

Methods
Seven groups of mixed-breed beef cattle weighing 182 – 273 kg were purchased from local auction markets between 2015 and 2020. Blood collected on arrival was stored in Tempus Blood RNA Tubes at -80 C. Cattle received conventional management and were treated for BRD if needed per standard protocols. After 60 – 85 days, cattle treated 0, 1, or 2 or more times (Healthy, Treated_1, or Treated_2+) were identified, and a sample of each group (n = 6 - 119) was selected for RNA sequencing or NanoString nCounter gene expression profiling of banked blood. For small sample sets (n = 12 - 48), RNA sequencing was performed. For larger sets (n = 115 - 119) NanoString gene expression was performed with captured polyadentylated mRNA. After data processing of sequenced reads via a HISAT2/StringTie pipeline, differentially expressed genes (DEGs; FDR $\leq$ 0.05) were identified using edgeR +/- DESeq2 likelihood ratio testing. NanoString gene expression output was normalized and statistically analyzed with nSolver Advanced Analysis Software v4.0 in accordance with the nSolver Gene Expression Data Analysis Guidelines ($p \leq 0.05$). The relationship between DEG and treatment group was evaluated with multiclass receiver operating characteristics (ROC) curve analysis, and a decision tree model was developed based on expression of 6 genes (*HERC6, IFI6, ISG15, MX1, LOC100297044,* and *CFB)*.

Results
Over all seven groups of cattle, genes related to production of specific proresolving mediators (SPM) and antimicrobial peptide production were upregulated at arrival in cattle that stayed Healthy, relative to Treated_1 and/or Treated_2+ cattle. Genes related to type I interferon production, complement factor B, and proinflammatory processes were upregulated in Treated_2 cattle relative to Healthy and/or Treated_1 cattle. Expression profiles in Treated_1 cattle were similar in some ways to Healthy, and in some ways to Treated_2+, suggesting that clinical diagnosis may have misclassified some Treated_1 cattle. The decision tree model classified Treated_2+ cattle with 90% accuracy.

Conclusions
Analysis of whole blood transcriptomes in cattle at arrival reveals differential expression of genes that consistently differentiate high-risk cattle that stay healthy from those requiring BRD treatment. Across multiple groups of cattle, genes related to production of specific proresolving mediators and antimicrobial peptides have been consistently differentially expressed at arrival in cattle not requiring treatment for BRD. These families of mediators warrant further investigation to define mechanisms that allow some cattle to resist BRD.

Notes:

186 - 3D organotypic cell cultures – an alternative *ex vivo* infection model for animal research

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Session: AAVI - Featured Speakers, 01/24/2023, 09:15 - 10:00

Historically, research based on both *in vitro* cell culture and *in vivo* models has been beneficial in understanding infectious disease processes. However, there is a significant gap in complexity between the highly controlled traditional reductionist *in vitro* cell cultures and the variability encompassing the largely undefined but relevant *in vivo* animal models. In recent years, stem cell research has made significant progress towards establishing *ex vivo* culture models ("organoids") that mimic biologically and physiologically the original tissue/organ *in vivo*, thus contributing to the advancement of translational and biomedical research.

Our laboratory has established and characterized 3D organotypic cultures for respiratory and intestinal mucosa. Specifically, respiratory epithelial cell cultures grown at the air-liquid interface can resemble the natural respiratory tract epithelium of the host, including the corresponding cell lineages and functionality. Likewise, intestinal crypt stem cell-derived cultures can differentiate into self-renewal intestine-like 3D cultures that recapitulate the i*n vivo* intestinal epithelium with most of its cell lineage composition and functionality. Using these models, our laboratory has investigated the molecular pathogenesis of the influenza A virus, coronavirus (PHEV, PEDV), and other respiratory and enteric infections. Our studies demonstrated that these viruses trigger early innate immune events that disrupt the homeostasis of mucosal epithelia, including immune changes at physiological barriers, such as mucociliary responses, and changes at the molecular level, such as toll-like receptors, NOD-like receptors, and RIG-I-like receptors causing antiviral, cytokine, and chemokine activation leading to the recruitment of immune cells towards epithelial mucosa.
My presentation will highlight the importance of 3D organotypic cell cultures to advance our understanding of complex mucosal epithelium immune responses toward viruses.

Notes:

187 - Use of reticuloruminal temperature to predict clinical mastitis in dairy cows challenged with *Streptococcus uberis*

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Session: Preventive Medicine, 1/24/2022, 08:30 - 08:45

Objective
Several automated monitoring devices (AMD) have been developed to aid in prediction of clinical mastitis (CM), however, optimal accuracy has not been reached. Algorithms that use variation in reticuloruminal temperature (RRT) have the potential to predict CM. Our objectives were to (1) determine variations in RRT relative to an experimental intramammary (IMM) challenge with *Streptococcus uberis* and (2) evaluate RRT-generated alerts to predict initial signs of CM based on severity of clinical signs and bacterial concentration in the infected quarter.

Methods
Clinically healthy Holstein cows (n = 37, parity 1-5, ≥120 DIM) were enrolled if test day SCC were < 200,000 cells/mL and the cow had no history of CM in the previous 60 d. Prior to the trial, cows received an intra-reticuloruminal temperature AMD which generated an alert when RRT departed 1 SD from the baseline temperature. A single mammary gland in each cow received an intramammary challenge with 2,000 cfu of *S. uberis* O140J. Interrupted time series analysis was performed to evaluate changes in RRT after challenge, while the ability of RRT-generated alerts to predict CM was assessed based on test characteristics.

Results
The IMM challenge increased RRT by 0.54°C (95%CI: 0.41, 0.66; P <0.001) at 24 h post-challenge. Alerts based on RRT correctly classified 78.3% (95%CI: 65.8 - 87.9) of first occurrences CM at least 24 h in advance, with a sensitivity of 70.0% (50.6 - 85.3) and a specificity of 86.7% (69.3 - 96.2). The correct classification for severe CM was only numerically higher (92.9%; 66.1 - 99.8) than mild and moderate cases. The sensitivity of RRT-alerts to predict initial signs of CM improved until bacterial count in the quarter reached 5.0 $\log_{10}$ cfu/mL, showing a sensitivity of 73.5% (55.6 - 87.1) and a specificity of 87.5% (71.0 - 96.5).

Conclusions
Results indicated that RRT was impacted by the IMM challenge with *S. uberis* and the RRT-alerts had similar accuracy as reported for other AMD. Further research that includes natural infections with other pathogens and different variations in RRT to determine CM status is warranted.

Financial Support
Diamond V Mills Inc.

Notes:

188 - Towards novel acaricide development against cattle fever tick: GPCR chemical hits and neuropeptide physiology

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Session: Preventive Medicine, 1/24/2022, 08:45 - 09:00

Objective
This new project advances cattle disease prevention through the discovery of acaricide chemical leads for neuropeptide G protein-coupled receptors as targets. Specific aims: 1) Evaluate anti-tick bioactivity of small molecules antagonists and peptidomimetics of tick kinins already validated on the receptor in dose-response assays. 2) Determine the physiological function of tick neuropeptides through tissue localization analyses, and by identification of gene networks affected by silencing the kinin receptor or kinin gene, using transcriptomics. Silencing periviscerokinin and pyrokinin receptors. 3) Discover small molecules targeting the pyrokinin receptor by high-throughput screens. This first-year report is on Aim 2.

Methods
2.a. RNAi in females of *R. microplus* was by injection of pyrokinin (PK) receptor (*Rhimi-PKR*) dsRNAs, and periviscerokinin receptor (*CAP$_{2b}$R*) dsRNAs. Controls were with dsRNAs for beta-lactamase (-) and actin (+). Phenotypic variables were evaluated, ticks were photographed daily, and silencing in tissues was verified by qRT-PCR. 2.b. Investigated the myotropic activity of tick endogenous PKs and a PK agonist analog on female feeding tissues of two species representing the Ixodidae lineages, *Ixodes scapularis* (Prostriata) and *Rhipicephalus sanguineus* (Metastriata).

Results
2.a. *Rhimi-PKR* and *Rhimi-CAP$_{2b}$R* silencing increased mortality and decreased the weight of females and egg masses, and delayed the egg incubation period ($P < 0.05$). PKR-silenced ticks were delayed in their repletion and pre-oviposition periods. *CAP$_{2b}$R*-silenced ticks had decreased egg hatching ($P < 0.05$). 2.b. PKs and PK analog elicited contractions of the pharynx-esophagus in both tick species. In accordance, the PK receptor relative transcript abundance was highest in the feeding tissues extracted from the capitulum of female *R. sanguineus*.

Conclusions
This is the first report on the activity of pyrokinins in ticks. Results point to the roles of *Rhimi-PKR* and -*CAP$_{2b}$R* in feeding and reproduction, making them potential targets for interference.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture; Texas A&M AgriLife Research - Insect Vector Diseases Grant Program

Notes:

189 - Pharmacokinetics of bumped kinase inhibitor-1708 in horses: a candidate for EPM treatment

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Session: Preventive Medicine, 1/24/2022, 09:00 - 09:15

Objective
Bumped Kinase Inhibitors (BKI) have proven efficacious in treating apicomplexan infections, and are now under investigation as therapeutics for equine protozoal myeloencephalitis (EPM) caused by *Sarcocystis neurona*. Here we report the intravenous (IV) pharmacokinetics (PK) of BKI-1708 in horses.

Methods
In vitro growth assays were performed to assess the effects of BKI-1708 on *S. neurona* invasion and growth in mammalian cells. The IV dose was based on allometric scaling informed by in vitro to in vivo extrapolation (IVIVE). BKI-1708 was dissolved in 90% polyethylene glycol-400/10% dimethylsulfoxide at 10 mg/mL and dosed at 1 mg/kg via IV catheters to two, three-year-old horses. Blood was sampled at 0 h, 5 min, 10 min, 15 min, 20 min, 30 min, 45 min, 1 h, 1 h 30 min, 2 h, 3 h, 4 h, 6 h, 8 h, 12 h, 24 h, 32 h, 48 h, and 72 h after administration and analyzed for BKI-1708 levels. Horses were monitored daily for adverse effects by physical exams, complete blood cell count and biochemistry panels. BKI-1708 quantitation was by LC-MS/MS with a limit of detection of 0.01 μM.

Results
BKI-1708 inhibits *S. neurona* proliferation by 50% at 42 nM. Administration of BKI-1708 at 1 mg/kg IV led to maximum levels in horse plasma of ~4 μM with an initial distribution phase followed by a terminal phase. A half-life of 2 hrs was observed for BKI-1708. BKI-1862, a metabolite of BKI-1708, was also detected in the plasma samples, but not at more than 0.3 μM. No adverse effects were observed.

Conclusions
Both the IV PK and *in vitro* efficacy results suggest BKI-1708 is a promising compound for treating EPM in horses. Since IV delivery would be suboptimal for EPM therapy, oral dosing experiments will be conducted to investigate the PK profile after gastrointestinal absorption and first pass metabolism. Assuming that the drug will maintain reasonable plasma levels after oral administration, assessment of central nervous system drug levels will be performed by placing intrathecal catheters for cerebrospinal fluid collection after oral dosing to estimate whether sufficient BKI-1708 distributes to the nervous system for therapy of EPM.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture; WCVV is an officer of ParaTheraTech Inc., a company engaged in research and development of BKIs for animal health

Notes:

190 - In vivo efficacy of tulathromycin and diclazuril against *Theileria haneyi* in horses

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Session: Preventive Medicine, 1/24/2022, 09:15 - 09:30

Objective
Equine theileriosis is caused by the hemoparasites *Theileria haneyi* and *Theileria equi.* These infections can lead to severe disease, and have resulted in strict international equine import and export restrictions. Imidocarb dipropionate is currently the only chemotherapeutic option for *T. equi* in the United States. Unfortunately, it has proven ineffective against *T. haneyi.*
The goal of this study was to assess the in vivo efficacy of the readily available drugs, tulathromycin and diclazuril, against *T. haneyi* in horses.

Methods
Fourteen horses were utilized for this study. Horses were inoculated intravenously with *T. haneyi*-infected erythrocyte stabilate. Infection was confirmed via blood smear cytology and nested PCR for *T. haneyi,* and horses were divided into four groups. The first (n=6) received 2.5 mg/kg tulathromycin (100mg/mL) intravenously once a week for eight weeks beginning one month after confirmation of *T. haneyi* infection. The second group (n=3) received 2.5 mg/kg 1.56% diclazuril orally daily for 60 days beginning one month after confirmation of *T. haneyi* infection. The third group (n=3) received 0.5 mg/kg diclazuril orally daily for one month prior to *T. haneyi* infection, and then 2.5 mg/kg per day for 60 days. The remaining two horses were used as untreated controls. Animals were assessed regularly via nPCR, physical exam, complete blood counts, serum chemistry panel, and blood smear cytology. To obtain data on the safety of tulathromycin in adult horses, necropsy and histopathology were performed at the end of the study.

Results
Horses treated with tulathromycin remained *T. haneyi*-positive. No adverse effects were detected via serum chemistry panel, physical exam, or tissue evaluation. Pre-treatment of horses with diclazuril failed to prevent *T. haneyi* infection, and horses treated with label doses of diclazuril remained positive for *T. haneyi*.

Conclusions
These results demonstrate that *T. haneyi* is not susceptible to tulathromycin or diclazuril at the dosages and routes of administration utilized in this study.

Financial Support
U.S. Department of Agriculture; Boehringer Ingelheim Animal Health

Notes:

191 - Dietary anti-IL-10 impacts on enteric disease outcomes in chickens without early *Salmonella* exposure

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Session: Preventive Medicine, 1/24/2022, 09:30 - 09:45

Objective
Early *Salmonella* Typhiumurium improves *Eimeria*+*Clostridium perfringens* (CP) challenge repeatability but may obscure effects on disease outcomes due to utilization of the IL-10 pathway. The study objective was to assess performance and immunometabolism in broiler chickens fed anti-IL-10 antibody and challenged with *Eimeria maxima* (EM)±CP without early *S.* Typhiumurium.

Methods
Ross 308 broilers were placed in 32 raised wire floor cages (15 birds/cage; 480 total) and assigned to basal diet±0.03% anti-IL-10 for 25d. On d14, a subset was gavaged with saline or 15,000 sporulated EM M6 oocysts. On d18 and 19, $1*10^8$ colony forming units of CP or culture media were administered to half the EM-challenged birds. Body weight (BW) and feed intake were recorded weekly. At baseline, 1, 3, 7, and 11 d post-inoculation (pi), 6 birds/treatment were euthanized for peripheral blood mononuclear cell isolation and immunometabolic analysis using Agilent real-time ATP and glycolytic rate assays (Santa Clara, CA). Data were analyzed with fixed effects of diet, challenge, and diet*challenge (SAS 9.4; $P \leq 0.05$).

Results
No baseline performance or immunometabolic differences were observed. The EM challenge main effect increased ATP production 25% at 1dpi and the ability to switch to glycolytic metabolism 17% at 3 dpi ($P \leq 0.03$). No additional immunometabolic changes were observed following CP inoculation. Within 7 dpi, the challenge main effect reduced BW gain (BWG) 43% in EM-challenged birds while EM+CP reduced BWG 54% ($P < 0.0001$). Control-fed EM+CP-challenged birds had a 50-point less efficient feed conversion rate (FCR) compared to their EM-challenged counterparts while no FCR differences were observed in EM- vs. EM+CP-challenged birds fed anti-IL-10.

Conclusions
Feeding anti-IL-10 did not protect birds from challenge-related BWG reductions but may have preserved FCR after secondary CP challenge. Anti-IL-10 diet did not alter immunometabolic outcomes within the first 3 dpi but initial EM challenge did. No additive immunometabolic changes after secondary CP inoculation suggest that immunometabolic responses may be site-specific.

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

Notes:

192 - Evaluating turkey-derived lactic acid producing bacteria as potential probiotics for use in commercial turkeys

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Session: Preventive Medicine, 1/24/2022, 09:45 - 10:00

Objective
Lactic acid producing bacteria (LAB) are widely used in the poultry industry as they have been associated with gut health and increased growth performance. Despite their wide use in poultry production, LAB have been highly variable in their ability to modulate poultry gut health and growth performance. Furthermore, most commercially available LAB probiotics are not specifically developed for use in turkeys. The objective of this study is to use probiotic screening assays to compare probiotic relevant phenotypic differences amongst different species of turkey-derived lactic acid bacteria in order to identify potential probiotics for use in turkey production.

Methods
We used different *in vitro* assays to compare the probiotic potential (phenotype) of each turkey derived LAB isolate. Twenty-four isolates representing 8 different species were used for our experiments. These assays include measuring each strain's acid tolerance, bile tolerance, and adhesion ability. Tolerance to acid was determined by comparing growth in acidic media between all isolates. Bile tolerance was determined by comparing growth in media containing bile salts between isolates as well as identifying bile salt hydrolase activity. Adhesion ability was measured by comparing percent adhesion to an avian intestinal cell line (BATC) between isolates.

Results
We were able to show that there was variability in assay performance between many of the strains in every assay performed ($P<0.05$). We also determined that isolates within the same species often varied in their performance between the assays. We were also able to identify isolates which performed highly in multiple assays.

Conclusions
Through these experiments we were able to show that LAB isolates vary phenotypically in probiotic performance assays. Variability was seen between and within different species of turkey-derived LAB. We have identified high performing isolates in multiple probiotic performance assays that we hope in the future could be used in performance trials with commercial turkeys to help develop turkey specific probiotics.

Notes:

193 - Dissemination of antibiotic resistance genes across the food chain of commercial antibiotic-free poultry farms

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Session: Antimicrobial Use 3, 1/24/2022, 08:30 - 08:45

Objective
Antimicrobial resistance (AMR) is a major concern for "One Health" and injudicious antimicrobial use (AMU) contributed to its spread. However, despite reducing AMU, antimicrobial resistant pathogens can persist in the environment promoting their spread. The objective of this study was to: i) determine AMR levels in the environment of antibiotic-free poultry farms in different stages of production; and ii) determine the potential for AMR spread between and within poultry farms.

Methods
A total of 16 antibiotic-free poultry farms were screened (3 pullet, 3 breeder, and 10 broiler). Litter samples were collected from inside the poultry house. Soil and any fecal samples found around the house were also collected. To determine the frequency of 2 mobile element genes (MEGs) and 15 antimicrobial resistance genes (ARGs) belonging to 8 classes of antimicrobials commonly used in poultry, we performed qPCR and analyzed the data using RStudio.

Results
On breeder and broiler farms, ARGs were most frequently found in litter followed by fecal and soil samples. On pullet farms, fecal followed by litter and soil had the highest AMR levels. Broiler farms had highest levels of AMR followed by breeder and pullet farms. Tetracycline and macrolide-lincosamide-streptogramin B genes were more abundant on broiler and breeder farms. On pullet farms, sulfonamide and tetracycline ARGs were the most frequent. In soil and litter samples, tetracycline and quaternary ammonium compound ARGs were most frequently observed. In fecal samples, tetracycline and sulfonamide ARGs were most frequently observed. MGEs were most frequently found on broiler farms and in litter samples from all farm types.

Conclusions
There is a potential for AMR spread between inside and outside environments and between farm levels in antibiotic-free poultry farms due to the high levels of MEGs and ARGs present in the environment. The understanding of dissemination of ARG facilitates identification of critical control points to tackle AMR in poultry production. Future studies will explore the molecular evidence of AMR within and between poultry farms.

Notes:

194 - SNP profiling of poultry litter resistomes following in-feed oxytetracycline administration

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Session: Antimicrobial Use 3, 1/24/2022, 08:45 - 09:00

Objective
Shotgun metagenomic sequencing allows for the assessment of microbial communities and their genetic content at the nucleotide level. Analyzing the single nucleotide polymorphism (SNP) profiles of antimicrobial resistance genes (ARGs) within shotgun data could provide information about the origin of unique genotypes within and between microbiomes. Thus, the goals of this project were to identify ARGs in poultry litter following exposure to in-feed oxytetracycline and to assess the SNP profiles of these ARGs as a function of antibiotic exposure and time.

Methods
Data used in this study comes from three successive flocks of broilers that were raised to 35 days of age on the same litter. Four treatments, each administered to five pens per flock cycle were analyzed: two controls and two in-feed oxytetracycline treatments. Pen-level litter samples collected at six time points per flock underwent shotgun metagenomic sequencing. Raw data was aligned to MEGARes v3.0 to identify ARGs using NGLess, and SNPs were identified using metaSNV v2. Bray-Curtis dissimilarity was calculated, and the impact of pen, treatment group, flock, and sampling day on dissimilarity was assessed using an additive PERMANOVA model.

Results
A total of 15,207 unique ARG SNPs were identified across all samples, and were contained within ARGs that can confer resistance to 44 unique ARG classes. PERMANOVA modeling revealed that treatment group, flock, and sampling day all had a significant impact on Bray-Curtis dissimilarity. Across all samples, SNVs were most commonly found in the combined drug and biocide resistance mechanisms, oxazolidinone, macrolide-lincosamide streptogramin, aminoglycoside, and tetracycline ARG classes.

Conclusions
As sequencing and bioinformatic tools advance, resistome-wide SNP analysis is likely to become more tractable for metagenomic datasets. Our preliminary findings suggest that these analyses may yield additional insights beyond analysis of gene- or mechanism-level changes. Future analysis will be conducted to evaluate potential SNP-level temporal changes and different antibiotic exposures.

Financial Support
U.S. Department of Agriculture National Institute of Food and Agriculture in part by grant no. 2015-68003-22972

Notes:

195 - Effect of enrofloxacin on cecal microbiota and metabolites of chickens challenged with *Campylobacter jejuni*

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Session: Antimicrobial Use 3, 1/24/2022, 09:00 - 09:15

Objective
Campylobacter is a leading cause of bacterial foodborne illnesses and is commonly present in the intestinal track of poultry. It is known that fluoroquinolone (FQ)-resistant *Campylobacter* quickly develops in chickens treated with enrofloxacin via drinking water, but it is unclear whether the treatment alters gut microbiota and metabolome and consequently provides a favorable environment for establishing FQ-resistant *Campylobacter* in the intestine. This study is aimed to evaluate the changes in microbiota and metabolites in the cecum contents of enrofloxacin (ENRO)-treated chickens infected with *C. jejuni*.

Methods
Campylobacter-free broiler chicks were orally inoculated with *C. jejuni* and divided equally into two groups. Birds in the treatment groups were given ENRO (50 ppm in drinking water) for 5 days, while chicks in the control group were given sham treatment. Birds were sacrificed periodically and cecal contents were collected for determining *Campylobacter* colonization levels, microbiota (16S rRNA gene-based) compositions, and untargeted metabolomics profiling.

Results
ENRO treatment significantly reduced *Campylobacter* colonization initially but quickly selected FQ-resistant mutants in the treated birds. The treatment significantly decreased the relative abundance of phylum *Actinobacteriota* and several genera, such as *Romboutsia*, *Proteus*, and *Blautia*. Untargeted metabolomics analyses showed notable differences in aromatic amino acid metabolites, benzoate metabolites, bile acids, phospholipid metabolites, and lysophospholipid metabolites between the control and the ENRO-treated groups. Interestingly, ENRO-treated group showed significantly higher concentration of phenethylamine.

Conclusions
These results indicate that gut microbiota diversity, composition, and metabolites were altered significantly by ENRO treatment, which may facilitate the rapid propagation of the FQ-resistant *Campylobacter* in the chicken gut in response to the treatment.

Financial Support
U.S. Department of Agriculture

Notes:

196 - Antimicrobial quantification on dairy farms before and after the implementation of farmworker stewardship training

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Session: Antimicrobial Use 3, 1/24/2022, 09:15 - 09:30

Objective
Antimicrobials are critical to preserve animal health. However, the development of antimicrobial resistance represents a public health threat. Veterinarians prescribe antimicrobials, but farmworkers are responsible for making on-farm treatment decisions and their training is vital to promote responsible antimicrobial use (AMU). The objectives of this study were to evaluate the impact of farmworker antimicrobial stewardship (AMS) training and describe AMU on dairy farms across OH and CA. We hypothesized that farms, where AMS training was administered, would have significantly lower AMU compared to farms where training was not administered.

Methods
We designed a quasi-experimental study with eighteen conventional dairy farms enrolled in Ohio and California. Twelve farms received AMS training and six farms did not. AMS training included a 12-weeks training program focused on increasing accurate diagnosis of cows requiring antimicrobial treatment. We quantified on-farm AMU by measuring the number of used antimicrobial bottles. Treatment incidence using animal daily-doses (ADD) and Poisson regression model were used to analyze AMU data.

Results
The highest mean ADD by antimicrobial classes was for cephalosporins at 5.8, followed by penicillins at 5.3 ADD/1000 cow-days. Mean ADD from the training group was at 10.7 and numerically lower when compared to the control group at 13.6 ADD/1000 cow-days. Therefore, AMS training farms had a rate ratio of 0.79 ADD/1000 cow-days lower when compared to farms in the control group. A decreased mean ADD was exhibited for farms in the AMS training group from 11.0 pre-intervention to 10.2 ADD/1000 cow-days post-intervention. However, Poisson regression mixed model showed that the reduction in the mean ADD post-intervention was not statistically significant (p= 0.916).

Conclusions
Cephalosporins followed by Penicillins were the most used antimicrobial classes for the enrolled dairy farms. Intramammary was the primary route of antimicrobial administration used by farm workers. Trained farms did not show a significant reduction in on-farm AMU compared to control farms.

Financial Support
U.S. Department of Agriculture

Notes:

197 - Quantifying antimicrobial use in peri-urban dairy system of Pakistan

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Session: Antimicrobial Use 3, 1/24/2022, 09:30 - 09:45

Objective
Antimicrobial resistance (AMR) is an emerging global health threat. Monitoring antimicrobial use (AMU) and its reduction in animal husbandry are one of the key strategic objectives to address the AMR crisis. The AMR-National Action Plan calls for the prudent use of antimicrobials in food-producing animals to prevent the AMR crisis. The present study aimed to quantify AMU in these peri-urban cattle colonies with a focus on finding the use of Critically Important Antibiotics (CIAs) and seeing regional AMU differences between these colonies.

Methods
A total of 9 peri-urban farms/dairy colonies from Punjab, KP, Balochistan, and Sindh provinces were selected for the monitoring AMU for a period of 4 summer months (May-August) in 2021. Data on antimicrobial brands used for treatment or prevention purposes were collected on data collection tool. The data collection began on May 1, 2021, and data on the cattle colony population, number of cow days under observation, and cow days treated were also collected.

Results
A total of 31 (104.2kg) antimicrobials were consumed in dairy herds under observation and treatment by antimicrobial prescribers. Penicillin (3.97 DDDA/1000 cow-days) followed by gentamicin (2.44 DDDA/1000 cow-days), enrofloxacin (2.26 DDDA/1000 cow-days), dihydrostreptomycin (1.66 DDDA/1000 cow-days) and amoxicillin (1.57 DDDA/1000 cow-days) were most used antimicrobials. Three antimicrobials (colistin, marbofloxacin, and ampicillin) were only used in Sindh whereas sulfamethoxypyridazine was only used in Balochistan. Approximately 63% of antimicrobial treatment incidence (ATIs) in cattle colonies of Pakistan were from CIAs either highest priority or high priority antimicrobials for human use as per the classification of the World Health Organization.

Conclusions
Inappropriate antimicrobial use in food-producing animals promotes the increase of AMR. This study showed that Pakistan is a large consumer of antimicrobials in the animal health sector because of emerging intensive livestock farming. It can guide policymakers to implement strategies to promote the judicious use of antimicrobials.

Notes:

199 - An investigation of the differential diagnoses of African swine fever in pigs slaughtered in central Uganda

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Session: Epidemiology 4, 1/24/2022, 08:30 - 08:45

Objective
African swine fever (ASF) is a highly infectious viral disease of pigs that is endemic in Uganda. Clinical diagnosis of ASF is difficult because it presents with clinical signs and lesions similar to several pathogens. Pathogens that are differential diagnoses for ASF include swine influenza A virus (S-IAV), porcine reproductive and respiratory syndrome virus (PRRSV), classical swine fever virus (CSFV) and *Salmonella* spp. Little is known about the occurrence of S-IAV, PRRSV, CSFV, and *Salmonella* spp. in pigs in Uganda. The objective of this study is to determine the seroprevalence of S-IAV and PRRSV antibodies, and the prevalence of CSFV, and *Salmonella* spp. in slaughterhouse pigs in Uganda.

Methods
We sampled 1316 pigs from six pig abattoirs in central Uganda (Wambizzi, Lusanja, Budo, Katabi, Buwate and Kyetume). The slaughterhouses were purposively selected, and the pigs were sampled following a weighted stratified systematic sampling method from May 2021 to June 2022. Serum samples were tested for antibodies using Ingenasa's S-IAV and PRRSV indirect ELISAs. Testing has not started for *Salmonella* spp. and CSFV, but we will use conventional PCR and RT-qPCR, respectively.

Results
Of the pig serum samples analyzed, 878 (89.7%, 95%CI: 87.61%, 91.44%) had antibodies against S-IAV and 10 (1.1%, 95%CI: 0.56%, 1.99%) had antibodies against PRRSV. Four of the pigs that were seropositive for S-IAV were also seropositive to ASFV. Five pigs that were seronegative to ASFV, were seropositive to PRRSV and had clinical signs and lesions typical of ASF. Molecular ASFV testing comparisons will be reported as well.

Conclusions
There is a high-level of exposure to S-IAV and low exposure to PRRSV among pigs at Kampala slaughterhouses during 2021-2022. The high occurrence of S-IAV in Uganda hinders syndromic surveillance for ASF. ASF surveillance programs in Uganda will require confirmatory laboratory diagnosis of ASFV.

Financial Support
U.S. Defense Threat Reduction Agency

Notes:

200 - Clinical and pathological presentation of African swine fever in pigs slaughtered in central Uganda

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Session: Epidemiology 4, 1/24/2022, 08:45 - 09:00

Objective
African swine fever (ASF) is an infectious disease of pigs caused by a DNA virus in the family *Asfarviridae*. The virulence of the infection depends on the infecting virus' genotype and host factors. Although ASF is endemic in Uganda and is a major constraint to pig production, there is not a comprehensive understanding of its clinical and pathological manifestations in the country. The objective of this presentation is to describe the clinical and pathological presentation of ASFV infected pigs in slaughterhouses in the Kampala metropolitan area.

Methods
We sampled 1316 pigs from Wambizi, Lusanja, Budo, Katabi, Buwate, and Kyetume pig slaughterhouses over a 13-month period from May 2021 through June 2022. Slaughterhouses were purposively selected and stratified systematic sampling method weighted by the estimated annual slaughter rate per slaughterhouse was used to sample pigs. Clinical and pathological scoring rubric was used to capture the ASF clinical signs and lesions in the pigs. Positive pigs were determined using a real-time PCR tested on blood samples.

Results
Of the 1297 pigs whose rectal temperatures were taken, 110 (8.5%) had mild fever (39.9 – 40.5 °C), 37 (2.8%) had moderate fever (> 40.5 – 41 °C), 38 (2.9%) had severe fever (>41 °C), and 1,112 (85.7%) had no evidence of fever. Out of 1227, 23.6% had skin discolorations and 1.1% of 1312 pigs had evidence of diarrhea. For the postmortem signs typical of ASF, 33.3% of 1274 pigs had enlarged spleens and 48.8% of 1249 pigs had hemorrhagic spleens. The gastro-hepatic lymph nodes were enlarged, hemorrhagic, and/or edematous in 56.7% of 1278 pigs. Petechial hemorrhages were found on 69.9% of 1274 pigs' kidneys.

Conclusions
Approximately half of the pigs slaughtered in central Uganda have lesions typical of ASF. This suggests sick pigs are sent to the slaughterhouse, potentially as part of outbreak sell-offs. This is a commonly reported management strategy to reduce economic losses due to ASF. Pig slaughterhouses in Uganda could be used as a surveillance site for identifying outbreaks in the major pig-producing areas of the country.

Financial Support
U.S. Defense Threat Reduction Agency

Notes:

201 - Analysis of IAV transmission in large U.S. swine production systems using active surveillance

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Session: Epidemiology 4, 1/24/2022, 09:00 - 09:15

Objective
Influenza A virus (IAV) is one of the three most frequently detected causes of respiratory disease in swine. The passive USDA swine surveillance system provides aggregated metrics to quantify spatial and temporal changes in genetic diversity; however, swine production is not homogenous. Production systems vary in size and management strategies that may affect the transmission and evolution of IAV. We utilize whole genome sequences (WGS) from active surveillance to elucidate the farm-level evolutionary dynamics.

Methods
We conducted active surveillance on selected sow farms and their linked downstream nurseries from 4 large US production systems for at least 12 monthly collections. From IAV positive samples, we obtained 85 complete HA sequences, and of these, we successfully assembled 61 whole genomes with linked production system metadata. To infer transmission and evolution, we conducted Bayesian phylodynamic analyses on the active surveillance data combined with control sequences sourced from passive swine and human surveillance.

Results
We detected 6 genetic clades from four HA lineages: the H1 1A classical swine, the H1 1B human-seasonal, and the H3 2010.1 and 1990.4 lineages. The 1B and H3 1990.4 strains showed evidence of transmission from sow farm to downstream nurseries. In contrast, 1A and H3 2010.1 viruses were detected in nurseries without upstream detection in linked sow farms. We also detected seven unique human-to-swine transmission events in the H1N1 pandemic clade (1A.3.3.2) in sow and nursery sites.

Conclusions
These data demonstrated that nursery sites were also infected with IAV not linked to the sow farm, possibly due to subclinical IAV below detection levels in the breeding herd, mixing of sow farm sources at the nursery, regional spread of new strains, or human-to-swine transmission. Detection of IAV in sow farms was predictive of transmission to nurseries in 3 out of 4 systems in our study, which can inform measures to decrease IAV virus movement among these production sites. Additional investigation is needed to understand nursery detections not linked to the sow farm.

Financial Support
U.S. Department of Agriculture, Agriculture and Research Services; Iowa State University; U.S. Department of Defense; U.S. National Institutes of Health

Notes:

202 - A review on the occurrence of foot-and-mouth disease virus serotypes in Uganda and Tanzania (2003 to 2015)

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Session: Epidemiology 4, 1/24/2022, 09:15 - 09:30

Objective
The progressive control pathway for FMD (PCP-FMD) specifies successive steps through which a country/region can reduce FMD Virus (FMDV) circulation and impact. These steps are reliant on understanding and obtaining knowledge on FMD epidemiology, to inform development of appropriate disease interventions like vaccination and quarantine programs. Currently, Uganda and Tanzania are in the early stages of the PCP-FMD. This review was undertaken to determine FMDV serotype distribution in Uganda and Tanzania between 2003 and 2015. The study also sought to compare the vaccine strains used in both countries for the same period viz avis the circulating virus topotypes.

Methods
Using a systematic review process and meta-analysis we studied published articles that had information on serotypes that were responsible for FMD outbreaks that had occurred in Uganda and Tanzania between 2003 and 2015. Maps were drawn using QGIS.

Results
The review highlights four (O, A, SAT 1 and SAT 2) and five (O, A, SAT1, SAT 2 and SAT 3) serotypes that occurred in Uganda and Tanzania respectively in the thirteen-year period. Observations revealed that reported circulating serotypes O and A in the two countries belonged to similar topotypes, East African 2 (EA-2) and AFRICA respectively. The SAT 1 viruses in Tanzania belonged to topotype I and differed from the Ugandan SAT 1s that belonged to topotype IV. Similarly, the SAT 2s in both countries belonged to different topotypes: IV in Tanzania and I in Uganda. This review additionally, underscores the spatial distribution of FMDV serotypes in Uganda and Tanzania and highlights regions in both countries that had high serotype diversity.

Conclusions
The paper shows similar serotypes but different topotypes especially for the SATs circulating in both countries, thus implications for cross border disease spread but also for regional disease control using vaccination. The study recommends definitive disease diagnoses, molecular serotype characterization and matched vaccination deployment to support improved disease control.

Financial Support
U.S. Defense Threat Reduction Agency

Notes:

203 - Neonatal calf diarrhea case definitions: a scoping review

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Session: Epidemiology 4, 1/24/2022, 09:30 - 09:45

Objective
This scoping review aimed to summarize case definitions used for neonatal calf diarrhea (NCD) in published literature and describe diarrhea-related outcomes to inform future efforts towards standardization and improve evidence synthesis capacity.

Methods
A literature search identified articles using 3 databases (Medline, CAB Direct, Agricola), along with Google and Google Scholar. This returned 16,854 unique articles which were then screened for eligibility by two independent reviewers, resulting in 555 being selected for data extraction.

Results
One or more references were cited for the NCD case definition by 49% of studies (n = 273/555), with the most common references being Larson et al. (1977) (n = 85), and McGuirk (2008) (n = 59). Studies used between 1 and 8 metrics to define NCD, with 933 unique metrics extracted in total. The most common metric was fecal consistency alone (30%; n = 281), or with at least 1 other metric (26%; n = 241). To define diarrhea, fecal consistency was either described qualitatively (e.g., "profuse liquid feces"), or semi-quantitatively, for example using a scoring system that frequently included 4 levels (n = 208). Some NCD case definitions included fecal color, volume, or odor (10%; n = 98), physical exam parameters (8%; n = 79), the duration of abnormal feces (7%; 67), the presence of abnormal contents (e.g., blood, 7%; n = 61), farm treatment records (6%; n = 54), fecal dry matter (1%; n = 12), or another metric (4%; n = 41). In total, 979 unique diarrhea-related outcomes were found, most commonly a binary categorization of calves having or not having diarrhea (49%; n = 483). Other articles reported statistical outcomes calculated from fecal scores (16%; n = 159), multiple diarrhea severities (10%; n = 95), or the age calves first developed NCD (8%; n = 76).

Conclusions
This review characterized substantial heterogeneity among NCD case definitions and outcomes, limiting interpretation and comparison of studies. Future work is required to develop and validate reporting standards for NCD to optimize knowledge synthesis and support rigorous calf health research.

Financial Support
Dairy Farmers of Ontario; U.S. Department of Agriculture, National Institute for Food and Agriculture; Ontario Research Fund – Research Excellence

Notes:

205 - Host-pathogen coevolution in a changing environment: pathogen exposure and host performance

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Session: Disease Pathogenesis 3, 1/24/2022, 08:30 - 08:45

Objective
As the climate continues to warm, we are seeing a variety of responses including species range shifts, changes in population demography, and altered species interactions. For host-pathogen interactions, climate change may either increase or decrease disease transmission depending on the ecological context. However, little is known about the evolutionary and coevolutionary effects of climate change on disease transmission. Our research focuses on the effects of a changing climate on the evolution and coevolution of a host's responses driven by the interaction between a host, its pathogen, and its environment.

Methods
Using an easily manipulated insect host-pathogen system -- the fall armyworm (*Spodoptera frugiperda*), an agricultural pest, and its lethal baculovirus -- we examined host-pathogen interactions across multiple generations and under multiple temperature regimes. The host is an agricultural pest and, like other outbreaking insects, fall armyworm population dynamics can be pathogen regulated. To examine host performance, we constructed a thermal performance curve by exposing each host population to a variety of temperatures. Then, to examine host susceptibility, we conducted a series of experiments where the hosts were exposed to a known quantities of the lethal pathogen. In addition, we quantified hemocyte production and pathogen production in exposed hosts.

Results
Our data show that temperature affects host development and survival. Our experiments also demonstrate how susceptibility to the virus depends upon the degree of exposure and as host and pathogens coevolve that level of exposure stays relatively constant.

Conclusions
The conclusions drawn from this research are not only applicable to this system but also other silvicultural and agricultural pathogen-susceptible pest species of which there are many. This research will, in turn, improve our ability to determine how best to use these pathogens as bioinsecticides. In future research, we will use these established host lines to examine how abiotic factors directly affect disease transmission in host-pathogen coevolution

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

Notes:

206 - Rumen dynamics of volatile fatty acids and *Epichloë coenophiala*-produced ergovaline in steers grazing tall fescue

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Session: Disease Pathogenesis 3, 1/24/2022, 08:45 - 09:00

Objective
Fescue toxicosis (FT) is caused by consumption of tall fescue infected with the ergot alkaloid, (i.e., ergovaline (EV))-producing endophyte *Epichloë coenophiala*. Lower weight gains are FT hallmark that cannot be entirely explained by feed intake reduction. With a multi-omics approach, we demonstrated that animals grazing toxic (E+) fescue had microbiota shifts suggestive of altered volatile fatty acid (VFA) status and also identified EV in the rumen with untargeted metabolomics. Here, we aimed to (*i*) characterize the VFAs in the rumen and in the feces and (*ii*) quantify EV using targeted LC-ESI-MS and determine its correlation with the EV profile in the rumen identified with the untargeted approach.

Methods
Twelve steers were placed on non-toxic (NT) or E+ fescue pastures for 28 days. Feces and rumen liquid were collected at several time points and analyzed for VFAs using GC-MS. EV was analyzed with LC-ESI-MS in the rumen liquid and in the plasma.

Results
Overall, E+ grazing resulted in decreased ruminal propionate (P) and increased acetate (A) and A:P ratio; across the 28 days of grazing, these effects were most pronounced on days 7 and 14. In the feces, there were similar non-significant trends for higher A:P ratio and lower P in the E+ steers. In the plasma, EV was undetectable, but it was readily detected in the rumen and exhibited similar dynamics (peaking on day 14 of E+ grazing) to that demonstrated via untargeted means.

Conclusions
Lower ruminal P and a high A:P ratio might be contributory for the decreased performance of E+ steers, but it could also lead to increased methane emissions. Detection of EV only in the rumen suggests its effects are mostly local and systemic toxicity of E+ grazing is indirect and/or due to EV metabolite(s). The reduction in EV after day 14 likely reflects pre-systemic, i.e., microbiota, shifts that favor EV breakdown. Moving forward, to improve detection and treatment of FT, we will evaluate the correlations between ruminal EV and VFA not only with changes in key microbiome taxa, but also with E+-specific urinary metabolites we identified previously.

Financial Support
U.S. Department of Agriculture

Notes:

207 - Development of an experimental model for liver abscesses in calves using an acidotic diet and bacterial inoculation

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Session: Disease Pathogenesis 3, 1/24/2022, 09:00 - 09:15

Objective
Holstein steers (n = 40; initial BW = 84.9 ± 7.1 kg) were used to determine the effects of ruminal acidosis and a bacterial inoculation on the development of liver abscesses.

Methods
Steers were individually housed in a climate-controlled barn for 50 d and randomly assigned to 1 of 3 treatments: 1) control diet (CON), 2) acidotic diet (AD), 3) acidotic diet plus intraruminal inoculation with *Fusobacterium necrophorum*, *Trueperella pyogenes*, and *Salmonella Lubbock* (1×10^9 CFU/mL in 100 mL; ADB). Steers in AD and ADB treatments were fed the acidotic diet for 3 d followed by the control diet for 2 d and repeated for 4 cycles, after which steers in the ADB treatment received intraruminal bacterial inoculations. Following inoculation, AD and ADB steers remained on the acidotic diet for the remainder of the study. Rumen pH boluses were randomly introduced to 19 steers to record ruminal pH. At harvest, gross pathology was observed and scored on lung, rumen, liver, and colon. Data were analyzed as a completely randomized design with animal as the experimental unit, where an α of ≤ 0.05 determined significance.

Results
Liver abscess prevalence was 42.9% in ADB vs. 0% in AD and CON treatments ($P < 0.01$). Mild rumen scores were greater in ADB vs. AD and CON treatments ($P < 0.01$). There was a treatment × time interaction for ruminal pH, where pH decreased in AD and ADB calves during each acidotic cycle ($P < 0.01$). Furthermore, there were no difference in the hematology variables measured ($P \geq 0.16$).

Conclusions
This challenge model successfully induced liver abscesses in Holstein steers and provides insight on possible mechanisms of liver abscess formation. These data suggest that acidosis in conjunction with intraruminal pathogen inoculation is a viable model to study liver abscess formation and evaluate novel interventions. Further research is needed to elucidate host pathogen interactions and determine the repeatability of this model.

Notes:

208 - Depot-specific adipose tissue transcriptional alterations in transition dairy cows with subclinical ketosis

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Session: Disease Pathogenesis 3, 1/24/2022, 09:15 - 09:30

Objective
Ketosis is one of the most prevalent postpartum diseases in dairy cows and involves adipose tissue (AT) dysfunction. The objective of this study was to determine transcriptional differences in the visceral (VAT) and subcutaneous adipose tissue (SAT) of dairy cows with or without subclinical ketosis.

Methods
Ten Holstein cows (DIM 8.0 ± 2.0, parity 3.2 ± 1.4, BCS 3.6 ± 0.3) were enrolled in a nonrandomized trial. Cows were blocked according to parity, body condition score and blood β-hydroxybutyrate (BHB) and assigned into two groups: subclinical ketosis (SCK, n=5, BHB > 1.0 mmol/L) and non-ketotic (NK, n=5, BHB ≤ 1.0 mmol/L). Abdominal SAT and omental VAT samples were obtained through laparotomy (right paralumbar fossa). Blood samples were collected before feeding and AT collection and biomarkers analyzed using the MIXED procedure of SAS. Significances were declared at $p \leq 0.05$. AT samples were sequenced on an Illumina Novaseq 6000 platform, and 150 bp paired-end reads were generated. DESeq2 R package was used for differential gene expression analysis (DEG). DEG ($p \leq 0.05$, $\log_2 FC \geq 1$) were evaluated for GO and KEGG enrichment analyses.

Results
As expected, serum BHB (1.06 *vs.* 0.68 ± 0.05 mmol/L, $p<0.01$) and NEFA (1.2 *vs.* 0.9 ± 0.07 mEq/dL, $p<0.05$) were greater in SCK than NK cows. Between SCK and NK cows, we observed 843 DEGs in SAT and 1458 in VAT. Extracellular matrix organization pathways were suppressed in SCK cows regardless of depot, suggesting that ketosis may affect remodeling of AT extracellular matrix. Pathways related to anti-inflammatory cytokine production (*IL4* and *IL10*) in SAT, and triglyceride biosynthesis and regulation of lipolysis in VAT were suppressed in SCK and agrees with the downregulation of lipid metabolism genes such as *ABHD11, RBP4*, and *TGRSP* in VAT of SCK *vs.* NK cows. Interestingly, inflammatory response and respiratory burst pathways were activated in VAT of SCK cows compared to NK.

Conclusions
These results highlight depot-specific transcriptional changes in AT during subclinical ketosis, and the active role that VAT dysfunction may play on the pathogenesis of ketosis.

Financial Support
U.S. Department of Agriculture

Notes:

209 - Investigating the role of IL-17A in lung repair following bovine respiratory syncytial virus infection

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Session: Disease Pathogenesis 3, 1/24/2022, 09:30 - 09:45

Objective
Bovine Respiratory Syncytial Virus (BRSV) poses a major economic burden in the cattle and dairy industries, and previous studies from our lab have shown that IL-17A exacerbates acute inflammatory signaling and increases pathogenesis during infections. Interestingly, we recently observed that IL-17A expression was still elevated 14 days post infection, a time frame associated with disease resolution. Therefore, we hypothesized that IL-17A mediated signaling may be playing a role in lung repair processes following BRSV infection, which we sought to test in a simplified *in vitro* model of lung repair.

Methods
Healthy and lesion lung samples from BRSV-infected neonatal calves, compiled from 3 separate studies, were analyzed using RT-qPCR to quantify the expression of genes associated disease resolution at 0, 7, and 14 days post infection. Bovine turbinates (BT), blood-derived monocytes, and BAL-derived macrophages were stimulated *in vitro* using recombinant cytokines for 6 (RT-qPCR analysis) or 48 (ELISA cytokine quantification) hours. BT cells were stained with Calcein AM for fluorescent imaging in a scratch-assay model of wound repair.

Results
PCR analysis showed a significant increase in IL-17A expression in lung lesions compared to healthy tissue as far out as 14 days post infection. This late expression of IL-17A coincided with altered expression of genes associated with tissue remodeling (MMP2, MMP9, and TIMP-1) and intercellular interactions (TJP1, OCCLUDIN, and AREG). *In vitro* experiments with BT cells confirmed that exposure to IL-17A alters the expression of lung repair genes.

Conclusions
Although IL-17A signaling is known to exacerbate BRSV pathogenesis during the acute phase of disease, it is intriguing that IL-17A remains upregulated after the infection has resolved. Based on our *in vitro* and *in vivo* experiments, we postulate a role for IL-17A in disease resolution, and future studies will seek to elucidate its role within lung repair and resolving bovine respiratory diseases.

Financial Support
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Notes:

210 - Global epigenomic changes observed postnatally elucidate lifelong defects following fetal BVDV infection

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Session: Disease Pathogenesis 3, 1/24/2022, 09:45 - 10:00

Objective
A vertically transmissible virus, Bovine Viral Diarrhea Virus (BVDV) induces profit loss in the form of spontaneous abortion, immunosuppression, and decreased performance. The impact of BVDV upon the fetus is dependent upon gestational age upon infection: fetal infection occurring prior to 125 of gestation generates a persistently infected (PI) calf that develops immunotolerance to the virus, while fetal infection occurring after 150 days of gestation generates a transiently infected (TI) calf capable of clearing the virus. Both TI and PI animals experience a lower body weight, higher incidence of postnatal illness requiring treatment, and altered complete blood count and immune cell profiles observed in peripheral mononuclear blood cells (PBMCs). We hypothesize that postnatal defects observed in animals experiencing a fetal BVDV infection occur due to dysregulation of the epigenome during gestation, a critical period of development that can impair postnatal health and growth.

Methods
To test this hypothesis, pregnant heifers were inoculated with non-cytopathic BVDV 2 or phosphate buffered saline (PBS) on day 175 of gestation to generate TI and control calves, respectively. Age-matched PI calves were identified at a cooperating ranch. DNA isolated from PBMCs collected from the calves at 4 months of age were subjected to reduced representation bisulfite sequencing (RRBS) via Zymo Research.

Results
When TIs were compared to controls, 3876 differentially methylated regions (DMRs) were identified, 1799 hypomethylated regions (inc. activity) and 2077 hypermethylated regions (dec. activity). When PIs were compared to controls, 4816 DMRs were identified, with 2723 hypomethylated regions and 2093 hypermethylated regions (p-adj.<0.01). Gene ontology characterization indicates affected pathways include immune function, metabolism, anatomical development, and reproduction.

Conclusions
Fetal BVDV infections induce postnatal epigenomic dysregulation contributing to the associated abnormalities in physiological function in both TI and PI animals.

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

Notes:

211 - Strategies for preventing rhodococcal foal pneumonia

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Session: AAVI - Featured Speakers, 01/24/2023, 10:30 - 11:15

Pneumonia caused by *Rhodococcus equi* remains an important cause of disease and death in foals worldwide. Although economic impact of the disease has not been quantified, costs to the horse industry are substantial for screening testing, prolonged treatment (medication and labor), and lost revenue from deaths of foals. Moreover, foals that develop *R. equi* pneumonia are less likely to race.

The insidious nature of the disease and the absence of a highly effective preventive methods led many veterinarians and farm managers to implement a program of thoracic ultrasonographic screening (TUS) and treatment of foals with pulmonary lesions presumed to be caused by *R. equi*. This practice, however, appears to have driven the emergence in the U.S. of an alarming prevalence of macrolide-resistant *R. equi*. This problem of antimicrobial resistance underscores the need for effective prevention.

Passive Immunization: To date, the only product licensed by the USDA for reducing the incidence of *R. equi* pneumonia is hyperimmune plasma (HIP). Transfusion of *R. equi* HIP, however, is not completely effective, expensive, labor-intensive, and carries some risk for foals. Thus, there is great need to explore alternative approaches for passive immunization. Our laboratory is collaborating with other scientists to develop mRNA encoded anti-*R. equi* antibodies as a method for preventing *R. equi* pneumonia. Preliminary findings will be presented.

Active Immunization: An effective vaccine for *R. equi* pneumonia remains elusive. This appears to be because foals are exposed and infected soon after birth, at an age when they are highly susceptible to infection. Neonatal foals respond weakly to immunization, and maternal antibodies likely interfere with immune responses. Some evidence exists that maternal immunization can protect foals against *R. equi* pneumonia, but positive results have not been repeatable. Approaches being explored in our laboratory for immunizing foals will be discussed.

Notes:

212 - Highly pathogenic avian influenza in the US and approaches to control

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Session: AAVI - Featured Speakers, 01/24/2023, 11:15 - 12:00

An outbreak of H5 highly pathogenic avian influenza (HPAI) virus began in the US in January 2022. As of October 2022, HPAI virus has been detected in wild birds and/or poultry in 49 states. The sustained and geographically wide spread number of cases indicate that the epidemiology of the outbreak is different from previous HPAI outbreaks. Infections have occurred in all sectors of the poultry industry including chickens, turkeys, domestic ducks and game birds. In gallinaceous birds HPAI virus causes a rapidly fatal disease. In contrast, domestic ducks (Pekin) and wild dabbling ducks can excrete virus for weeks but may not exhibit any clinical signs, which may be due to differences among species in immune responses. Notably, this particular lineage of HPAI virus have a low infectious dose for domestic avian species and mallard ducks.
In poultry, genetic resistance to HPAI virus infection and disease is not well understood. However, it is now feasible to engineer chickens with precise genome modifications such as those that would prevent viral infection or disease, and reduce or eliminate transmission. Several methods using prior knowledge of viral receptors, host-derived resistance and key proteins involved in innate immunity against HPAIV, are now being investigated as potential options to help mitigate HPAI.
Vaccines are another valuable tool for HPAI prevention and control, but are not currently used in the US. However, vaccines for HPAI have been used elsewhere. Vaccines are effective at eliminating morbidity and mortality, and reducing virus excretion. Numerous vaccine platforms are available for poultry (inactivated whole virus, replicating viral vectored and virus like particles) and are being evaluated for efficacy against the current strains in the US. Finally, management factors, for example the impact of viral immunosuppression by infectious bursal disease virus, have been shown to abrogate vaccine efficacy. Therefore, good management and control of other diseases can improve disease outcomes after HPAIV exposure in vaccinated animals.
Currently, control of HPAI in domestic birds in the US relies heavily on farm biosecurity to prevent virus introduction and post-outbreak decontamination to eliminate recrudescence.

Financial Support
US Dept of Agriculture

Notes:

214 - The role of Hfq and sRNAs on gene expression and virulence in *Histophilus somni*

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Session: Bacteriology, 1/24/2022, 10:30 - 10:45

Objective
Histophilus somni is one of the primary bacterial agents responsible for bovine respiratory disease in cattle. Hfq is a chaperone for small RNAs (sRNA), which are known to be important in gene regulation. We sought to identify those sRNAs that interact with Hfq, their potential contribution to regulation of *H. somni* virulence factors, and the role of Hfq in gene expression and virulence.

Methods
The wildtype and an Hfq mutant of *H. somni* strain 2336 (2336Δhfq) were compared by RNA-Seq, intracellular survival in bovine monocytes, serum susceptibility, and virulence in mouse and calf models. Hfq-associated sRNAs in *H. somni* were isolated by co-immunoprecipitation, and confirmed by electrophoretic mobility shift assay followed by RNA sequencing. Bioinformatic analyses was used to identify putative gene targets of sRNAs.

Results
Of 180 sRNAs that bound Hfq, 17 were unique to strain 2336, and 9 were similar to quorum sensing RNAs of Vibrio sp. Many sRNAs bound the 5'-UTR of uspE, uspA, and narQ (needed for *H. somni* biofilm formation). Some sRNA putatively regulate expression of master regulators csgD, ydaM and rpoS. Significantly upregulated genes (832) outnumbered significantly downregulated gene (809) between the mutant and wildtype. Differentially expressed genes in 2336Δhfq were associated with virulence and polysaccharide synthesis. Mutant 2336Δhfq had a truncated lipooligosaccharide and was more susceptible to intracellular killing and serum than the wildtype. The mutant was attenuated in a mouse seticemia model and following calf intrabronhial challenge. *H. somni* was recovered more often from nasopharyngeal swabs, endotracheal aspirates, and lung tissue from calves challenged with the wildtype than with 2336Δhfq. The percentage of abnormal lung in calves challenged with 2336Δhfq was lower than with the wildtype.

Conclusions
In conclusion, Hfq and Hfq-associated sRNAs from *H. somni* may have important regulatory roles in virulence and biofilm formation. Further characterization of these factors is warranted in order to control respiratory and other diseases due to H. somni.

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

Notes:

215 - Identification of virulence genes of *Mycoplasma bovis* by transposon mutation coupled with in vivo infection

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Session: Bacteriology, 1/24/2022, 10:45 - 11:00

Objective
Mycoplasma bovis is one of major contagious mastitis pathogens in the United States. Its virulence factors and pathogenesis are not well understood which hampered the development of effective prevention and control tools. The objective of this study was to determine important virulence factors of *M. bovis* by transposon mutagenesis and evaluation of pathogenicity of the mutant clones using experimental infection of dairy cows.

Methods
Transposon mutant library of *M. bovis* strain PG45 (wild type) was created and screened for loss of pathogenicity *in vitro* on mammary epithelial cells and *in vivo* on mice model. Two less pathogenic clones (Mutants 1 and 2) were selected and further tested by intramammary infection in dairy cows. A total of 12 Holstein dairy cows in 1st-4th parity were divided into 4 groups of 3 cows each. Groups 1 and 2 (controls) were given intramammary infusion of sterile phosphate buffered saline (PBS, pH 7.4) and wild type strain respectively into contralateral quarters. Similarly, Groups 3 and 4 received mutant 1 (M1) and 2 (M2) respectively. Systemic and local clinical signs of mastitis, milk somatic cells count, and *M. bovis* counts in milk were monitored for 7 days post challenge. After 7 days, cows were euthanized and gross pathological changes recorded. Udder tissue samples were collected for histopathology and swab samples were collected from uterus, joints, lung, and middle ear for culture.

Results
Wild type strain and mutant 1 caused mastitis in challenged quarters whereas PBS and mutant 2 infused cows did not develop mastitis. Joint and uterus swab samples from wild type and mutant 1 infected cows were positive for challenge strain *M. bovis*.

Conclusions
Mutated gene in mutant 2 may be critically important to cause mastitis but this need to be confirmed by challenge infection with mutation complemented mutant 2.

Financial Support
University of Tennessee; BARD project

Notes:

216 - Egg components inhibit the transfer of antimicrobial resistance plasmids *in vitro*

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Session: Bacteriology, 01/24/2023, 11:00 - 11:15

Objective
The animal gut acts as a potent reservoir for the lateral transfer of antimicrobial resistance (AR) and virulence genes between bacteria. This transfer has been observed in both food producing animals as well as in humans. Dietary interventions with the ability to inhibit this process in the gut are desirable for both humans and food animals. Eggs are dietary sources of antimicrobials and other bioactive compounds that regulate the gut microbiota. This study investigated the use of poultry eggs components inhibiting the transfer of large AR plasmids *in vitro* between *Enterobacteriaceae* strains.

Methods
Fresh eggs obtained from local grocers were separated into egg albumin and yolk fractions and completed 1:1 with sterile water. Fractions were then added in 1:10 dilutions into bacterial conjugation reactions consisting of 1:1 mixture of donors (*E. coli* SP915 or APEC-O2-211) and recipients (*E. coli* HS-4). The donor strains carry the multidrug resistant broad host range pKJK5-GM (IncP1ε) and narrow host range pAPEC-O2-211A-ColV (IncFIIβ) plasmids, respectively.

Results
In conjugations between donors and recipient where either pAPEC-O2-211A-ColV or pKJK5-GM were being transferred, significant reductions in transconjugants were observed in yolk supplemented treatments compared to the control. Furthermore, in conjugations where pKJK-5 was transferred and treated with egg albumin, a significant reduction in transconjugants was observed compared to non-treated groups.

Conclusions
The supplementation of egg components *in vitro* results in a significant decrease in conjugation of two large AR plasmids. This indicates that egg components may serve as an important dietary additive to reduce the prevalence and emergence of novel antimicrobial resistance strains in both humans and food animals. Additional studies are required to determine if this effect is consistent amongst other plasmids and *in vivo*, as well as to determine the key factors involved in this regulation.

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Notes:

217 - Prevalence and characterization of *Salmonella* in mesenteric lymph nodes of bob veal calves

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Session: Bacteriology, 1/24/2022, 11:15 - 11:30

Objective
Multi-drug resistant (MDR) *Salmonella* continues to emerge in food-producing animals. The marketing dynamics of surplus calves that involves transportation from the dairy farms to the auction or livestock markets where they commingle with calves and animals from other farms provide an optimal environment for *Salmonella* spread. Bob veal calves, or surplus dairy calves marketed at less than three weeks of age, have a higher prevalence of *Salmonella* than formula-fed veal samples. However, fecal sampling is less sensitive in identifying *Salmonella* and *Salmonella* are known to have predilection for lymphoid tissues. Therefore, estimating *Salmonella* prevalence in lymph nodes in bob veal can be used as a surveillance tool to reveal the early-life exposures of surplus dairy calf population. The objectives of this study were to estimate the prevalence, strain types, and antimicrobial resistance of *Salmonella* present in mesenteric lymph nodes of bob veal calves.

Methods
Ten cohorts of calves were enrolled and mesenteric lymph nodes were collected from randomly selected 30-32 calves per cohort. A *Salmonella* isolation protocol was applied and all the recovered isolates were characterized by serogrouping. Antimicrobial susceptibility to a panel of 14 antimicrobials was tested by disc diffusion method. Five randomly selected isolates per cohort were whole genome sequenced using Illumina NextSeq platform. Additionally, source information of the calves was collected.

Results
Results showed that the number of different sources for calves per cohort ranged between 1 and 19 and cohort-level *Salmonella* prevalence ranged from 16.7 and 84.8%. A majority of the recovered *Salmonella* belonged to either B (26.7%), or E/G (26%) serogroups. Eleven percent of the isolates were MDR, which was much lower than the estimates from special-fed veal calves.

Conclusions
Overall, the results indicated that the prevalence of *Salmonella* in bob veal was higher when compared to special-fed veal calves. Hence, identifying *Salmonella* in bob veal can be used as a proxy for estimating *Salmonella* prevalence in surplus calf production.

Notes:

218 - Comparison of MDR *Salmonella enterica* serovar Heidelberg outbreak isolates linked to dairy beef calves

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Session: Bacteriology, 1/24/2022, 11:30 - 11:45

Objective
Over the last decade, six multistate outbreaks caused by *Salmonella enterica* subspecies *enterica* serovar Heidelberg (*S.* Heidelberg) have occurred in the United States, primarily associated with poultry-related products. However, the latest multidrug-resistant *S.* Heidelberg outbreak was linked to contact with dairy beef calves. Traceback investigation revealed calves infected with outbreak strains of *S.* Heidelberg showed symptoms of disease frequently followed by death from septicemia. Two variants were identified that differed in morbidity and mortality. SX 245 affected calves had high morbidity and mortality, while SX 244 affected calves did not even though there was no difference in antimicrobial susceptibility between the two variants. The aim of our study was to investigate the emergence of *S.* Heidelberg as a pathogen in bovine through characterization of selected bovine-origin outbreak isolates.

Methods
For genomic comparison via pan-genome and SNP analyses, whole-genome sequencing was performed on two bovine-origin *S.* Heidelberg isolates with distinct PFGE patterns received at the Wisconsin Veterinary Diagnostic Laboratory at the University of Wisconsin-Madison during the 2015 to 2017 multistate outbreak. RNA-sequencing was performed to determine differentially expressed genes (DEGs) and predict their functional consequences, and invasion assays were performed with the HEp-2 cell line to assess pathogenicity.

Results
Percent invasion of isolate SX 245 (JF6X01.0523) was 2-fold greater than SX 244 (JF6X01.0590). Genomic comparison revealed SX 245 lacked over 200 genes present in SX 244, including genes associated with Inc1 plasmid and phages, whereas genes related to multidrug transporter MdfA and IS1110 transposase were absent in SX 244. Thirty-five common genes, primarily fimbriae-related, displayed higher expression in SX 245.

Conclusions
Fimbriae-related genes and unique mobile genetic elements may play a role in the increased pathogenicity and host range expansion of the *S.* Heidelberg isolates involved in the bovine-related outbreak.

Notes:

219 - Specific bacteria biotransform bile acids to influence *Campylobacter jejuni in vitro* growth

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Session: Bacteriology, 1/24/2022, 11:45 - 12:00

Objective
Campylobacter jejuni is one of the prevalent foodborne pathogens with limited mechanism understanding. Chenodeoxycholic acid (CDCA) and cholic acid (CA) differentially influenced mouse campylobacteriosis in *Il10*$^{-/-}$ mice. CDCA and CA derivatives metabolized from mouse small intestinal (SI) microbiota differentially impact *C. jejuni in vitro* growth. We reasoned specific bacteria in the microbiota possessed bile acid metabolizing genes to biotransform bile acids and to influence campylobacteriosis. The objective of this study was to investigate specific bile acid-metabolizing bacteria on influencing *C. jejuni in vitro* growth.

Methods
The bile acid metabolites in CDCA and CA cultured with mouse SI were analyzed by untargeted metabolomics of HPLC/MS-MS. To identify which bacteria biotransformed the CDCA or CA into the bioactive metabolites, PCR was used to evaluate the presence of bile acid metabolizing genes in *Il10*$^{-/-}$ mouse microbiota, including *3αHSDH, 3βHSDH, 5AR,* and *5βR*. Based on the metabolic pathways of bile acids, *Parabacteroides merdae* (Pm), *Eggerthella lenta* (El), and *Clostridium scindens* (Cs) were selected. The bacteria were individually or combinedly cultured with 1.5 mM CDCA or CA in 0.5% arginine BHI for three days. *C. jejuni* at 0.01 OD_{600} was inoculated into 1 ml supernatants centrifuged and filtered from the culture above for coculturing.

Results
We found that CA was metabolized by mouse SI into isoCAs at large amount and DCA and isoDCA at small amount. CDCA was metabolized into six metabolites. Based on the retention time and MS of targeted metabolomics of bile acids, they were determined to be UDCA, iso/isoallo-CA, iso-CDCA/UDCA/DCA, and 3-oxoCDCA. Interestingly, *3αHSDH, 3βHSDH, 5AR,* and *5βR* genes were detected in the contents of mouse SI, cecum or colon. Notably, the supernatant from CDCA cultured with Pm+El or Pm+El+Sc increased *C. jejuni* growth, while the supernatant from CA cultured with Pm+El+Sc mildly reduced *C. jejuni* growth.

Conclusions
The results suggest that Pm+El or Pm+El+Sc biotransform CDCA into bioactive metabolites to promote *C. jejuni* growth and infection.

Financial Support
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Notes:

220 - Antimicrobial genes in thermophilic *bacillus paralicheniformis* associated with mobile elements

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Session: Antimicrobial Use 4, 1/24/2022, 10:30 - 10:45

Objective
To examine antimicrobial resistance genes and their genomic locations relative to transposons and bacteriophage in *Bacillus paralicheniformis* isolated from hot springs.

Methods
Bacteria were collected in Yellowstone National Park in 2020. Water temperatures in collection sites averaged 65.1 °C. For propagation, samples were divided into three types of liquid media: malt yeast, ATCC Medium 1554 (mineral salt), and peptone-yeast-glucose. Samples were incubated aerobically and anaerobically at 37°C, 60°C, and 70°C for 10 days. Broths positive for growth were subcultured to blood agar plates, bacterial colonies were isolated, DNA was extracted, and stocks were frozen. 16SrRNA genes were amplified by PCR and sequenced using the Sanger technique. A strategy was developed to identify difficult to place elements including transposons, bacteriophage, and plasmids. We used a short-read-first hybrid assembly method (short-read assembly followed by long-read bridging and polishing). SPAdes v3.14.0 was used to assemble the Illumina Hiseq short reads into an assembly graph using a variety of *k-mer* sizes, evaluating the graph at each step to select the graph with the lowest contig count and dead-end count. The SPAdes contigs were then polished using Raconv1.5.0 and Miniasm v 0.3 with MinION nanopore long uncorrected reads.

Results
Genes associated with resistance to bacitracin (*bcrA-1, bcrA-2, bcrA_3, bcrA_4, bcrB*), beta-lactamase and its accessory proteins (*BlaR1, BlaI, penP*), virginiamycin (*vgb*), oleandomycin (*oleD_1, oleD_2*) , bicyclomycin (*bcr_1, bcr_2*), linearmycin (*lnrL_1, lnrL_2, lnrL_4, lnrL_5, lnrN, lnrL_M*),chloramphenicol (*CAT*) and rifamycin (*rpnC_1, rpnC_2*) were identified. Multiple copies of transposons *Tn3, IS1595, IS1182,* and *IS200/IS605* were located in several positions. These transposons, which have been associated with horizontal gene transfer, were found in close proximity to clusters of resistance genes.

Conclusions
These findings and the identification of bacteriophage and plasmids suggest the potential for the exchange of resistance genes between organisms in this extreme environment.

Financial Support
University of Tennessee Center of Excellence in Livestock Diseases and Human Health (COE)

Notes:

221 - Metaphylaxis increases prevalence of multidrug resistant *Mannheimia haemolytica* while improving health in stockers

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Session: Antimicrobial Use 4, 1/24/2022, 10:45 - 11:00

Objective
The objective of this study was to determine the effect of macrolide metaphylaxis on: 1) morbidity and mortality in stocker cattle over 21 days and 2) isolation rate and antimicrobial susceptibility of *Mannheimia haemolytica* (MH) at arrival (d0) and d21.

Methods
Commercial beef cross heifers (n=335, 232 ± 17.8 kg) were purchased from auction markets for 4 trials from October 2019 to October 2021. Cattle were randomized to receive tulathromycin at 2.5 mg/kg subcutaneously (META, n=168) or not (NO META, n=167). Nasopharyngeal swabs were obtained on d0 and d21 for aerobic culture and susceptibility testing. Groups were separated with no contact; any calves requiring additional antimicrobial (AM) treatment were separated. Logistic mixed models were constructed to evaluate effect of group (META, NO META), AM treatment, and previous MH isolation with isolation of multi-drug resistant (MDR, MICs classified as not susceptible to AM in 3 classes) MH on d21 as the outcome variable, with trial as a random effect.

Results
Over all trials, total and bovine respiratory disease (BRD) morbidity were significantly lower in META (14.9 %) animals than NO META (29.3 %) (χ^2, P=0.002); however, difference in BRD morbidity was observed only in the Spring and Fall 2021 trials (χ^2, P=0.002 & P=0.037, respectively). There was no difference in mortality, or d21 MH isolation risk between groups. Risk of isolation of MDR MH was significantly higher (χ^2, P=0.0004) at d21 (69/139) compared to d0 (17/72), and odds of recovery of MDR MH at d21 was significantly higher from META animals (OR=164.03, 95% CI= 23.27-1156.23) compared to NO META (Reference).

Conclusions
Tulathromycin metaphylaxis was associated with increased risk of MDR *M. haemolytica* isolation in high-risk heifers on day 21. Isolation of MH at d21 was not decreased in META cattle, possibly due to antimicrobial resistance (AMR). Metaphylaxis maintained efficacy in reducing morbidity, possibly through non-antimicrobial mechanisms; thus, future work investigating such mechanisms are warranted to develop approaches that decrease BRD without increasing AMR.

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

Notes:

222 - Characterizing the influence of metaphylaxis for bovine respiratory disease on host transcriptome responses

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Session: Antimicrobial Use 4, 1/24/2022, 11:00 - 11:15

Objective
Bovine respiratory disease (BRD) is a complex disease resulting from interactions among the host immunological response, environmental conditions, and polymicrobial components. Currently, control against BRD primarily consists of mass administration of an antimicrobial upon arrival to a stocker or feedlot facility, termed "metaphylaxis". The objective of this study was to determine the influence of six different antimicrobials on the whole blood host transcriptome in healthy steers upon and following arrival to the feedlot.

Methods
One hundred and five steers were stratified by arrival body weight (BW = 247 ±28 kg). Cattle were randomly assigned equally to one of seven treatments: negative control, enrofloxacin, florfenicol, tulathromycin, tildipirosin, ceftiofur, or oxytetracycline. In each pen, ten cattle received a treatment, and five cattle did not, serving as sentinel controls. On day 0, whole blood samples and BW were collected prior to the one-time administration of the assigned antimicrobial. Blood samples were collected again on day 3, 7, 14, 21 and 56. A subset of cattle (n=6) per treatment group in each treatment pen were randomly selected for RNA sequencing across all time points. Isolated RNA was sequenced (NovaSeq 6000; ~30M paired-end reads/sample), where sequenced reads were processed with ARS-UCD1.3 reference-guided assembly (HISAT2/StringTie2). Differential expression analysis was performed with glmmSeq and edgeR (FDR <0.05). Functional enrichment was performed with WebGestalt API (FDR <0.05).

Results
Gene expression patterns were similar at day 0 but became increasingly distinct across treatment groups over the next several timepoints; cattle were similar in gene expression profiles by the end of study. Differential expression between treatment groups enriched for immunological and inflammatory mediating mechanisms.

Conclusions
Our research demonstrates immunomodulation and potential secondary therapeutic mechanisms induced by commonly used antimicrobials for metaphylaxis. These findings provide new concepts related to therapeutic success against BRD.

Notes:

223 - Predictive modeling of MICs of cephalosporin antibiotics from beta-lactamase resistance genes in *Enterobacteriaceae*

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Session: Antimicrobial Use 4, 1/24/2022, 11:15 - 11:30

Objective
Extended-spectrum cephalosporins (ESCs) are commonly used in human and veterinary medicine. However, increased resistance to these antimicrobial groups has been reported, mediated by different types of beta-lactamase resistance genes. The study aims to predict the phenotypic antimicrobial susceptibility of Ceftriaxone, Cefoxitin, and Ceftiofur in *Escherichia coli* and *Salmonella enterica* from different beta-lactamase resistance genotypes.

Methods
Using datasets retrieved from the retail meat surveillance program of the United States National Antimicrobial Resistance Monitoring System for Enteric Bacteria, we used multivariable linear regression and weighted least square models to explore the relationship between MICs of ESCs and different types of beta-lactamase resistance genes.

Results
MIC of ceftriaxone increased significantly in the presence of bla$_{CMY-2}$, *bla*$_{CTX-M-1}$, *bla*$_{CTX-M-55}$, *bla*$_{CTX-M-65}$, and *bla*$_{SHV-2}$ by 55.16 mg/ml, 222.70 mg/ml, 250.81 mg/ml, 204.89 mg/ml, and 31.51 mg/ml, respectively. MIC of cefoxitin increased significantly in the presence of *bla*$_{CTX-M-65}$ and *bla*$_{TEM-1}$ by 1.57 mg/ml and 1.04 mg/ml, respectively. In the presence of *bla*$_{CMY-2}$, MIC of cefoxitin increased by an average of 8.66 mg/ml over 17 years. MIC of cefoxitin in *Salmonella enterica* isolates decreased significantly by 0.67 mg/ml compared to the MIC of *Escherichia coli* isolates. Moreover, the MIC of cefoxitin decreased by 0.89 mg/ml in isolates (*E. coli* and *Salmonella enterica*) from ground beef compared to chicken breast. On the other hand, MIC of ceftiofur increased in the presence of *bla*$_{CTX-M-1}$, *bla*$_{CTX-M-65}$, *bla*$_{SHV-2}$, and *bla*$_{TEM-1}$ by 8.51 mg/ml, 9.20 mg/ml, 7.36 mg/ml, and 1.05 mg/ml, respectively. In the presence of *bla*$_{CMY-2}$, MIC of ceftiofur increased by an average of 8.53 mg/ml over 14 years.

Conclusions
The ability to predict ESC phenotypic antimicrobial susceptibility information directly from beta-lactamase resistance genes may help reduce the reliance on routine phenotypic testing with higher turnaround times in diagnostic, therapeutic, and surveillance of antimicrobial resistant *E. coli* and *Salmonella enterica.*

Notes:

224 - Evaluation of *Mycoplasma hyopneumoniae* antimicrobial resistance associated mutations in US contemporary isolates.

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Session: Antimicrobial Use 4, 1/24/2022, 11:30 - 11:45

Objective
To assess single nucleotide polymorphisms (SNPs) previously associated to antimicrobial resistance (AMR) in different genes of *M. hyopneumoniae* US contemporary isolates.

Methods
Eleven *M. hyopneumoniae* US contemporary isolates obtained from pigs with clinical signs suggestive of *M. hyopneumoniae* infection, and no previous antibiotic treatment were used in this study. Isolates were characterized for genes associated to AMR. Gene segments coding for DNA gyrases (*gyrA*, *gyrB*), DNA topoisomerases (*parE*, *parC*) and 23S rRNA, were amplified. Amplicons were purified and Sanger sequenced. Forward and reverse reads were used to create consensus sequences that were aligned to the reference *M. hyopneumoniae* J strain (Accession AE017243.1). The assembled sequences were evaluated for SNPs manually using MEGA 11.

Results
Sequences were obtained for all genes in all evaluated samples and shared a homology ranging from 97% to 100% with those of the reference. Both, silent (S) and missense (M) SNPs were detected in all genes, except in 23S rRNA. Mutations detected included, 1M and 5S for *gyrA*, 7M and 11S for *gyrB*, 4M and 2S in *parC* and, 3M and 10S in *parE*.

Conclusions
Detection of SNPs in *M. hyopneumoniae* genes previously associated with AMR was evaluated in contemporary US isolates. Different mutations were detected in the evaluated genes. However, only one mutation previously associated to AMR was identified in the *parC* gene (C248A), which represents an amino acid change (Ser83Tyr) and has been previously correlated to increased resistance to fluoroquinolones in *M. hyopneumoniae* and other mycoplasmas *in vitro*. The SNP was present in five isolates (45%). Development of AMR in *M. hyopneumoniae* has been hypothesized to be responsible for continuous infections in the field. This study highlights the importance of regular testing and monitoring to detect potential AMR and improve treatment of swine mycoplasmosis.

Financial Support
Minnesota Rapid Agricultural Fund; Minnesota Agricultural Experiment Station

Notes:

225 - Molecular evaluation of *Mycoplasma hyopneumoniae* antimicrobial resistance in field specimens

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Session: Antimicrobial Use 4, 1/24/2022, 11:45 - 12:00

Objective
Antimicrobial resistance (AMR) is hypothesized to be responsible for continuous *Mycoplasma hyopneumoniae* infections in the field. The goal of this study was to assess directly in field specimens, single nucleotide polymorphisms (SNPs) associated to *M. hyopneumoniae* AMR.

Methods
A 5,000-sow farm positive to *M. hyopneumoniae* where elimination by herd closure and medication has been attempted, was selected for the study. Field specimens consisted of deep tracheal secretions, collected from sows with clinical signs suggestive of *M. hyopneumoniae* infection (n=251). Specimens were tested by species-specific PCR. Specimens with Ct values ≤30 were selected for molecular characterization (n=51) of gene segments coding for DNA gyrases (*gyrA*, *gyrB*), DNA topoisomerases (*parE*, *parC*) and 23S RNA. Amplicons were purified and used for Sanger sequencing. Forward and reverse reads were used to create consensus sequences that was aligned to the reference *M. hyopneumoniae* J strain (Accession AE017243.1). Sequences were evaluated for the presence of SNPs by manual examination using MEGA 11.

Results
A total of 46 *gyrA*, 28 *gyrB*, 47 *parC*, 44 *parE* and 17 23S RNA gene sequences were obtained out of the 51 specimens tested. Sequences shared 98.88% to 99.83% nucleotide identity with the reference strain. A total of 8 SNPs were detected in *gyrA* (2 missense and 6 silent), 11 in *gyrB* (8 missense and 3 silent), 4 in *parC* (3 missense and 1 silent), 7 in *parE* (4 missense and 3 silent) and, 3 in 23S RNA (missense) gene segments.

Conclusions
This study revealed the detection of antimicrobial resistance mutations directly using clinical samples, overcoming the low sensitive bacterial culture and isolation process of *M. hyopneumoniae*. Two SNPs, C248A in *parC* (45/45) and A2071G in 23S RNA (17/17) were detected, which have been previously associated with fluoroquinolone and macrolide and lincosamide AMR, respectively, both in *M. hyopneumoniae* and other mycoplasma species.

Financial Support
Minnesota Rapid Agricultural Fund; Minnesota Agricultural Experiment Station

Notes:

226 - Outcomes affected by metaphylaxis from a clinical trial of feedlot calves at medium-risk for respiratory disease

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Session: Epidemiology 5, 1/24/2022, 10:30 - 10:45

Objective
The primary objectives of this study were to evaluate impacts of pull-and-treat or metaphylaxis health programs for control of bovine respiratory disease (BRD) on health, beef production, and antimicrobial use related outcomes in commercial feedlot calves with an uncertain BRD risk status.

Methods
A total of 2,366 crossbred steer and heifer calves [261 kg initial weight (± 11.0 SD)] were allocated to the trial at one of two commercial feedlots in Kansas (6 pens) and Nebraska (10 pens) in a randomized complete block design. Within feedlot, cattle were blocked by origin and arrival, and within a block, allocated to one of two pens which were randomly assigned to treatment. Treatments were pull-and-treat (PT; no metaphylaxis given; treated when clinical signs were observed) or metaphylaxis (META) with tulathromycin. The antimicrobials, doses used for BRD, and their post-treatment intervals for further treatment were identical for PT and META, with the lone difference being when tulathromycin was first administered (at first BRD treatment for PT or at initial processing for META). Intent to treat analyses were used with general and generalized linear mixed models accounting for clustering.

Results
Total BRD morbidity was lower for META than PT (7.3% vs 17.2% respectively; $P < 0.01$). While BRD mortality ($P = 0.34$) did not significantly differ between treatments, total mortality was reduced for META compared to PT (1.1% vs 2.5% respectively; $P = 0.03$). Attributable risk estimates indicate if PT was used in lieu of META, 1.4 excess deaths occurred per 100 calves. Number of antimicrobial doses for BRD per animal was greater for META than PT (1.10 vs 0.21 respectively; $P < 0.01$). Carcass weight per animal enrolled was 9 kg heavier for META than PT ($P = 0.08$), while the number of antimicrobial doses used per 45.4 kg of carcass was 0.03 for PT, and 0.13 for META ($P < 0.01$).

Conclusions
While the pull-and-treat program resulted in less antimicrobial use than metaphylaxis, significantly more cattle became morbid and died, and less total beef production occurred when metaphylaxis was not used.

Notes:

227 - Comprehensive assessment of feedlot health interventions using outcomes research in a sustainability context

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Session: Epidemiology 5, 1/24/2022, 10:45 - 11:00

Objective
When outcomes research approaches are effectively applied to animal health research, the value of interventions or health management options are quantified to enable more informed decisions by stakeholders. In animal agriculture, stakeholders spanning from food consumers through corporate and government entities are increasingly interested in sustainability of food production systems. Sustainability involves multiple components that broadly include social, economic, and environmental aspects. Although animal well-being, food production efficiency/availability, greenhouse gas emissions, economic viability, antimicrobial use, and other issues may be important, there is no standard set of metrics to measure and compare the sustainability of different health or management strategies in a production system. Our objective was to use data from a commercial feedlot trial evaluating different antimicrobial use strategies to demonstrate approaches to comprehensively estimate value and assess sustainability, while outlining how this type of approach may be relevant and applied by animal disease researchers.

Methods
We provide examples quantifying animal well-being (e.g. morbidity and mortality), the number of antimicrobial doses used, the efficiency and volume of beef production, net-economic returns from partial budgets, and a standardized measure of greenhouse gas production for each treatment group of the pen-level feedlot trial.

Results
The results demonstrate the potential "trade-offs" that may need to be understood if sustainability is considered in a comprehensive context. As stakeholders have increased the use of sustainability terms and goals, the need for an ability to measure change and impacts becomes more apparent. There are multiple ways of determining value in a sustainability context and those elements may be determined by stakeholders' perceptions.

Conclusions
Considering sustainability is a broad topic with multiple components an outcomes research approach may provide a framework to quantify values for comprehensive assessments of animal health and management strategies.

Notes:

228 - Effect of genetic selection for reduced heart failure risk on feedlot performance and carcass characteristics

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Session: Epidemiology 5, 1/24/2022, 11:00 - 11:15

Objective
Bovine congestive heart failure (BCHF) has emerged as a burdensome disease in feedlots throughout the Western Great Plains. Variation in two genes, *ARRDC3* and *NFIA*, has been associated with an 8-fold increase in risk of terminal BCHF in feedlot cattle with one risk factor, and a 28-fold increase in risk for animals with both risk factors. A common concern with single trait DNA marker-based genetic selection, like that for BCHF, is the possibility for inadvertent selection against desired performance traits like weight, backfat, ribeye area, and marbling. Therefore, the objective of this study was to determine if selection for reduced BCHF risk coincides with a detectable difference in feedlot performance and carcass traits.

Methods
Three calf crops from a western Nebraska herd with a high annual incidence of BCHF were used in this study. Genotypes for BCHF risk were determined for non-replacement animals born in 2019-2021 (n = 1771). Individual pre-harvest ultrasound measurements were collected and analyzed within BCHF risk groups. The 2019 and 2020 calf crops served as a baseline for results without genetic selection, whereas the 2021 calves were produced by selecting sires with less genetic risk for BCHF. Statistical analysis was conducted using an analysis of variance to detect differences between BCHF risk groups.

Results
Despite reducing the number of calves at highest risk of developing BCHF by over 62%, significant differences in feedlot performance and carcass characteristics were not observed.

Conclusions
Feedlot performance and carcass characteristics did not differ in the cohorts of cattle selected for a decreased risk for developing BCHF when compared to cattle without genetic selection.

Financial Support
University of Nebraska; U.S. Department of Agriculture; Nebraska Beef Industry Endowment

Notes:

229 - Investigation of chronic *Anaplasma marginale* infection and breeding soundness in beef bulls

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Session: Epidemiology 5, 1/24/2022, 11:15 - 11:30

Objective
In breeding beef bulls, clinical anaplasmosis transiently eliminates satisfactory breeding potential. Cattle that recover from acute clinical anaplasmosis transition to chronic carriers of *Anaplasma marginale* and serve as transmission reservoirs. The impact of chronic *A. marginale* infection on reproductive performance parameters in carrier bulls is unknown. We hypothesize that bulls with chronic *A. marginale* infection will have lower reproductive performance metrics compared to uninfected bulls due to chronic infection or sustained damage from initial clinical disease. The objectives of this study were: i) evaluate the prevalence of chronic *A. marginale* infection in bulls from eastern Kansas; and, ii) compare breeding soundness parameters and overall satisfactory breeding potential rates between *A. marginale* infected and uninfected bulls.

Methods
Eastern Kansas, client-owned beef bulls (n=537) undergoing a routine breeding soundness examination (BSE) were enrolled in this study. Complete BSEs were performed by recruited local veterinarians according to the Society for Theriogenology Manual for BSE of Bulls, 2nd edition. BSE parameters included sperm morphology and motility, palpation of external genitalia and internal accessory sex glands, and overall physical soundness. Blood samples were collected for packed cell volume determination and analysis of *A. marginale* infection status via quantitative PCR and cELISA. Logistic and linear regression methods were used to evaluate factors associated with *A. marginale* infection status and BSE parameters.

Results
Prevalence of chronic *A. marginale* was 45.7% (245/537) among the sampled eastern Kansas bulls. Of the bulls with unsatisfactory BSE results, 53.6% (37/69) were chronically-infected with *A. marginale*. Common reasons for unsatisfactory BSE results were poor sperm motility or increased abnormal sperm morphology.

Conclusions
Prevalence of *A. marginale* infection among bulls tested is similar to eastern Kansas cow infection rates. Many bulls with chronic *A. marginale* infection retain overall satisfactory breeding potential.

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

Notes:

230 - Prevalence of methicillin-resistant & susceptible *Staphylococci* spp. in bulk tank milk from dairy farms in Michigan

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Session: Epidemiology 5, 1/24/2022, 11:30 - 11:45

Objective
The purpose of this study was to determine the prevalence of methicillin-resistant Staphylococci spp. in bulk tank milk obtained from 300 MI dairy farms.

Methods
Bulk tank milk samples (n = 300) were collected and frozen by dairy field personnel and submitted to the MSU Milk Quality Laboratory. Frozen bulk milk samples were thawed at room temperature and pre-enriched by adding 1 mL of milk to 4mL of Mueller-Hinton broth supplemented with 6.5% NaCl and incubated at 37ºC with shaking for 24 h. Subsequently, 10µL of milk were plated on mannitol salt agar (MSA) and a Mueller-Hinton agar supplemented with 2.5% NaCl and containing 2 mg/L of oxacillin and 20 mg/L of aztreonam (MHA). Colonies on the selective media were sub-cultured on blood agar and species identified using MALDI-TOF MS. Phenotypic methicillin resistance was tested using cefoxitin disk diffusion as defined in the Clinical and Laboratory Standards Institute guideline (Vet01S-Ed5 Table 7D). Conventional PCR was used to detect *mecA* and *mecC* (Silva Abreu *et al.*, 2021).

Results
A total of 550 isolates were obtained from MSA plates and 10 isolates from MHA plates. MALDI-TOF MS identified 16 species of non-aureus Staphylococci (NAS) that accounted for 84% of all Staphylococci, and *S. aureus* accounted for the remaining 16%. Four *S. aureus* from 4 farms (1.3%) demonstrated phenotypic resistance to methicillin but none carried *mecA* or *mecC* genes. Forty-five NAS from 40 farms (13.3%) demonstrated phenotypic resistance to methicillin. However, only 13 NAS isolates from 13 farms were positive for *mecA* while all were negative for *mecC.* Thus, the prevalence of *mecA* positive NAS was 4.3%.

Conclusions
This study demonstrated a low prevalence of methicillin resistance NAS from bulk milk samples collected from Michigan dairy farms.

Reference
Silva Abreu, A.C., *et al.* Antimicrobial resistance of Staphylococcus spp. isolated from organic and conventional Minas Frescal cheese producers in São Paulo, Brazil. *Journal of dairy science* 2021;104(4):4012-4022.

Financial Support
Michigan Alliance for Animal Agriculture

Notes:

231 - Multilevel modeling: association between bovine leukosis proviral load and dairy production parameters

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Session: Epidemiology 5, 1/24/2022, 11:45 - 12:00

Objective
Bovine leukemia virus integrates the host's genome and forms provirus in lymphocytes. Proviral load (PVL) is the number of proviruses per infected cell. The level of PVL is a good indicator of infectivity with high PVL cows more likely to infect naïve animals. Culling cows with a high PVL is a practical control strategy to reduce prevalence and incidence in herds with a high prevalence of BLV. The objective of this study is to evaluate if there is any association between high PLV and milk production, occurrence of ketosis, mastitis or fertility in BLV infected cows.

Methods
Out of 3386 cows tested, 1167 were identified as BLV positive by individual milk testing. The PVL was quantified from positive cows using a quantitative qPCR. For each BLV positive cow, 305 days milk yield, average somatic cell count, fat/protein ratio in routine milk testing at 4th week after calving, number of days from calving to conception, and number of days in milk at first breeding were collected from medical record from dairy comp software (DC_{305}). Three level Mixed linear regression model (province and herd random effect) was built to assess the relationship between PVL milk production, subclinical mastitis, ketosis and reproduction performance.

Results
Accounting for region, and herd random effects, high PVL was strongly associated with reduced milk production (each increase of 1 copy of provirus per lymphocyte there was a decrease of 312 liters of the total 305milk) (p-value <0.005) and reduced reproduction performance (for every increase of 1 copy of provirus per lymphocyte there was increase of 25 days from calving to conception and an increase of 22 days in milk at first breeding (p-value <0.002 for both). PVL was also associated with subclinical mastitis but not with ketosis (P-value of 005 and 0.35, respectively).

Conclusions
BLV infected cows with a high PVL produced less milk and had a reduced reproduction performance than cows with low PVL load. This result supports implementing a control program to prioritize culling high PVL cows.

Notes:

232 - Infrequent intra-household transmission of *Clostridioides difficile* between pet owners and their pets

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Session: Zoonoses, 1/24/2022, 10:30 - 10:45

Objective
Companion animals have been shown to carry *Clostridioides difficile* strains that are similar or identical to strains found in people, and a small number of studies have shown that pets carry genetically identical *C. difficile* isolates as their owners, suggesting interspecies transmission. However, the directionality of transmission is ultimately unknown, and the frequency with which animals acquire *C. difficile* following their owners' infection is unclear. The goal of this study was to assess how often pets belonging to people with *C. difficile* infection carry genetically related *C. difficile* isolates.

Methods
We enrolled pet owners from two medical institutions (University of Pennsylvania Health System (UPHS) and The Ohio State University Wexner Medical Center (OSUWMC)) who had diarrhea with or without positive *C. difficile* assays and tested their feces and their pets' feces for *C. difficile* using both anaerobic culture and PCR assays. When microorganisms were obtained from both the owner and pet and had the same toxin profile or ribotype, isolates underwent genomic sequencing.

Results
Fecal samples were obtained from a total of 59 humans, 72 dogs, and 9 cats, representing 47 complete households (i.e., where a sample was available from the owner and at least one pet). Of these, *C. difficile* was detected in 30 humans, 10 dogs, and 0 cats. There were only two households where *C. difficile* was detected in both the owner and pet. In one of these households, the *C. difficile* isolates were of different toxin profiles/ribotypes (A+/B+ / RT 499 from the owner, A-/B- / RT PR22386 from the dog). In the other household, the isolates were genetically identical (1 SNP difference). Interestingly, the dog from this household had recently received a course of antibiotics (cefpodoxime and metronidazole).

Conclusions
Our findings suggest that interspecies transmission of *C. difficile* occurs infrequently in households with human *C. difficile* infections.

Financial Support
U.S. National Institutes of Health; National Agency for Agricultural Research - Czech Republic; The Thomas B. and Jeannette E. Laws McCabe Fund, University of Pennsylvania; Ohio State University College of Veterinary Medicine

Notes:

233 - Investigating multistate *Salmonella* outbreaks linked to backyard poultry - detection, data, and partners

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Session: Zoonoses, 1/24/2022, 10:45 - 11:00

Objective
Salmonella illness outbreaks linked to contact with backyard poultry continue to increase in size. Backyard poultry can carry multiple *Salmonella* serotypes even if the birds look healthy and clean. Investigation of multistate *Salmonella* illness outbreaks is necessary to identify sources of illness and prevention strategies. We describe backyard poultry-associated outbreaks and the partners involved in managing the risk of *Salmonella* infection.

Methods
Public health investigators used PulseNet, the national molecular subtyping network for enteric disease surveillance, to identify illness outbreaks. An outbreak-associated case was defined as *Salmonella* infection yielding an isolate highly related to one of the outbreak strains by whole genome sequencing with isolation dates beginning February 3, 2022. Public health officials interviewed people about animal exposures during the week before illness onset, collected information about poultry purchase locations, and conducted environmental sampling for *Salmonella*.

Results
Public health officials investigated 11 backyard poultry-associated outbreaks of *Salmonella* infections with serotypes of Enteritidis, Hadar, I 4,[5],12:i:-, Indiana, Infantis, Mbandaka, and Typhimurium. As of August 1, 2022, 884 people infected with one of the outbreak strains were reported from 48 states and the District of Columbia. Five of the outbreak strains were isolated from poultry and their environments at sick people's homes and at retail stores that sell backyard poultry. Of 269 people who reported contact with backyard poultry, 169 (63%) reported buying backyard poultry in 2022. They reported purchases from 177 different locations, and at least nine hatcheries supplied backyard poultry to these locations.

Conclusions
Epidemiologic and laboratory data provided evidence that contact with backyard poultry made people sick. CDC and partners routinely work with hatcheries and stores that sell poultry to educate new poultry owners and control the spread of *Salmonella*, which is crucial in preventing outbreaks linked to backyard poultry contact.

Notes:

234 - Understanding close contact between humans and white-tailed deer in Illinois: a survey study

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Session: Zoonoses, 1/24/2022, 11:00 - 11:15

Objective
First manifesting in 2019, SARS-CoV-2, has been found in multiple species, including dogs, cats, minks, and cervids by using seroprevalence surveillance. Multiple regions in the United States, including Illinois, have identified SARS-CoV-2 infections in the wild white-tailed deer (WTD) population. The seroprevalence in Illinois was estimated at 6.9%; with an overall seroprevalence of 40% across 4 different states. Although the exact path for spillover into wild cervids is unknown, the virus has been shown to replicate within and spread among WTD during experimental inoculation. There are several areas of concern, including the possibility for spillover into the human population from a wildlife reservoir and the potential for viral evolution. However, transmission requires contact between WTD and humans. The aim of this cross-sectional survey is to understand the frequency and type of close contact between the general public in the state of Illinois and WTD.

Methods
The survey will be distributed electronically using convenience sampling, recruiting participants from list serves, social media, and community partners or extension liaisons. Questions will address frequency and distance of contact with WTD and encompass live animals and bodily fluids.

Results
Summary statistics will be calculated stratified by region of the state, property type, and demographics.

Conclusions
The survey is part of a broader study that seeks to establish whether disease spread in wild cervids correlates to disease spread in humans.

Notes:

235 - Hunters' behavior change preferences based on risk of tuberculosis and brucellosis from wood bison in Canada.

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Session: Zoonoses, 1/24/2022, 11:15 - 11:30

Objective
Although Canada is officially free of bovine tuberculosis and brucellosis, wood bison in Wood Buffalo National Park are a known reservoir of these and other zoonotic diseases. Several issues complicate the situation: wood bison are a threatened species; the species is an important cultural and food resource for indigenous communities; and the zoonotic diseases pose an economic and public health risk. As part of a larger project, we wanted to know if hunters would change behavior based on proximity to infected bison.

Methods
We investigated potential influences on Alberta hunters' stated willingness to change (WTC) their hunting practices in response to a hypothetical case of a zoonosis in a species they hunt. We anticipated significant predictors would include demographics, perception of risk, and knowledge of zoonoses. A questionnaire link was distributed to 100,000 hunters in Alberta exploring opinions on ways to manage the wood bison health; 139 useable responses were evaluated. Hunters were asked how close an animal infected with tuberculosis (TB) or brucellosis could be before hunters would change their hunting practices. Risk awareness was calculated as an aggregated score from questions addressing TB and brucellosis impact on health and economic livelihood; knowledge was similarly based on questions evaluating knowledge of TB and brucellosis.

Results
Preliminary results of multiple variable linear regression models show significant predictors of WTC ($p<0.05$) include income, knowledge of brucellosis and tuberculosis, and threats to hunting opportunities. Age and education were not significant predictors.

Conclusions
Although hunters show WTC practices, they were not as sensitive to risk of zoonoses as we expected. Part of the reason may be a false sense of ability to recognize a TB+ve animal without lab confirmation. Our research provides important findings addressing potential policy support that engages hunters in wildlife conservation and their willingness to engage in the particular supportive behaviors.

Financial Support
Government of Alberta; Government of Canada

Notes:

236 - Emergence and mitigation of carbapenem-resistant *Escherichia coli* in a small animal veterinary teaching hospital

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Session: Zoonoses, 1/24/2022, 11:30 - 11:45

Objective
Describe the detection of a carbapenem-resistant *Escherichia coli* in clinical and environmental samples at a small animal veterinary teaching hospital (SA-VTH) and report the infection control measures undertaken to reduce environmental contamination and nosocomial transmission.

Methods
Carbapenemase-positive *E. coli* was detected in clinical samples from two dogs at the University of Georgia (UGA) SA-VTH in July 2022. Electrostatic dust collection wipes were used to collect environmental samples where these patients were known to have been housed or treated, as well as other high-traffic areas in the SA-VTH. These samples were cultured for *E. coli*, and positive isolates were tested for antimicrobial susceptibility and carbapenemase production. Subsequently, the SA-VTH was temporarily closed for thorough cleaning and disinfection, and environmental sampling was repeated.

Results
Pre-cleaning, imipenem-resistant *E. coli* was detected in 54% (28/52) of environmental samples, 82% (23/28) of which were confirmed to be carbapenemase-positive. Environmental contamination was reduced post-cleaning, with carbapenemase-positive *E. coli* detected in only 8% (4/52) of samples. However, in the following month, carbapenemase-positive *E. coli* was isolated from clinical samples in three additional dogs, prompting the implementation of increased barrier nursing precautions for high-risk patients. Two distinct antimicrobial resistance profiles were detected among clinical and environmental carbapenem-resistant *E. coli* isolates, distinguished by their susceptibility to gentamicin.

Conclusions
The UGA SA-VTH detected multiple distinct strains of carbapenem-resistant *E. coli* among hospitalized patients that resulted in widespread environmental contamination. Electrostatic dust collection wipes were a convenient and effective sampling tool to conduct environmental surveillance for this pathogen. Rigorous cleaning and disinfection were effective in reducing environmental contamination, but strict adherence to hand hygiene and barrier nursing protocols are critical to preventing transmission.

Notes:

P001 - Novel therapeutic leads: Demonstration of efficacy, safety, and applicability of anti-APEC molecules in chickens

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Session: Antimicrobial Use

Objective
Avian pathogenic *Escherichia coli* (APEC) causes colibacillosis in chickens and other avian species. It continues to be the most significant health threat to poultry industry, results in multi-million-dollar annual losses, and reflects lack of efficacy of current prophylactic measures. In order to identify the best therapeutic alternative to antibiotics, we evaluated promising anti-APEC leads [growth inhibitor (GI-7) and quorum sensing inhibitor (QSI-5)] in an appropriate oral challenge model of infection. In addition, we investigated the mechanisms of action of these novel anti-APEC leads.

Methods
We used oral challenge model (1 x 109 CFU/chicken at 2 days of age) to evaluate the efficacy of small molecules GI-7 and QSI-5 and their combination (GI-7 + QSI-5) administered orally in drinking water in controlling APEC infection in chickens. In addition, to identify the mechanism of action of these small molecules *in vitro*, we performed a thermal proteome profiling (TPP) assay and designed biotin-linked probes for GI-7 and QSI-5 for pull-down assay to identify specific targets.

Results
Compared with the currently used antibiotic sulfadimethoxine (SDM), the GI-7 and QSI-5 individually or in combination, possessed better efficacy against APEC when administered in drinking water. In addition, the TPP assay unveiled potential targets, such as ABC transporter ATP-binding protein, carboxypeptidase/penicillin-binding protein 1A, and other cytoplasmic proteins responsible for the transcription and translation. A series of biotin-linked probes are being investigated to identify the targets of GI-7 and QSI-5 using pull down assay.

Conclusions
GI-7 and QSI-5 displayed promising effects, thus represent potential antibiotic-independent approach to control APEC infections in chickens. When used in combination, the SMs can potentiate each other's anti-APEC activities. Moreover, pull-down and TPP assays would allow for the identification of potential targets and understanding mechanism of action of these novel inhibitors.

Financial Support
U.S. Department of Agriculture

Notes:

P002 - Macrolide resistant enterococci of Canadian beef cattle origin - a risk profile using the Codex framework

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Session: Antimicrobial Use

Objective
Enterococci, a genus of commensal bacteria present across the Canadian beef farm-to-fork continuum, can acquire antimicrobial resistance (AMR). It is well established that AMR is a food safety issue and a public health concern. Macrolide resistance is of particular interest, given the use of macrolides in beef production and the expression of macrolide, lincosamide, and streptogramin B (MLSB) resistance. This risk profile aims to evaluate the current state of knowledge specific to enterococci macrolide resistance in the Canadian beef production system and recommend appropriate actions.

Methods
The risk profile follows the reporting format recommended by *Codex Guidelines for Risk Analysis of Foodborne Antimicrobial Resistance – Appendix 1*. Information is derived from a scoping review conducted in tandem, as well as grey literature and expert consultation. Quality assessments are taken of included material to evaluate confidence in information and recommendations gained from the source.

Results
Preliminary findings identified that macrolide-resistant enterococci are present across the farm-to-fork continuum; however, the predominate enterococci species differ by stage of production due to host specificity. Resistance is correlated with the species of enterococci and the timing of sampling related to antimicrobial treatment and other factors. Resistance trends are repeatedly identified as having multiple causes. There are also knowledge gaps in AMR enterococci prevalence in retail meat preparation.

Conclusions
Preliminary evidence suggests that current surveillance programs, veterinary prescribing requirements for antimicrobials, and public education campaigns regarding safe meat handling are essential mitigation strategies that should be continued. The Codex guideline is practical for data summarization and risk evaluation and provides a standardized and replicable structure.

Financial Support
Government of Alberta; Public Health Agency of Canada

Notes:

P003 - *In vitro* characterization of anti-microbial peptides (AMPs) isolated from the probiotics against the *Salmonella*

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Session: Antimicrobial Use

Objective
Salmonella is the leading cause of death associated with foodborne illness in the USA. Salmonellosis, a disease caused by non-typhoidal *Salmonella*, is a pressing issue due to the evolution of multidrug-resistant strains such as a fluoroquinolone, and extended-spectrum β-lactamase, which are difficult to treat with the existing antibiotics. To ameliorate this problem, we identified different sets of small antimicrobial peptides from the culture supernatant of *Lacticaseibacillus rhamnosus* GG and *Bifidobacterium lactis* Bb12 using LC-MS/MS. Among them, we intend to characterize the effect of peptides (PN3 and PN5) against *Salmonella in vitro*.

Methods
To evaluate the efficacy of PN3 and PN5, we performed the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) assays by growing the *Salmonella* Typhimurium (ST) in different concentrations of peptides followed by plating of culture with no visible growth. Peptides were tested for effectiveness against 8 different serovars of *Salmonella*. The Minimum Biofilm Eradication Concentration (MBEC) assay and gentamicin protection assay was performed to assess the ability of peptides to remove the biofilm-embedded ST and intracellular ST in chicken macrophage (HD-11) cells. The Lactate dehydrogenase (LDH) assay was conducted to determine the cytotoxicity of the peptides to the human intestinal (Caco-2) and HD-11 cells. In addition, to check whether the *Salmonella* would gain resistance to the peptides, lethal and sublethal resistance assays were conducted.

Results
PN3 and PN5 were effective against ST. MIC and MBC of the PN3 were 18 and 24mM, whereas of PN5 were 21 and 30mM, respectively. MIC of these peptides inhibited the growth of 8 different serovars of *Salmonella* and completely eradicated ST inside the biofilm. 1.5X MIC of the PN3 and 1X MIC of PN5 removed the intracellular ST in HD-11 cells. LDH assay showed no cytotoxicity of peptides, and the resistance assay demonstrated no resistance by ST.

Conclusions
Overall, both PN3 and PN5 exhibited a promising effect against *Salmonella* and its serovars.

Notes:

P004 - Delivery of antimicrobials by the probiotic *Limosilactobacillus reuteri* to target *Streptococcus suis*

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Session: Antimicrobial Use

Objective
Streptococcus suis is a swine pathogen that represents worldwide a major economic burden. As of today, vaccination success rates are low, and we are mostly dependent on antibiotics to eradicate *S. suis*. Given the increased in antibiotic resistance, there is an urgent need to develop alternative treatment strategies. This project aims to engineer the probiotic *Limosilactobacillus reuteri* to release bacteriophage-derived antimicrobial enzymes—endolysins—to kill *S. suis*.

Methods
To quantify and optimize production of recombinant proteins in *L. reuteri*, we first developed a luminescent tagging system. Recombinant proteins were tagged with HiBiT, which facilitates luminescence when mixed with a substrate. We optimized conditions to detect and quantify recombinant proteins in vitro and in vivo. *L. reuteri* was engineered to release the endolysin PlySs, which encodes a catalytic and cell-wall binding domain (CWB). To construct a PlySs derivative with enhanced binding affinity, we constructed *L. reuteri* harboring different CWBs tagged with HiBiT. To maximize the release of recombinant proteins from *L. reuteri*, we developed a lytic-trigger for increased lysis and thus therapeutic release.

Results
The HiBiT system works in *L. reuteri* and quantified recombinant proteins, including PlySs, in the 2 – 9 log-linear range. Production of six different CWBs was confirmed. Lastly, we successfully leveraged expression of an antirepressor gene to trigger prophage-mediated lysis in *L. reuteri.* Optimization of induction levels and the timing of induction boosted the release of recombinant protein by 2-fold when compared to the concentration of protein with the addition of the induction peptide at the early exponential phase.

Conclusions
We are now in the exciting position to exploit our developed luminescent tagging system to monitor and normalize (chimeric) endolysin proteins when testing their antimicrobial potential. In addition, this creates the opportunity to perform high-throughput cloning strategies to build a library of antimicrobials to be tested in a genetically diverse panel of *S. suis* isolates.

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

Notes:

P006 - Characterization of antimicrobial resistance in indicator and pathogenic bacteria from retail meat in Wyoming

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Session: Antimicrobial Use

Objective
The objective of this study was to determine the prevalence, antibiotic resistance (AMR) phenotypes, and AMR genetic determinants in *Escherichia coli*, *Salmonella*, and *Enterococcus* isolates from retail meat samples marketed in southeastern Wyoming.

Methods
Retail meat samples were collected between August 2021 and May 2022 at retail supermarkets in Laramie, Wyoming. 180 samples including beef, pork, chicken, and turkey were collected. The NARMS retail meat methodology was employed to obtain bacterial isolates. MALDI-TOF was employed for isolate identification. Susceptibility testing of all isolates was conducted via broth microdilution followed by whole-genome sequencing of isolates resistant to azithromycin or ceftriaxone or displayed reduced susceptibility to ciprofloxacin. Statistical analyses were carried out using SAS software.

Results
Salmonella was found in 6.11% of the samples. 6 *Salmonella* isolates were resistant to multiple drugs (MDR), including β-lactam/β-lactamase inhibitors, penicillins, cephems, phenicols, aminoglycosides, sulfonamides, and tetracyclines. 15 unique AMR genes such as genes for resistance to carbapenems (bla_{CMY-2}, bla_{SHV-12}, and bla_{TEM-1}), quinolones (qnrB2), and sulfonamide (sul1) were found. The recovery rate of *E. coli* was 23.3%. 80.4% of the *E. coli* isolates were MDR. 44 unique AMR genes were found in *E. coli* isolates, including those encoding resistance to β-lactamases ($bla_{CTX-M-55}$ and bla_{TEM-1}) and aminoglycosides (aph(3')-Ia). *Enterococcus* was found in 75% of the samples. *E. faecalis* (78.2%) and *E. faecium* (15%) were the top two *Enterococcus* species isolated. 36.4% of the *Enterococcus* isolates were MDR. All were susceptible to first-line therapies for enterococcal infections, thus, none were sequenced.

Conclusions
This study established a baseline for antimicrobial resistance levels in the indicator bacteria *Salmonella* and *E. coli* from retail meats marketed in southeastern Wyoming. The presence of relevant AMR genes conferring resistance to medically important antimicrobials often found on mobile elements indicates a heightened risk for transfer.

Financial Support
Wyoming Agricultural Experiment Station

Notes:

P007 - The RpoE stress response pathway mediates polymyxin resistance in Gram-negative bacteria

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Session: Antimicrobial Use

Objective
Recent emergence of polymyxin resistant Gram-negative bacteria of animal origins has drawn worldwide attention. Polymyxins, including colistin, are the drugs of last resort to treat multidrug-resistant bacterial infections in humans. In-depth understanding of molecular basis and regulation of polymyxin resistance would help us search for new antimicrobials to combat increasing polymyxin resistance. Here we aimed to identify novel targets that are essential for polymyxin resistance using *E. coli* BL21(DE3), a unique model strain that displays clinical resistance to polymyxin.

Methods
The BL21(DE3) was subjected to *in vivo* random transposon mutagenesis for screening the mutants with increased susceptibility to colistin. The insertion sites of desired mutants were mapped by direct sequencing. The key genes of interest were also inactivated in diverse Gram-negative bacteria to examine functional conservation in polymyxin resistance. Specific genes in the known PmrAB and PhoPQ regulatory network were inactivated to examine crosstalk among different pathways. Lipid A species and phospholipids were analyzed by normal phase liquid chromatography/mass spectrometry.

Results
Among 8 mutants with increased susceptibility to colistin, 5 mutants (MIC = 0.25 μg/mL) contained different mutations in three genes (*rseP*, *degS* and *surA*) belonging to the RpoE (σ^E) stress response pathway. Complementation of the mutants restored colistin resistance. Inactivation of *rpoE*, *pmrA*, *pmrB*, *eptA*, and *pmrD* led to significantly increased susceptibility to colistin; however, inactivation of PhoPQ did not change colistin MIC. RpoE mutation in different *E. coli* and *Salmonella* strains all led to significantly reduced MIC in colistin. Inactivation of *rpoE* did not change lipid A profile but significantly influenced phospholipids profile.

Conclusions
The RpoE stress response system plays an essential role in polymyxin resistance in Gram-negative bacteria. The molecular and lipidomics findings from this study also suggested novel mechanism mediated through RpoE stress response pathway for polymyxin resistance.

Financial Support
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Notes:

P009 - Antimicrobial susceptibilities of *E. coli* from calves treated with chlortetracycline for anaplasmosis control

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Session: Antimicrobial Use

Objective
In the U.S., chronic bovine anaplasmosis is commonly managed by protracted use of chlortetracycline (CTC)-medicated (0.5-2 mg/lb/BW/day) feed products, with no limit on duration of use. Prolonged antibiotic use may have unintended consequences, including development of antimicrobial resistance in off-target microbes. The aim of this study was to evaluate changes in *Escherichia coli* antimicrobial susceptibilities from cattle provided CTC for chronic anaplasmosis control.

Methods
Holstein-Jersey cross cattle with chronic anaplasmosis were blocked by weight, randomly allocated to one of the CTC treatment group (0-, current FDA-approved doses 0.5-, and 2-, and experimental dose, 10 mg/lb/BW/day) and fed their respective treatment for 120 days. *Escherichia coli* were isolated from fecal samples collected pre-treatment, after 58 and 114 days of consecutive treatment, and 21-days post-treatment cessation. Sensititre™ NARMS gram negative plates were used to evaluate *E. coli* antimicrobial susceptibility to 14 antibiotics using CLSI breakpoints. The log-transformed minimum inhibitory concentration (MIC) data were subjected to linear mixed model analysis. Tests were performed at the 0.1 level with Tukey's multiplicity adjustment.

Results
The median MIC for tetracycline (TET) did not significantly change by treatment or over time. Median MICs for chloramphenicol, sulfisoxazole, trimethoprim/sulfamethoxazole, ampicillin, and streptomycin significantly increased within groups, sometimes crossing breakpoint classifications. Cefoxitin, azithromycin, gentamicin, nalidixic acid, and ceftiofur media MIC did not or minimally changed.

Conclusions
Under the conditions of this study, FDA-approved CTC dosages for active anaplasmosis control had minimal effect on increasing *E. coli* TET resistance, however, most isolates were already TET resistant. Increased *E. coli* resistance to other antibiotics did occur, however, indicating that long-term antibiotic use may broadly influence microbial antimicrobial susceptibility and highlighting the need for judicious antimicrobial use.

Financial Support
U.S. Department of Agriculture, Agriculture and Research Services; Agriculture and Food Research Initiative Competitive Grant no. 2018-68003-27463, National Institute for Food and Agriculture

Notes:

P010 - Antimicrobial resistance in *Campylobacter* spp. and its association with antimicrobial use on Canadian turkey flocks

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Session: Antimicrobial Use

Objective
The emergence of antimicrobial resistance in *Campylobacter* isolates poses a public health risk. On-farm data collected by the Canadian Integrated Program for Antimicrobial Resistance Surveillance were analyzed to determine the prevalence of antimicrobial resistance (AMR) in *Campylobacter* isolates and its association with antimicrobial use (AMU) in Canadian turkey flocks.

Methods
From 2013 to 2021, veterinarians visited turkey flocks once each year, collected four pooled fecal samples at each visit, and administered a questionnaire to collect AMU data. The AMU indicator used for this analysis was mg antimicrobial per kg turkey pre-slaughter weight. From the pooled fecal samples collected from 365 turkey flocks, 1334 *Campylobacter* were isolated and tested for susceptibility to 9 antimicrobials by the broth microdilution method. Hierarchical clustering analysis was performed to identify differences in AMR between *C. jejuni* and *C. coli*. Logistic regression models were built to assess associations between AMR and AMU by using the Generalized Estimating Equations method to account for farm-level clustering.

Results
Overall, a high resistance to tetracycline (42%, 95% CI: 39-45%) and fluoroquinolones (29%, 95% CI: 27-32%) and low resistance to lincosamides (5%, 95% CI: 4-6%) and macrolides (7%, 95% CI: 6-8%) were detected. High resistance to tetracyclines (47.9%) was observed among *C. jejuni* isolates, whereas *C. coli* showed high resistance to fluoroquinolones (43.1%) and moderate resistance to macrolides (19.3%) and lincosamides (12.8%). Multidrug resistance (resistance to ≥3 antimicrobial classes) was observed in 6 *C. coli* and 3 *C. jejuni* isolates. Resistance to fluoroquinolones was associated with the use of streptogramins (OR:1.009, 95% CI:1.003-1.017) and bacitracin (OR:1.031, 95% CI:1.009-1.053), and resistance to tetracyclines with the use of flavophospholipids (OR:1.269, 95% CI:1.005-1.601) and streptogramins (OR:1.028, 95% CI:1.008-1.049).

Conclusions
Ongoing monitoring of AMR and AMU is needed to detect how resistant *Campylobacter* strains are evolving in turkey populations in Canada.

Notes:

P011 - Assesing Illinois small animal veterinarians' opinions on AMR and factors that influence their AMU pratices

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Session: Antimicrobial Use

Objective
The emergence of antimicrobial resistance (AMR) is a global public health issue. Inappropriate antimicrobial use (AMU) has been identified as the major factor impacting the occurrence of AMR. The opinion of veterinarians on AMR may influence their antimicrobial prescription practices. To prevent the development of AMR it is essential to understand their opinions and factors influencing their decision to prescribe antimicrobials.

Methods
To better understand this issue this study developed an online questionnaire using the Qualtrics^{xm} survey tool and administered it to licensed small animal veterinarians in Illinois through the Illinois State Veterinary Medical Association. The survey was open for two months between September and November 2022.

Results
A total of 95 responses were recorded, of which 83 were included in the analysis. The majority of respondents (74.7%) were veterinarians working in primary care hospitals, followed by veterinarians employed in academic teaching hospitals (14.5%) and emergency hospitals (7.2%). The majority of veterinarians believed that inadequate doses of antimicrobials (87.9%), and empirical antibiotic therapies without performing a culture and susceptibility tests (72.3%) contributed most to the increase of drug-resistant bacteria. A total of 43.4% of veterinarians stated that they were aware of the current local antimicrobial resistance profiles of major bacterial pathogens. Only 24.1% of respondents had AMU guidelines at their practice. The main factors influencing veterinarians' decision to prescribe antimicrobials were: disease severity (88%), antimicrobial susceptibility test results (84.4%), administration route (72.3%), previous experience with similar cases (68.7%), antimicrobial prescription guideline recommendations (68.7%), and the patient's medical and previous AMU history (68.7%).

Conclusions
Understanding the current local status of AMU and AMR in Illinois will aid Illinois small animal veterinarians in developing an effective antimicrobial stewardship program to mitigate the emergence of drug-resistant bacteria.

Notes:

P012 - Developing qPCR assays to identify and characterize pathogens causing canine skin and soft tissue infections

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Session: Antimicrobial Use

Objective
In supporting judicious use of our antimicrobial products, Zoetis regularly monitors the activity of antibiotics against labeled pathogens. Zoetis has conducted an ongoing surveillance program to evaluate the in vitro activity of antimicrobial agents against companion animal bacterial pathogens seen at primary/general care practices exhibiting naturally occurring skin and soft tissue infections. These infections are often caused by *Staphylococcus* species. We limit our analysis to labeled pathogens, so correctly identifying these isolates is important. However, differentiating these isolates can be complicated, as our normal workflow does not consistently differentiate closely related Staphylococci. We therefore use a multiplex PCR assay to confirm the species of our Staphylococcus isolates. In addition to antibiotic susceptibilities, we monitor these pathogens for the prevalence of methicillin resistance conferred by the *mecA* gene by another PCR assay. Zoetis has relied on these PCR assays for several years. Though they function well, the assays are laborious and time consuming due to the need for gel-based visualization. To improve our workflow and replace these PCR assays, we are developing qPCR-based assays for species and *mecA* identification.

Methods
We used publicly available qPCR and PCR assays to design qPCR single-plex Taqman assays. These primer/probe sets were run on isolates from our culture collection and results were validated against those of the traditional PCR workflow. The validated single-plex assays were then used to make duplex reactions for further validation.

Results
We developed 7 qPCR primer probe sets for use in this workflow and validated them against 152-290 Staphylococcus isolates. All assays show >94% sensitivity and >93% specificity and are more sensitive than the traditional PCR assays.

Conclusions
These qPCR assays have been grouped into duplex reactions and represent an effective and significant improvement over our previous workflow. The use of these assays has reduced our time requirements by more than 60% and eliminated the need for electrophoresis gels.

Financial Support
Zoetis

Notes:

P013 - Antimicrobial susceptibility of swine pathogens isolated in North America 2016-2020

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Session: Antimicrobial Use

Objective
The Zoetis Antimicrobial Susceptibility Surveillance Program collects bacterial pathogens from Canada and the USA. This report documents the antimicrobial susceptibility of *Actinobacillus pleuropneumoniae, Bordetella bronchiseptica, Pasteurella multocida,* and *Streptococcus suis* strains isolated from diseased pigs during the period from 2016-2020.

Methods
We performed in vitro broth microdilution susceptibility MIC testing using ten antimicrobials: ampicillin, ceftiofur, danofloxacin, enrofloxacin, florfenicol, penicillin, tetracycline, tilmicosin, trimethoprim-sulfamethoxazole, and tulathromycin. These were tested against 250 *A. pleuropneumoniae*, 602 *B. bronchiseptica,* 874 *P. multocida*, and 1223 *S. suis* isolates following CLSI methodology.

Results
We found that *A. pleuropneumoniae* isolates were 100% susceptible to ceftiofur, florfenicol, and tulathromycin and that *P. multocida* isolates were 100% susceptible to ceftiofur. High susceptibility (95%-99%) was observed for *A. pleuropneumoniae* isolates to tilmicosin, *P. multocida* isolates to ampicillin, enrofloxacin, florfenicol, penicillin, tilmicosin, and tulathromycin, *S. suis* to ampicillin and florfenicol and for *B. bronchiseptica* to tulathromycin. Tetracycline had low susceptibility to all four species.

Conclusions
We found that under the limitations of this surveillance program, the predominant swine pathogens in the USA and Canada, *A. pleuropneumoniae, P. multocida, S. suis,* and *B. bronchiseptica,* display high susceptibility to most veterinary antimicrobials.

Financial Support
Zoetis

Notes:

P014 - Integron-encoded antimicrobial resistance genes and virulence determinants among *S.* Typhimurium isolated from swine

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Session: Antimicrobial Use

Objective
Compare virulence factor and antimicrobial resistance gene classes between *Salmonella* Typhimurium isolates with and without class I integrons.

Methods
Several veterinary diagnostic laboratories in the United States provided swine *Salmonella* Typhimurium isolates (n=36) for integron detection, using PCR and gel electrophoresis. Of the 36 swine isolates, 32 were included in whole genome analysis and then screened for the presence of antimicrobial resistance genes and virulence factors via a BLAST approach. Plasmid sequences were extracted using the SPAdes algorithm. Gene identification by integron size and location was entered in MS Excel and summarized.

Results
We observed a higher count of both virulence factors and AMR genes among isolates with class I integrons localization on chromosomes when compared to that on plasmids. Three major gene classes: tetracycline, phenicol, and beta lactam, were found across 100% of integron-containing isolates with integrons localized on chromosomes. Antimicrobial resistance and anti-phagocytosis were two of the three gene classes with 100% presence across isolates with integrons on plasmids.

Conclusions
The results from this study can be applied to predict the pathogenicity of *S.* Typhimurium isolates based on the presence of integrons and identification of AMR and virulence factor genes on isolates that pose a disease risk to food animals as well as to public health.

Financial Support
U.S. Department of Agriculture; College Research Council

Notes:

P015 - Avian mycoplasmosis-use antibiotics smartly with rapid PCR

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Session: Antimicrobial Use

Objective
Instead of time-consuming culture method, we use PCR for timely mycoplasma detection at farm site. Two case studies showed how to use antibiotics smartly with the help of on-site Polymerase chain reaction (PCR) test in Asian region.

Methods
In this study, 10 tracheal swabs were collected from 50,000 chicken population at day of age, day 7, day 14, day 21 and day 28 in 2 different farm. 5 swabs were pooled into 1 vial of taco Sample storage solution as sample for PCR detection. The dose and duration for antibiotic treatment were recorded as well. 200 ul sample were added into the sample well of POCKIT Central Extraction Cartridge and analyzed with POCKIT Central Nucleic Acid Analyzer.

Results
In case 1, *Mycoplasma gallisepticum* (MG) was detected at the day of age, although Tilmicosin was treated, insufficient dosage did not solve the problem and MG was still detected until day 28 despite several drugs were treated continuously. In case 2, MG and *Mycoplasma synoviae* (MS) were detected at the day of age. Sufficient Doxycycline was treated immediately and Mycoplasmosis turned negative at day 7 until harvesting. Although mycoplasma was detected early at the day of age, following treatments were different in two case studies.

Conclusions
Treatment with insufficient dose did not solve the problems but increase the cost of antibiotics. This flock may have antimicrobial resistance MG strains. Instead, on-site PCR detection and treatment with sufficient antibiotics at earliest helps farmer to identify the Mycoplasmosis problem as well as reduce the use of antibiotics, a suitable method for early detection and treatment evaluation is very important. On-site PCR provides evidence for the presence of mycoplasma. What more exciting is that PCR can also be a good tool to evaluate the efficacy of antibiotics treatment. For next-generation Mycoplasmosis control in modern farm, on-site PCR can help farmer and veterinarian to use antibiotics smartly.

Notes:

P016 - Presence and expression of antimicrobial resistance genes in respiratory bacterial isolates of weaned dairy heifers

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Session: Antimicrobial Use

Objective
Bovine respiratory disease (BRD) is the leading cause of morbidity, mortality and antimicrobial drug (AMD) use in weaned dairy heifers, however there is minimal literature describing antimicrobial resistance (AMR) related to BRD in this group. This study aims to define the prevalence of AMR genes in *M. haemolytica*, *P. multocida* and *H. somni* isolates from weaned dairy heifers, compare isolate genotype to phenotype, and assess if AMR gene content of isolates represents AMR expression in the entire source nasopharyngeal microbiome.

Methods
Samples were obtained in a cross-sectional study; 341 weaned dairy heifers, with and without signs of BRD, on 6 calf rearing facilities in California were sampled by 2 deep nasopharyngeal swabs. One swab was used for selective culture and antibiotic sensitivity testing of *M. haemolytica*, *P. multocida* and *H. somni;* isolates were banked and then analyzed for AMR gene content using whole genome sequencing (WGS) (n=326 isolates). The other swab was banked, and then analyzed for AMR gene expression using metatranscriptomics in a subset of heifers (4/6 farms)(n=204 swabs).

Results
Twenty-six AMR genes were identified by WGS; the most prevalent encoded resistance to AMD of veterinary importance including tetracycline, aminoglycoside, sulfonamide, penicillin, cephalosporin, phenicol, macrolide class AMD. The WGS results were particularly discordant with sensitivity testing for cephalosporin and fluoroquinolone class AMD. Over 800 AMR genes were expressed in the metatranscriptomes; the most frequently expressed encoded resistance to tetracycline, penicillin, cephalosporin, macrolide, aminoglycoside, phenicol, fluoroquinolone, sulfonamide class AMD.

Conclusions
AMR genes are highly prevalent and frequently expressed in the nasopharynx of the study population. Discordance between WGS and sensitivity testing for cephalosporin and fluoroquinolone class AMD are discussed. Diversity of AMR gene expression in the nasopharynx suggests the AMR potential of this biologic niche is much larger than represented by analysis of selective isolates.

Financial Support
California Department of Agriculture, Antimicrobial Use and Stewardship Group

Notes:

P018 - Buffered peptone water promotes the transfer of antimicrobial resistance genes to *Salmonella* in swine cecal samples

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Session: Antimicrobial Use

Objective
Buffered peptone water (BPW) is often used as a non-selective pre-enrichment media for the resuscitation of *Salmonella* prior to selective enrichment and isolation. However, BPW enriches bacterial populations that readily transfer genetic elements to *Salmonella*, and the potential for antimicrobial resistance transfer during pre-enrichment is not well understood. Here, the objective was to evaluate the transfer of antimicrobial resistance to *Salmonella* in swine cecal samples incubated in BPW.

Methods
Swine cecal samples were spiked with nalidixic acid resistant and ampicillin sensitive *Salmonella enterica* serovar Typhimurium X4232 (10^4 CFU per gram) and incubated in BPW, Gram-negative broth, or tetrathionate broth for 24 hours. X4232 was differentially recovered, and the percentage of cells resistant to ampicillin was enumerated. In addition, the diversity of plasmids in *Salmonella* isolates resistant to ampicillin and the microbial profiles after enrichment were evaluated.

Results
The proportion of *Salmonella* resistant to ampicillin was significantly higher in cecal samples incubated in BPW and Gram-negative broth compared to tetrathionate broth. Upwards of 4% of recovered *Salmonella* were resistant to ampicillin in some cecal samples incubated in BPW. Plasmid sequencing of the isolates resistant to ampicillin demonstrated the transfer of multiple plasmid types that encoded known resistance genes. Further, sequencing of the bacterial communities of the enrichments confirmed that BPW increased the relative abundance of bacteria that are known to transfer plasmids to *Salmonella*.

Conclusions
These findings demonstrate that BPW can promote the transfer of plasmids when the bacterial donor populations are present with compatible plasmid types. Given that BPW is commonly used as a pre-enrichment media, our findings suggest that the antimicrobial resistance profiles of some recovered isolates could be due to the acquisition of plasmids during pre-enrichment. The results of this study should be confirmed with more diverse sample types and *Salmonella* strains.

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

Notes:

P019 - Context matters for antibiotic resistance gene transfer to *Salmonella* in the chicken gastrointestinal tract

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Session: Antimicrobial Use

Objective
Antimicrobial resistance (AMR) has made treatment of infections a significant problem. AMR *Salmonella* is a serious concern, as is the potential for AMR genes to transfer between commensals and pathogens. The objectives of this study were to determine which AMR genes could be acquired by *Salmonella* Heidelberg SH2813 from the gut microbiota *in vivo*, and to identify potential microbial AMR donors.

Methods
Forty day-old chicks were orally inoculated with 2X10^8 cfu of nalidixic acid resistant SH2813. Cecal contents from 10 birds were collected at various timepoints for total *Salmonella* enumeration and determination of acquired tetracycline (tet) or ampicillin resistance. Additionally, to determine if the AMR donors were present on the eggshell prior to hatch, eggs were hatched conventionally (germ-replete) or after disinfection in non-sterile rooms; a set of conventional eggs was hatched in a sterile isolator (n=36 per group). Three days after hatch, chicks were orally inoculated with SH2813, and cecal contents were collected for *Salmonella*e numeration as well as determination of acquired tet resistance as described above.

Results
A high percentage of *Salmonella* recovered were tet resistant. Sequence data from plasmids acquired by SH2813 suggested some acquired plasmids may have originated from *E. coli*. *In vitro* conjugation between SH2813 and tet resistant *E. coli* demonstrated resistance gene transfer to *Salmonella*. With the egg study, no AMR transfer was detected in SH2813 isolated from chicks despite the presence of tet resistant *E. coli* in cecal contents. *In vitro* conjugation experiments confirmed the lack of tet transfer from *E. coli* isolates to SH2813. Genomes from the *E. coli* strains confirmed the tet genes were chromosomally located or were on plasmids that lacked transfer genes.

Conclusions
Ongoing research aims to explore the genetic diversity of antibiotic resistance reservoirs within the microbiota and the ability for AMR genes to be acquired by *Salmonella*. Understanding factors that impact the transfer of resistance genes to pathogens will inform interventions to impede this process.

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

Notes:

P020 - Identification of gut microbiota species antagonistic to *Shigella flexneri* and their use as antibiotic alternative

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Session: Antimicrobial Use

Objective
Shigella flexneri is a major public health concern in both developed and developing nations as it causes mild to severe as diarrhea. Since many strains of *Shigella* are already multi-drug resistant, treating shigellosis with antibiotics is becoming difficult. In this study, we screened healthy human gut microbiota culture collection to identify species antagonistic to *S. flexeneri*. Ability of a subset of these strains to reduce *S. flexeneri in vivo* colonization and pathogenicity was tested using a *Caenorhabditis elegans* model.

Methods
Our group previously developed a culture collection of gut bacteria from healthy humans that consists of over 1200 strains representing more than 75% functional capacity of gut microbiome. A co-culture assay was used to screen 82 species representatives from this library to identify species that show growth antagonism to *S. flexneri*. Following *in vitro* testing, a *C. elegans* model was utilized for standardizing anaerobic colonization of the top inhibiting strains, challenge with *S. flexneri*, and qualitative assessment with assays such as Lifespan and Smurf. For the first time, we also developed a histopathological assay to score pathological response of C. elegans to *S. flexneri* infection

Results
Of the 52 species tested *in vitro*, genus *Bifidobacterium* had the 4 strongest inhibitory strains against *S. flexneri*. Initial lifespan assays with continuous feeding of *S. flexneri* showed significant reduction of lifespan where majority of animals died within 11 days. Initial smurf assays display regional enlargement of intestinal tracts within *C. elegans* indicating intestinal integrity loss. Successful colonization of the top four S. flexneri inhibiting species within *C. elegans* also has been done.

Conclusions
Our in vitro screen identified several species antagonistic to *S. flexneri*. For the first time, we were able to perform anaerobic in vivo colonization resistance assay using *C. elegans* as an *in vivo* model. Future studies will include challenge with *S. flexneri* against *C. elegans* colonized with the top in-vitro inhibitors.

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

Notes:

P021 - Intralesional species-level diversity of *Staphylococcus* spp. and their antimicrobial resistance profiles in dogs

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Session: Antimicrobial Use

Objective
Staphylococcus spp. is associated with >90% of canine skin and soft-tissue infections, the majority (>50%) of which are multidrug resistant. The objective of this study was to investigate intralesional species level diversity among *Staphylococcus* spp. and their antimicrobial resistance.

Methods
Skin swabs were collected from 60 dogs housed in 10 shelters (6 dogs/shelter) across the Cumberland Gap Region of TN, KY, and VA. From each dog, one swab from a lesion and one from the lesion-free area of the skin were collected and cultured for isolation of *Staphylococcus* spp. For each sample, ≥2 colony morphotypes were subjected to species-level identification using MALDI-TOF and tested for antimicrobial resistance (AMR) by broth microdilution method using a companion animal antibiotic panel.

Results
A total of 198 *Staphylococcus* spp. including 166 (84%) *S. pseudintermedius* and 32 (16%) other *Staphylococcus* spp. (*S. epidermidis, S. simulans*, *S. aureus, S. sciuri, S. xylosus, S. intermedius*, and *S. cohnii*) were isolated. In 20% (11/55) of lesions, AMR testing on ≥ 2 isolates of *S. pseudintermedius* isolated from the same lesion showed a distinct antimicrobial resistance profile. In 5% (3/60) lesions, more than one species of *Staphylococcus* was isolated. Eleven out of 21 (52.3%) non-*S. pseudintermedius Staphylococcus* spp. isolated from lesions were multi-drug resistant (resistant to ≥ 3 antibiotic classes).

Conclusions
Our data shows that, in many cases, the skin lesion in dogs may contain more than one strain of *S. pseudintermedius* with distinct AMR phenotypes, and in some cases, a highly resistant non-*S. pseudintermedius Staphylococcus* spp. Thus, a conventional diagnostic approach of testing a single colony morphotype for detection and AMR profiling of *Staphylococcus* spp. isolated from canine skin and soft tissue infections may be misleading. Testing of more than one colony morphotype of *Staphylococcus* spp. should be considered for accurate diagnosis and to implement of evidence-based antibiotic treatment to achieve favorable treatment outcomes.

Financial Support
Lincoln Memorial University

Notes:

P022 - Modulation of *Saprolegnia parasitica* growth using copper and ionophores

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Session: Aquaculture

Objective
Saprolegnia parasitica is an aquatic pathogen responsible for saprolegniasis in fish. Eggs, embryos, and injured/juvenile fishes are susceptible to *S. parasitca* infection which can lead to death, poor fish quality and impact production levels. Some chemicals such as malachite green, formalin and peroxides that are used to treat saprolegniasis have been reported to exhibit carcinogenic and mutagenic effects. Other chemical agents such as metal chelators and ionophores are being evaluated as alternatives in the treatment of saprolegniasis. In this study, we described the role of copper (Cu, in the form of copper sulfate) and the addition of metal-binding small molecules (*i.e.* ionophores) in controlling the growth of *S. parasitica.*

Methods
In vitro screening and analyses were carried out to determine the effects of the ionophores and Cu on the pathogen.

Results
The results from the ionophores screening demonstrate that tetraethylthiuram disulfide (TDD), ciclopirox olamine (CLP), 2-mercaptopyridine N-oxide (MPO), 5-chloro-8-hydroxy-7-iodoquinoline (CHI), 5,7-dichloro-8-hydroxyquinoline (DHQ) and 8-Quinolinol (8QN) altered *S. parasitica* growth in a copper-dependent manner. At concentrations below the toxic level of individual ionophore, increasing the dose of copper resulted in the suppressed growth and eventual death of *S. parasitica*. However, the addition of bathocuproine sulfate (BCS) to maintain copper in the extracellular growth media reversed the suppression of *S. parasitica* growth for some ionophores including TDD, CLP, MPO and 8QN. BCS addition to CHI and DHQ, however, resulted in the death of *S. parasitica*.

Conclusions
Our data suggest that ionophores in combination with low levels of Cu can effectively curve *S. parasitica* growth *in vitro*. Our current research directions are aimed at assessing the effectiveness of Cu-ionophore combinations to inhibit *S. parasitica* growth on catfish eggs.

Financial Support
U.S. Department of Agriculture

Notes:

P023 - Detection of leptospiral DNA and antibodies in fish

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Session: Aquaculture

Objective
Leptospirosis is an important zoonotic disease that accounts for significant morbidity and mortality in animals and humans. It is maintained in populations due to chronic kidney infection of reservoir mammals. Previous work has identified rodents, voles, shrews, chipmunks and several species of amphibians and reptiles as reservoirs of *Leptospira* spp. in the Cumberland Gap Region of Kentucky, Tennessee, and Virginia. However, fish have not been studied as potential reservoirs in this area. The aim of this study is to determine if fish contribute to the maintenance of leptospirosis in the aquatic environment.

Methods
Fish (n= 238), of various species were collected from six locations along the Powell River in Tennessee. These included 34 Rockbass (*Ambloplites*), 40 Sunfish (*Lepomis*), 1 Catfish (*Pylodictus*), 22 Hogsuckers (*Hypentelium*), 12 Common Log Perch (*Percina caprodes*), 30 Stonerollers (*Campostoma*), 5 Longnose Gar (*Lepisosteus*), 34 Chubs (*Erimystax, Nacomis, Semotilus, Hybopsis*), 1 Bluntnose Minnow (*Pimephales*), 13 Shortnosed Redhorse (*Moxostoma*), 16 Bass (*Micropterus*), 6 Darters (*Percina aurantiaca, Etheostoma)*, and 24 Shiners (*Cyprinella, Notemigonus, Luxilus*). Fish kidneys were harvested and screened for leptospiral DNA using a highly sensitive and specific TaqMan quantitative PCR (qPCR) assay. Blood samples were collected for measuring leptospiral antibodies using microscopic agglutination test (MAT).

Results
Of the 238 kidneys screened, leptospiral DNA was detected in 5 (2.1%) samples: 2 Sunfish, 1 Common Log Perch, 1 Rockbass, and 1 Shiner. Thus far, 125 fish sera were screened with MAT and 6 samples (4.8%) had antibodies to at least one leptospiral serovar: 3 Rockbass and 1 Sunfish reacted with serovar Icterohaemorrhagiae, 1 Sunfish with serovar Grippotyphosa, and 1 Shortnosed Redhorse with serovars Pomona, Hardjo, and Grippotyphosa. Additionally, PCR amplification and sequencing of multiple genes will be used to genotype leptospires present in positive kidneys.

Conclusions
This study will provide information on the role of fish in the epidemiology of leptospirosis in the region.

Financial Support
Lincoln Memorial University

Notes:

P025 - Investigation of a novel 3D bovine airway model with bacterial concentrated culture supernatant

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Session: Bacteriology

Objective
To investigate biofilm formation during bovine respiratory disease (BRD), we have designed a novel 3D co-culture model that incorporates bovine epithelial (BEs) and airway endothelial cells (BAEs) to study the effect of signaling factors from *M. haemolytica* and *H. somni* concentrated culture supernatant (CCS).

Methods
3D bovine airway co-culture models were assembled as follows: BEs were encapsulated in a 1.1 mg/ mL type 1 collagen gel. BAEs were encapsulated at a ratio of 3 BEs:1 BAE in type 1 collagen and then seeded on top 24h later. Three media conditions were then administered to cultures 24h after co-culture assembly, which included (i) cell media (CM) (ii) 50% CM and 50% bacterial CCS (50/50) and (iii) bacterial CCS. DNA analysis of cells was conducted 24 h, 72 h, and 108 h after CCS administration. For the cultures stopped at 72 h and 108 h, five media conditions were continued, which included (1) CM, (2) 50/50 revert, where cell media was administered from 24 h onwards, (3) 50/50 (continuous), (4) bacterial CCS revert, and (5) bacterial CCS.

Results
DNA analysis shows BE monocultures are not affected by *M. haemolytica*, but do not proliferate with *H. somni* CCS. If *H. somni* CCS is reverted to CM, BE monocultures proliferate. BAE monocultures exhibit the opposite effect, and seem to be resilient against *H. somni* CCS. However, with *M. haemolytica*, BAEs do not proliferate with continuous and 50/50 CCS. If *M. haemolytica* CCS is reverted to CM, BAEs recover and proliferate. Co-cultures do not proliferate with *H. somni*, but show recovery once the CCS is reverted to CM. Initially, co-cultures are affected by *M. haemolytica*. However, from 24 h to 108 h, cells proliferate even with continuous *M. haemolytica* CCS.

Conclusions
DNA analyses of mono- and co-cultures of BEs and BAEs provide an understanding of cell proliferation with bacterial CCS. Future work will include the characterization of the epithelial barrier, cytokine secretion, and investigation of C3D bovine airway model to study biofilms of BRD pathogens.

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

Notes:

P026 - Proliferation and apoptosis in growing heifer mammary glands experiencing *Staphylococcus aureus* infection

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Session: Bacteriology

Objective
Intramammary infections are common in non-lactating dairy heifer mammary glands and can occur during periods of marked mammary epithelial cell (MEC) accumulation, an important determinant of milk production. We recently reported a reduced amount of epithelial tissue area in rapidly growing heifer mammary glands with *Staph. aureus* intramammary infection but the cellular processes producing this observation are unknown. The objective was to delineate the degree of cellular proliferation and apoptosis in infected and uninfected rapidly growing mammary glands.

Methods
Eight nulligravid Holstein heifers received supraphysiological injections of estradiol and progesterone for 14 d to stimulate rapid mammary growth. On d 8, 1 randomly selected mammary gland of each heifer was infused with *Staphylococcus aureus* (CHALL) while another mammary gland served as an uninfected control (UNINF). Mammary tissues were collected on the last day of hormonal injections from center and edge parenchymal regions in the gland. Tissues were formalin-fixed, paraffin-embedded and subject to proliferation and apoptosis assessment via Ki-67 staining and TUNEL assay.

Results
Cellular proliferation percentages did not differ between quarter treatments, but MEC proliferation was marginally greater in edge parenchyma than center parenchyma. Coincidently, CHALL quarters had a greater percentage of apoptotic MEC and lower percentage of apoptotic stromal cells than UNINF quarters. Stromal cell apoptosis was marginally greater in edge parenchyma than center parenchyma.

Conclusions
These results indicate that *Staph. aureus* IMI impairs MEC accumulation while reducing stromal cell regression during rapid mammary gland growth. Such changes are posited to impede epithelial expansion in the fat pad and reduce the total number of MEC at parturition. These factors are expected to negatively impact first lactation milk yield.

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

Notes:

P027 - Complement fixation and rose bengal test as a combined method for diagnosing brucellosis in animals in Armenia

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Session: Bacteriology

Objective
Brucellosis is currently one of the most important zoonotic bacterial diseases worldwide, and accurate diagnosis is imperative to control the spread of disease. Brucellosis can be caused by various *brucella* species that are transmitted from animals to humans by ingestion through infected food products, direct contact with an infected animal or inhalation. We sought to determine if adding the complement fixation test (CFT) as a secondary confirmatory test to rose bengal test (RBT) would improve our ability to accurately diagnose brucellosis in the Republic of Armenia.

Methods
In 2020, blood samples from 594998 cattle and 410678 small ruminants (SR) were tested in Armenia. We screened all blood samples for brucellosis by RBT. The RBT is an inexpensive assay for the detection of brucellosis and is used for initial screening. It is a very sensitive assay, but can result in false positives. To confirm RBT results, we tested all positive samples by CFT, which is a highly sensitive and specific assay. We also screened an additional 10 positive and 10 negative samples by fluorescent polarization assay (FPA) and ELISA to ensure the accuracy of CFT results.

Results
We determined that 2577 (0.433%) of cattle and 2324 (0.566%) SR were positive by the RBT assay. CFT results confirmed that only 1952 cattle and 1829 SR were positive for brucellosis. The FPA and ELISA test results confirmed the CFT results. Based on the confirmation by CFT, the total prevalence of infected cattle was 0.328% and 0.445% for SR.

Conclusions
We determined that CFT is more accurate than RBT, and the combination of both methodologies provide a precise diagnosis of brucellosis in animals compared to a single method. Based on the data, it is recommended to continue using RBT as a low-cost primary screening test followed by confirmation by CFT. We will continue parallel professional development of specialists of regional laboratories by organizing interregional proficiency tests (PT). It is also suggested to add a third diagnostic test to get more accurate results from indeterminate samples.

Financial Support
U.S. Defense Threat Reduction Agency

Notes:

P028 - Study of *Yersinia*-like organisms among small mammals in 2019-2021, country of Georgia

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Session: Bacteriology

Objective
The genus *Yersinia* includes three species of zoonotic pathogens including *Y. pestis*, the agent of plague, and the enteropathogens *Y. pseudotuberculosis* and *Y. enterocolitica*. Plague has continuously circulated in the Caucasus region for many centuries. *Yersinia spp*. was widely found in Georgia in the regions overlapping with the natural plague reservoirs. The goal of this study was to identify the yersiniosis among rodent species in different urban areas of the country.

Methods
In total, 223 rodents were collected in eastern and southern Georgia during 2019-2021. Tissue samples (intestines) were collected from all captured rodents and investigated for the presence of *Yersinia spp*. Bacteriological methods were applied for the laboratory study – culturing the tissue samples to obtain bacterial growth with further biochemical characterization.

Results
Out of 223 collected rodent samples, the following strains were isolated: 36 isolates of *Y. enterocolitica*, 12 isolates of *Yersinia intermedia* and 9 isolates of *Yersinia frederinii* were obtained. Species identification was confirmed by API20 and BD Poenix50 tests. Positive samples were obtained from *Rattus norvegicus, Mus musculus, Meriones libycus, M. macedonicus, Apodemus ponticus, Microtus socialis, M. arvalis, Crocidura leucodon, Apodemus spp*.

Conclusions
This finding suggests a wide distribution of *Yersinia species* in these parts of Georgia. Isolation of numerous cultures of *Y. enterocolitica* from various species of wild mammals in Georgia strongly suggests a wide circulation of this species of *Yersinia* in natural habitats across all landscape zones of the Caucasian region. This discovery was unexpected because previously *Y. enterocolitica* has been primarily known as a human enteric pathogen with some involvement of domestic animals (pigs) with rare reports from commensal rats living closely to pig farms. Until recently, frequent reports of isolation of strains, which were identified as *Y. enterocolitica* from wild rodents collected in plague endemic areas in the Caucasus and Central Asia remained undocumented in English language literature.

Financial Support
U.S. Defense Threat Reduction Agency

Notes:

P029 - Characterizing *Mycobacterium* isolates from dogs and cats

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Session: Bacteriology

Objective
Infections caused by nontuberculous *Mycobacterium* spp. has been an increasing cause of morbidity in humans and animals, ranging from localized granulomatous infections of the skin and subcutis to disseminated disease. These infections are often challenging to diagnose and treat due to the diversity of species causing these infections, and hence the variation in antimicrobial susceptibility patterns.

Methods
In this study, we characterized mycobacterial isolates obtained from dogs and cats representing both in house and referral cases submitted between 2016 and 2022 at the University of Tennessee Veterinary Medical Center. We performed a variety of tests, including Gram staining, Acid-fast staining, Matrix-Assisted Laser Desorption/Ionization Time-of-flight mass spectrometry (MALDI-TOF MS), 16s rRNA amplification and sequencing with two different sets of primers for the identification of these isolates and antimicrobial susceptibility testing on all the isolates.

Results
We observed a discrepancy in identifications made by MALDI-TOF and those made by DNA sequencing with the two different sets of primers. Of the 25 samples with an identification by both MALDI-TOF and sequencing using the primer pair-1, only 9 samples (36%) had a correct match. Of the 24 samples with an identification by both MALDI-TOF and sequencing using primer pair 2, 15 samples (62.5%) had a correct match. Antimicrobial susceptibility testing by broth microdilution test identified variations in patterns between isolates.

Conclusions
A comprehensive approach using multiple modalities may be required to accurately identify Mycobacterium spp. Characterization of genotypic and phenotypic attributes of mycobacterial isolates in combination with their antimicrobial susceptibility pattern could serve as an important tool for improved diagnostic care and more effective clinical management.

Notes:

P031 - Carapace microbiota of American lobsters associated with epizootic shell disease

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Session: Bacteriology

Objective
Epizootic shell disease (ESD) is associated with dysbiotic shift in the carapace bacterial community. This means that ESD may not be only caused by a single species, but by polymicrobial infections. However, there is little information about carapace microbiota associated with ESD. This study aims to characterize the structure and function of carapace microbiota of American lobsters with ESD using 16S metagenomic sequencing.

Methods
American lobsters were obtained from 3 regions: Eastern Long Island Sound (ELIS), Western Long Island Sound (WLIS), and 50 miles south of Montauk (offshore). Lobsters with shell lesions were classified as diseased (ESD, n=8), and lobsters with no apparent signs of carapace lesions were considered healthy (HTH, n=10). Microbial genomic DNA was extracted from shells of lobsters using the DNeasy Blood & Tissue kit according to the manufacturer's protocols. 16S V3-V4 amplicon sequencing library preparation and Illumina MiSeq sequencing were conducted at GENEWIZ, Inc. (South Plainfield, USA). Sequencing data were analyzed with various bioinformatics tools.

Results
We found that lobsters with ESD harbored specific carapace microbiota characterized by high abundance of Aquimarina, which was significantly different from healthy lobsters. According to PICRUSt analysis, ESD-associated carapace microbiota was found to be significantly more abundant in gene families involved in valine, leucine and isoleucine biosynthesis, fatty acid biosynthesis, and renal cell carcinoma. We also found that Aquimarina, Octadecabacter, and Tenacibaculum were found in all lobster carapaces with ESD, suggesting that they are core carapace bacteria that may contribute to ESD.

Conclusions
Our data suggest that ESD may be associated with alterations in the structure and function of carapace microbiota. The genus Aquimarina, Octadecabacter, and Tenacibaculum were among the core bacteria associated with ESD. Further study is warranted to elucidate the roles of Aquimarina, Octadecabacter, and Tenacibaculum in the pathogenesis of ESD.

Financial Support
Foundation for Food and Agricultural Research

Notes:

P032 - Phenotypic and genomic comparison of human outbreak and cattle-associated *Escherichia coli* O157:H7

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Session: Bacteriology

Objective
Shiga toxin-producing *Escherichia coli* O157:H7 (O157) are a common cause of human foodborne illnesses. Cattle are an asymptomatic reservoir of O157, with fecal contamination of meat and produce a major concern. Currently, the relationship between phenotype and genotype of O157 isolates that pose health risks to humans or exist primarily as environmental isolates is unclear. The objective was to compare diverse O157 isolates from human outbreaks or cattle origin to identify relationships between phenotype (cattle shedding, biofilm formation, cell attachment) and genotype in an attempt to predict phenotype from genome information alone.

Methods
Cattle colonization and fecal shedding, biofilm formation, cell adherence, Shiga toxin (Stx) production, and phylogenetic relationship (LSPA-6) were evaluated across four diverse O157 isolates. O157 isolates included the human outbreak isolates EDL933, TW14588, and RM6067W, along with the cattle isolate FRIK1989.

Results
Differences in biofilm formation, cell adherence, and Stx production were observed across the four examined O157 isolates. Of note, EDL933 had strong cell adherence and strong biofilm formation, while TW14588 had poor cell adherence and poor biofilm formation. While no significant differences in cattle colonization or shedding were observed, TW14588 did have the lowest observed average colonization and fecal shedding counts. FRIK1989 produced the greatest Stx-induced Vero cell cytotoxicity. All four of these O157 isolates are closely related to human clinical O157 isolates deposited in the NCBI Pathogen Detection database.

Conclusions
The isolates displayed variations in phenotype and genotype, yet no significant difference in cattle colonization or fecal shedding was observed, indicating that cattle colonization and shedding is a dynamic process that warrants further study. These results suggest the relation between biofilm, cell adherence, and virulence is uncertain. Future work with additional phenotypic panels and a greater number of isolates is needed to better draw conclusions on O157 genome and virulence.

Financial Support
U.S. Department of Agriculture

Notes:

P033 - Segmented filamentous bacteria reduce *Salmonella* infection and total *Enterobacteriaceae in vivo*

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Session: Bacteriology

Objective
Segmented filamentous bacteria (SFB) are keystone species in the maturation of the gut immune system during the early life of humans, mice, and chickens. Our lab has demonstrated the ability to isolate and introduce SFB to chickens after hatch to increase early SFB gut colonization, which increases immune maturation and resistance to bacteria, like *Salmonella*. The objective of this study was to demonstrate the ability of SFB treatment to reduce *Enterobacteriaceae* and *Salmonella* colonization in layer hens *in vivo*.

Methods
One-day-old specific pathogen-free layers (n = 12 per group) were treated with either PBS (CON) or SFB orally. At 4 days post-inoculation, both CON and SFB groups were orally challenged with *Salmonella* Typhimurium. Feces were examined for total *Enterobacteriaceae* and *Salmonella* from all birds at 3, 6, and 10 days post-challenge (dpc). At 14 dpc, all birds were euthanized, total *Enterobacteriaceae* and *Salmonella* levels were examined in the ileum, cecum, and spleen, and levels of SFB were determined from ilea scrapings via microscopy and qPCR.

Results
No significant difference in weight gain was observed between groups at any timepoint. At 6 and 10 dpc, a significant decrease in total *Enterobacteriaceae* was observed in feces of the SFB group. At necropsy, the level of SFB was significantly higher in the SFB group than in the CON, while a significant decrease in total *Enterobacteriaceae* and *Salmonella* was observed in the ceca of the SFB group.

Conclusions
The introduction of SFB at hatch as a prophylactic treatment may benefit commercial partners as well as consumers by reducing the incidence of *Enterobacteriaceae* in food animals. Reduction of these bacteria in animals would in turn, increase animal health, productivity, and safety for consumers. Ongoing studies are optimizing the treatment for poultry industry applications.

Financial Support
Kent Nutrition Group

Notes:

P035 - Effects of β-glucan on *Salmonella enterica* serovar Typhimurium swine colonization and microbiota alterations

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Session: Bacteriology

Objective
With the elimination of medically important antibiotics for growth promotion of food animals by the 2017 Veterinary Feed Directive, nutritional supplements to enhance animal health and reduce pathogen colonization are sought after by food animal producers. β(1-3)(1-6)-D-glucan (β-glucan) is a soluble fiber with prebiotic characteristics that has been shown to modulate immune and intestinal functions when administered in the diets of growing pigs. In the current study, the effects of a β-glucan product on gut microbial community structure as well as *Salmonella* shedding and intestinal colonization was evaluated.

Methods
Five-week-old pigs were fed a β-glucan amended diet at 500g/ton (n=13) or a non-amended control diet (n=14) for three weeks, followed by inoculation of the 27 pigs with 1×10^9 colony forming units of *Salmonella enterica* serovar Typhimurium strain UK1. Fecal samples were collected at 2, 4, 7, and 16 days post-inoculation (dpi) for enumeration of *Salmonella*. Cecal contents and the Peyer's patches region of the ileum were obtained for *Salmonella* quantitation at necropsy on 16 dpi.

Results
While remaining on the respective diets, fecal samples collected at respective time points were similar for *Salmonella* shedding counts between the two diets. At 16 dpi, *Salmonella* counts were significantly lower in the cecal contents of the β-glucan-fed pigs (P=0.0339) and a trend towards a reduction was observed in the Peyer's patches (P=0.0790) compared to the control pigs. Pigs fed β-glucan for three weeks exhibited an increase in members of the *Clostridia* class in their fecal microbial communities, and after inoculation with *Salmonella*, several potentially beneficial microorganisms were enriched in the microbiota of β-glucan-fed pigs (*Lactobacillus, Ruminococcaceae, Prevotellaceae, Veillonellaceae, Bifidobacterium* and *Olsenella*).

Conclusions
β-glucan diet supplementation altered the swine gut microbiome and reduced *Salmonella* colonization in the cecal contents.

Financial Support
U.S. Department of Agriculture; Phileo by Lasaffre

Notes:

P036 - Are multiple pathogens associated with infectious bovine keratoconjunctivitis?

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Session: Bacteriology

Objective
Infectious Bovine Keratoconjunctivitis (IBK) is a highly contagious ocular disease of cattle resulting in substantial economic losses. *Moraxella* spp. are thought to be the causative agents, but research suggests involvement of other microorganisms. This project investigated pathogens involved in IBK.

Methods
Ocular fluid samples (n=102) were swabbed from 45 Black Angus and mixed breed Angus cattle at the University of Kentucky from June 10 to July 8, 2022. Sampled eyes were grouped into 1 of 5 categories: Control Animal (CA; 28 samples), Control Eye (CE; 13), Infected (I; 30), Healing/Healed (H; 28), and Possibly Infected (PI; 3). One swab was used for bacterial isolation and identification using MALDI-TOF, and one for genetic identification by PCR targeting *Moraxella bovis* (MoB), *Moraxella bovoculi* (MoBo), *Mycoplasma bovoculi* (MyBo), *Mycoplasma bovis* (MyB), Chlamydia, and Bovine Herpes Virus. Sampled eyes were graded on IBK severity (0-4 scale).

Results
PCR detected MoB, MoBo, and MyBo in a subset of samples. In 28 CA samples the following were detected: 9 (32.1%) MoB, 11 (39.3%) MoBo, and 24 (85.7%) MyBo. In 13 CE: 6 (46.2%) MoB, 6 (46.2%) MoBo and 1 (84.6%) MyBo. In 30 Infected: 20 (66.7%) MoB, 22 (73.3%) MoBo, and 27 (90.0%) MyBo. In 28 H: 12 (42.9%) MoB, 12 (42.9%) MoBo, and 24 (85.7%) MyBo. In 3 PI: 1 (33.3%) MoBo, and 3 (100%) MyBo. Culture isolation and MALDI-TOF revealed MoB, MoBo, and *Trueperella pyogenes*. In 28 CA samples the following were isolated: 4 (14.3%) MoB and 3 (10.7%) MoBo. In 13 CE: 2 (15.4%) MoB and 5 (38.5%) MoBo. In 30 Infected: 9 (30.0%) MoB, 22 (73.3%) MoBo, and 1 (3.33%) *T. pyogenes*. In 28 H: 7 (25.0%) MoB and 1 (3.57%) MoBo. No known pathogens were isolated in the PI samples. Antimicrobial susceptibility testing found 5 samples of MoBo resistant to Trimethroprim/Sulfamethoxazole.

Conclusions
Our study indicates the involvement of multiple pathogens in IBK. Future research should use a more sensitive technique evaluating these and other factors involved in the pathogenesis. Information from this study will aid in the development of future management protocols.

Financial Support
Lincoln Memorial University College of Veterinary Medicine

Notes:

P037 - Liver abscesses in feedlot cattle: further studies on etiology and pathogenesis

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Session: Bacteriology

Objective
Liver abscesses are a polymicrobial infection. The causative agents include two subspecies of *Fusobacterium necrophorum, necrophorum* and *funduliforme*, *Trueperella pyogenes*, and *Salmonella enterica*. Additionally, other bacterial pathogens are isolated sporadically. However, their prevalence in liver abscesses has not been determined. Also, an aspect of the pathogenesis that has not been investigated is whether the pathogens could originate from the colon? Our objectives were to isolate and identify subsp. *necrophorum* and *funduliforme*, *T. pyogenes*, *S. enterica*, *E. coli*, *Klebsiella* spp., and *Pseudomonas aeruginosa* from liver abscesses and ruminal and colonic tissues.

Methods
Liver abscesses, matched with ruminal and colonic epithelial tissue samples from 71 steers and heifers, originating from 11 feedlots, were collected at slaughter. Samples were subjected to anaerobic and aerobic bacterial isolations, including enrichment and selective media.

Results
Overall, prevalence of subsp. *necrophorum*, subsp. *funduliforme*, *T. pyogenes* and *S. enterica* in liver abscesses were 85.9% (61/71), 21.1% (15/71), 30.9% (22/71) and 8.4% (6/71), respectively. Prevalence of *E. coli*, *Klebsiella*, and *P. aeruginosa* in liver abscesses were 73.2% (52/71), 21.1% (15/71), and 8.4% (6/71), respectively. Subsp. *funduliforme* was more prevalent in ruminal and colonic tissues than subsp. *necrophorum* and the later was isolated more frequently from colonic tissues (12/71; 16.9%) than ruminal epithelial (4/71; 5.6%) tissues. Prevalence of *Salmonella* was higher in colonic tissues 8.4% (6/71) compared to rumen epithelial tissues 4.2% (2/71). No *T. pyogenes* was isolated from GI tract tissue samples. Prevalence of *E. coli*, *Klebsiella* spp., and *P. aeruginosa* in rumen epithelial and colonic tissues were 87.3%, 52.1%, and 21.1%, and 87.3%, 14.0%, and 5.6%, respectively.

Conclusions
In conclusion, *E. coli* was the second most dominant pathogen, next only to *F. necrophorum*, and the frequencies of *F. necrophorum* and *S. enterica* isolations from colonic tissues suggest that hindgut could be a source of pathogens involved in liver abscesses.

Financial Support
Foundation for Food and Agricultural Research

Notes:

P038 - Occurrence of canine infectious respiratory disease-associated pathogens in the shelter dog population

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Session: Bacteriology

Objective
Canine infectious respiratory disease (CIRD) is a common disease of dogs caused by one or more viral and bacterial pathogens. Multiple bacteria (Bordetella bronchiseptica, Mycoplasma canis, Mycoplasma cynos, Streptococcus equi subsp. Zooepidemicus) and viruses [canine adenovirus (CAH), canine herpesvirus (CHV), canine influenza A virus (CIAV), canine respiratory coronavirus (CrCoV), canine distemper virus (CDV) and canine parainfluenza virus (CPIV)] are associated with CIRD in dogs. CIRD occurs more commonly in shelters, kennels, or other settings where large groups of dogs are housed with the frequent addition of new animals. The objective of this study was to determine the prevalence of CIRD pathogens in the respiratory tract of the shelter dog population.

Methods
We tested non-symptomatic shelter dogs for the presence of 11 respiratory pathogens, including SARS-CoV. Nasopharyngeal swabs were collected from a total of sixty dogs from 10 shelters (6 dogs/shelter) across the Cumberland Gap Region of TN, KY, and VA, and tested for the presence of the above-mentioned respiratory pathogens using conventional or real-time PCR assays.

Results
The highest percent positivity was detected for CPIV (15%; 9/60), followed by B. bronchiseptica and CrCoV (6.67%; 4/60), CAV (5%; 3/60), CIAV and M. canis (3.33%; 2/60), CHV and M. cyanos (1.67%; 1/60). S. equi subsp. zooepidemicus, CDV, and SARS-CoV were not detected in any of the tested dogs. None of the tested pathogens was detected in dogs from 1 (10%) shelter. Co-infection with 3 pathogens (CAV, CrCoV, CPIV) was detected in three dogs from the same shelter. Co-infection with 2 pathogens (B. bronchiseptica - CPIV and B. bronchiseptica - M. canis) was detected in 2 dogs from different shelters

Conclusions
These data show that dogs housed in shelters can carry multiple respiratory pathogens associated with CIRD. The relative importance of these pathogens, alone or in combination, in respiratory infections in shelter settings, should be further investigated.

Financial Support
Lincoln Memorial University

Notes:

P039 - Evaluation of an electrostatic precipitator on PRRS virus aerosol removal and inactivation

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Session: Biosecurity and infection control

Objective
Airborne diseases pose a threat to both human and animal health. The rapid spread of airborne pathogens makes them difficult to contain and protect against in animal premises, including swine barns. Although air filtration is widely used to reduce incidence of airborne infections in swine farms, there are still shortcomings, such as high costs and pressure drop due to resistance to the air flow.
Electrostatic precipitators (ESP) are filterless air cleaning devices that utilize charged electrodes to ionize airborne particles and remove them. ESPs in general generate reactive oxygen species and ions that are deemed effective in virus inactivation. We have developed a novel ESP for application as a biosecurity technology against aerosol infections. In this study, we aim to assess the effect of ESP on reducing and inactivating airborne porcine reproductive and respiratory virus (PRRSV) using experimentally generated aerosols.

Methods
The newly designed ESP was tested under different conditions, including high and low air flow rates, two voltages of 12 and 14 kV and operating it with and without secondary electrodes. A suspension of PRRSV was aerosolized in a custom single-pass wind tunnel. Air samples were collected by Andersen cascade impactors simultaneously upstream and downstream, with the ESP located in the middle of the tunnel. Samples were tested for PRRSV by RT-qPCR to quantify total RNA, and titrated in cell culture using the $TCID_{50}$ method to quantify viral viability. Physical collection was assessed by tagging the aerosol with fluorescein and quantified by fluorimetry. Swab samples from the collection electrodes were collected to examine viability of PRRSV.

Results
To quantify the performance of the ESP in virus removal and inactivation, we are in the process of testing. We will calculate the log reduction of PRRSV for each of the conditions tested.

Conclusions
In this study, we will determine the effectiveness of the ESP on virus removal and inactivation which will help identify the potential of the ESP to prevent and reduce the spread of airborne diseases in swine.

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

Notes:

P040 - Anthrax outbreak investigation in the Republic of Armenia in 2021

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Session: Biosecurity and infection control

Objective
Prior to 2021, anthrax was last diagnosed in Armenia in 2019. Anthrax is a zoonotic disease which is caused by gram-positive endospore-forming bacterium, *Bacillus anthracis.* Anthrax has been sporadically observed in livestock in Armenia, occasionally resulting in human cases. We sought to identify the source of infection of the outbreak.

Methods
In June 2021, human anthrax cases were reported in the Shirak region by the RA Ministry of Health. Field teams were sent to the region to conduct interviews with the patients, their family members, and other contacts. We collected 6 meat samples and 11 other food products from patient's homes and sent to the Republican Veterinary Sanitary and Phytosanitary Laboratory Services Center (RVSPLSC) for laboratory testing using classical microbiologic and molecular (Real-Time PCR) methods.

Results
The investigation revealed that on June 29, 2021, one cattle and one sheep died in the Isahakyan village of Shirak region. The cow was slaughtered by the animal owner, and the carcass was processed and used by 36 people from 8 families. Two days after using the shared meat, 10 people went to the infectious hospital with skin ulcers and the patients were diagnosed with anthrax disease. Leftover meat was placed in a refrigerator with other foods. Visual inspection of the meat showed non-coagulated blood and pinkish flesh, which is typical in anthrax contamination. On July 1, 2021, laboratory testing of the meat confirmed the presence of *B. anthracis.* Other food samples collected from the refrigerator were also positive for *B. anthracis,* revealing cross-contamination. The human morbidity rate from this outbreak was 27.8%.

Conclusions
Following confirmation, disinfection and monitoring of the outbreak region was conducted, which contained the spread of disease. This illustrates how anthrax can spill over into the human population from contaminated meat. Meat should only be purchased from authorized vendors whose meat is subject to appropriate sanitary practices and testing. This study also reinforces the importance of vaccinating susceptible animals in Armenia.

Financial Support
U.S. Defense Threat Reduction Agency

Notes:

P041 - Understanding the biosecurity perspectives of Ontario equestrian competition organizers

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Session: Biosecurity and infection control

Objective
Horses are frequently transported across North America to compete, creating an opportunity for the spread of pathogens. While there are biosecurity measures in place for the prevention of infectious pathogen spread at equestrian competitions, it is important to understand the perspectives of those who are responsible for implementing these equine biosecurity measures. This study aims to identify the specific factors that competition organizers consider influential when executing biosecurity measures.

Methods
Individual, semi-structured interviews are being conducted with Ontario competition organizers. Organizers were selected for recruitment from a variety of equestrian disciplines and each participant completed an eligibility questionnaire to gather relevant demographic information. Individuals who had organized an equestrian competition in the past five years were then contacted for a virtual interview. Interview questions focus on organizers' understanding of equine biosecurity, their attitudes, and their future recommendations. Transcripts will undergo thematic analysis to identify major themes and draw comparisons between participants.

Results
Interviews are underway with majority completed or scheduled. The remaining interviews and data analysis are to be completed in fall 2022. The identification of primary themes and subthemes will help to highlight key areas of focus for future biosecurity recommendations and their implementation. Biosecurity is only as successful as its uptake and the role competition organizers play in biosecurity makes the understanding of their perspective invaluable to equine health and welfare. The results can convey areas of interest and gaps in the knowledge for future research projects.

Conclusions
This research study investigating the perspectives of Ontario equestrian competition organizers will highlight common areas of concern and success regarding equine biosecurity at equestrian events. These results will contribute to the further refinement of biosecurity recommendations to maintain equine health and welfare for competition horses.

Financial Support
Natural Sciences and Engineering Research Council of Canada; Canada Research Chairs (CRC) Grant

Notes:

P042 - Evaluation of biosecurity practices in pig farms in African swine fever hotspots of central Uganda

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Session: Biosecurity and infection control

Objective
African Swine Fever virus (ASFV) is a member of the *Asfarviridae* family that causes a highly infectious and fatal hemorrhagic disease of both domestic and wild pigs. The virus is endemic in Uganda, where it is a threat to both food access and economic security. Transmission of the virus can occur through contact with infected pigs, feed, fomites, and soft ticks from the *Ornithodoros genus*. Farms that practice biosecurity measures such as limiting contact with animals, fomites, and prevention of feed contamination can potentially mitigate the spread of the virus. The goal of this study is to understand biosecurity practices amongst ASFV hotspots in Uganda.

Methods
Pigs that presented clinical and pathologic signs were systematically sampled at peri-Kampala slaughterhouses, and were collected between May 2021 through June 2022. Five districts were identified as possible hotspots from this data, such as: Kamuli, Masaka, Wakiso, Luwero, and Mpigi. Twenty farmers were interviewed from each of these districts between June to July of 2022, where a biosecurity questionnaire was administered in either the appropriate local language or in English. Along with the gathering of this primary data, an observational checklist on each surveyed farm was completed and a key informant survey was also done to triangulate the biosecurity questionnaire responses.

Results
Results are currently being compiled and will be presented by district more thoroughly, but biosecurity issues identified include: garbage feeding, relaxed pig husbandry systems such as free-range and tethered pigs, difficulty in exclusion of wildlife, and mortality management.

Conclusions
ASFV is limited in transmission by sound biosecurity practices. Given its endemic nature in Uganda, biosecurity practices play a key role in protecting farmer livelihoods. Disease prevention and control can be enhanced by: diverting the feeding of food leftovers to plants, secure housing for all pigs including piglets, reduced sharing of farming and cleaning equipment if it must be shared, and proper use of designated footwear and clothing for choring pigs.

Financial Support
U.S. Defense Threat Reduction Agency

Notes:

P043 - Improving biosecurity of small-scale and backyard farmers in the US

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Session: Biosecurity and infection control

Objective
The number of small-scale and backyard (SS&BY) farms raising livestock and/or poultry species has increased over the past decades in the US. Challenges associated with their rapid growth include limited access to veterinary care and technical information on biosecurity practices and animal health management. This may lead to an increased risk of occurrence of endemic and foreign animal diseases with potential spread to commercial farms. Therefore, there is a need to develop simple, practical biosecurity plans that are adapted for SS&BY farms. Our goal was to improve uptake of biosecurity measures in these farming systems, through an outreach program targeting farmers and other professionals, such as extension educators and veterinarians (i.e., train-the-trainer approach).

Methods
A website was developed to provide relevant tools and resources targeting SS&BY systems for extension educators, veterinarians, and farmers. A comprehensive online workshop of eight webinars introduced key biosecurity concepts and practices that participants could use to create a biosecurity plan for their operation. After each webinar, attendees were asked to complete a survey to evaluate the knowledge gained and provide feedback. Participants were also given the option of having our team of subject-matter experts and students visit their operation to develop a customized biosecurity plan

Results
Results indicate that we reached our target audience insomuch as participants included a mix of producers, veterinarians, and extension educators. Additionally, participants reported a high level of satisfaction and their knowledge significantly increased on each topic evaluated.

Conclusions
This project implemented several levels of training for farmers and trainers to maximize efforts and create consistent messaging for SS&BY operations. The sequence of webinars introduced key biosecurity concepts that enabled participating farmers to build daily and enhanced biosecurity plans for their operation.

Financial Support
University of California Division of Agriculture and Natural Resources; U.S. Department of Agriculture - National Animal Disease Preparedness and Response Program

Notes:

P044 - Evaluation of intramammary infection status in dairy heifers using two different sample collection techniques

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Session: Diagnostic testing

Objective
The objective of this study is to determine intramammary infection status of dairy heifers using two different sampling techniques, fine needle aspiration (FNA)/cisternal sampling compared to traditional teat end secretion sampling. The goal is to determine the frequency that bacteria cultured from mammary gland secretion samples represent an IMI versus contaminants from the streak canal or teat end skin.

Methods
This cross-sectional study will be conducted at the University of Missouri Foremost Dairy Research Center using approximately 200 heifers. Heifers will be selected across all age groups, starting at weaning age up to 14 days pre-partum. Samples will be collected in the following order: quarter-level mammary gland secretion samples collected via the teat orifice followed by mammary gland FNA/cisternal sample. Samples will be processed using standard laboratory techniques described by the National Mastitis Council. All morphologically distinct bacterial colony types will be identified using MALDI-TOF mass spectrometry. Species identification cutoff values will be applied according to the manufacturer's instructions, in which a score of ≥ 2 indicating a species-level identification. An alternative cutoff value will be used for *Staphylococcus* spp. in which ≥ 1.7 will be used for a species-level identification. The prevalence of bacterial species identified will be cross-tabulated by sample collection method. To investigate if the prevalence of each species is different between each sample type, Chi-squared or Fisher's exact tests will be conducted, as appropriate.

Results
We anticipate finding a difference when comparing the two sampling methods because we expect teat canal inhabitants will influence results of the teat orifice secretion samples.

Conclusions
These data will further our knowledge base on heifer teat canal colonization and intramammary infections. These results will inform future research evaluating mastitis prevention measures in heifers.

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

Notes:

P045 - Proof-of-concept computer vision identification of canine dermatologic conditions

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Session: Diagnostic testing

Objective
Canine dermatologic issues are a common presenting complaint in veterinary medicine. Correct diagnosis of conditions is essential for proper treatment. Artificial intelligence approaches in human dermatology are capable of dermatologist-level performance. Similar models in veterinary medicine would enhance veterinary medicine by providing additional diagnostic support. The objective of this work was a proof-of-concept for an image classification model for common canine pedal dermatological issues (healthy vs neoplasia vs pododermatitis).

Methods
We obtained a total of 573 canine paw images (321 healthy, 63 neoplasia, 189 pododermatitis) from a combination of web scraping, clinical images, and individual submissions. Images were reviewed and classified by a board-certified veterinary dermatologist. Data were split into 80% train and 20% test datasets and random data augmentation (crop, rotation, flip, translation, zoom, contrast) was applied to the training set. No augmentation was performed on the test set. An image classification model was trained in TensorFlow using transfer learning, an ImageNet pretrained ResNet-150 model, and a custom model top. To account for imbalanced classes, losses were weighted by class. The ResNet-150 layers were locked during training and only the dense model top was trained. Model performance was calculated by class for the test set.

Results
Overall model accuracy was 83%. Accuracy by class was 88% Healthy, 56% neoplasia, and 80% pododermatitis.

Conclusions
The trained model was able to successfully differentiate between multiple dermatologic conditions, despite the limited dataset size. Model performance could be improved by including new images in the training data. Additional samples would make it possible to fine-tune convolutional layers.

Notes:

P046 - Epizootology of foot and mouth disease in Georgia

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Session: Diagnostic testing

Objective
FMD disease virus is an animal pathogen that infects domestic animals (cattle, sheep, goats, pigs). Georgia is an exporter of sheep to such countries as Turkey, Iran, Iraq, Jordan, Saudi Arabia. The high demand for sheep from Georgia is explained by the fact that they are grass-fed and therefore healthier and tastier than other suppliers. In order to ensure the health of livestock, the NF Agency throughout Georgia monitors and vaccinates animals. The entire livestock is examined for brucellosis. Positive samples are discarded. The remaining livestock, negative for brucellosis, is examined for foot and mouth disease. This contributes to the growth of confidence in Georgia, and this, in turn, significantly increases the export potential

Methods
During the period 2015-2021 in Georgia 1105,386 samples of sera of small ruminants were studied. Laboratory diagnosis begins with a study of the entire herd for brucellosis serological-detection of immunoglobulins (Bengal Rose test). All positive samples are discarded. Samples negative for brucellosis are tested by enzyme-linked immunosorbent assay (ELISA), in total for the period 2015-2021 was studied 210,783 samples. All sera were analyzed for the presence of antibodies against viral nonstructural proteins (NSPs). This information is of great importance for improving disease control strategies and for selecting a vaccine strain for Georgia in the future. A positive result of the detection of antibodies to FMD unstructured proteins indicates that these samples were obtained from naturally infected animals. A seroepidemiological study of this virus showed that there are 3 different types (A, O and Asia-1) of FMD in Georgia.

Results
From samples collected in 3,83 % serum was positive for anti-NSP antibodies. The highest serological prevalence was for serotype A, followed by serotypes Azia-1 and O.

Conclusions
These outbreaks reinforce the concerns about how readily the disease can pass across international borders, and stimulate the development and improvement of new assays for the detection and characterisation of FMDV.

Notes:

P047 - Development of test kits for diagnosis brucellosis: comparison of diagnostic methods

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Session: Diagnostic testing

Objective
The purpose of this study was to produce reagents for RBP and meet the needs of the country.

Methods
To obtain a Brucella color antigen for Roze-Bengal (RBP), a culture of the strain Brucella abortus 19 was grown for three days on a dense nutrient medium - liver-Martin agar with the addition of Hottinger's medium. The grown Brucella culture was washed off with sterile 0.5% phenolized saline and inactivated in a water bath at 80°C for 1 h. saline and lactic acid. The amount of pink bengal dye was determined by titrimetry, bringing the staining of the antigen to the standard.

Results
Veterinary Scientific Research Institute made a special set of "Brucella Antigen Test" for the agglutination reaction. The results were compared with competitive Enzyme Linked Immunosorbant Assay (cELISA). We used the bovine serum.

Conclusions
Our findings show that, the sensitivity of the test was the 100% (35/35 positive) and the specify was 83%.
We recommend using a local reagent which is cost effective.

Notes:

P048 - *Mycobacterium bovis* screening using a novel phage-based assay compared to pathogen specific biomarkers

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Session: Diagnostic testing

Objective
Bovine Tuberculosis (bTB), caused by *Mycobacterium tuberculosis* variant bovis (MBO) is a contagious cattle disease that accounts for $3 billion in agricultural economic losses worldwide. Bovine tuberculosis is generally first screened for with a Caudal Fold Test (CFT) and confirmed with mycobacterial culture. However, a CFT test lacks specificity and can generate false positive results, while confirmatory bacterial isolation tests are slow, delaying timely diagnosis and therefore mitigation of bTB in cattle herds. We evaluated Actiphage, a novel phaged-based assay, that has been shown to detect *Mycobacterium bovis* from whole blood samples. Actiphage is based on the principle of mycobacteremia which, if present, will be lysed by a broad host range bacteriophage D29 and is detected by IS6110 Polymerase Chain Reaction (PCR).

Methods
Eighty-Six blood samples from Michigan (n=42) and New Mexico (n=44) cattle herds were evaluated by Actiphage. Twelve of these 86 samples were from CFT positive animals, and one animal was confirmed positive by culturing MBO from lymph node lesions. The 12 CFT positive samples were further analyzed with pathogen specific biomarkers (Mb2515c, Mb1895c, and pks5) using an indirect elisa.

Results
None of the samples were positive by the Actiphage procedure. Suggesting a lack of mycobacteremia. Four of these 12 samples were reactive to Mb2515c (a LuxR family transcription regulator) and Mb1895c (molybdenum binding protein). Detection of pks5 is currently underway.

Conclusions
The Actiphage technology did not increase MBO diagnostic sensitivity when compared to current diagnostic strategies. However, further evaluation of pathogen specific protein biomarkers and antigen-specific cytokine transcriptional profiles and/or protein arrays, in conjunction with bacterial and Interferon Gamma Release Assays (IGRA), offer a promising new avenue for early detection of bTB. We anticipate this new multipronged approach to lead to a more robust and versatile detection method.

Financial Support
Michigan Alliance for Animal Agriculture; U.S. Department of Agriculture, Agriculture and Research Services; Michigan State University

Notes:

P049 - Direct reverse transcription real-time PCR to detect porcine reproductive and respiratory syndrome virus

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Session: Diagnostic testing

Objective
Direct real time reverse transcription polymerase chain reaction (dRT-rtPCR) is emerging as a time-saving and cost-effective alternative to customary extraction-based RT-rtPCR. A dRT-rtPCR assay would be invaluable to high-throughput diagnostic laboratories, increasing testing faculties and lowering cost of consumables. Thus, a pilot study was conducted to determine if a model virus, Porcine Reproductive and Respiratory Syndrome Virus (PRRSV), could be tested via dRT-rtPCR.

Methods
The effect of heat treatment (HT) at 65º, 80º, 95º C for 5 minutes, vs. no HT, was assessed with PRRSV positive isolates (cell culture, n=8) and clinical specimens (processing fluids, oral fluids, and serum; n=15). To combat congealing, clinical samples were tested undiluted, 1:2, 1:3, or 1:4 in phosphate-buffered saline (PBS) with HT alone or HT supplemented with proteinase K (PK, 2.0 mg/mL) or N-Acetyl Cysteine (NAC, 1.0mM). Subsequently, PK (0.5 mg/mL, 1.0 mg/mL, or 2.0 mg/mL) and sample volume (3 µL, 5 µL, or 8 µL) were evaluated to establish optimum reagent concentration and template volume. To enhance detection, five PCR enhancing additives were individually incorporated: Triton X-100 (1.0%), Sodium dodecyl sulfate (SDS, 0.05%)/Tween-20 (2.0%), Tween-20 (2.0%), Bovine Serum Albumin (BSA, 0.4 mg/mL), or PK (0.5 mg/mL). All treatments were compared to magnetic bead-based extraction.

Results
65ºC and 80ºC HT significantly reduced PRRSV detection in dRT-rtPCR compared to standard RT-rtPCR ($p<0.0001$ and $p<0.05$, respectively). No difference was observed for 95ºC. Congealing was eliminated in processing fluids and serum following 1:2 dilution in PBS and HT with PK. 5uL and 8uL of template hindered PRRSV detection ($p<0.001$), however, PK dose did not have a measurable effect. Of the PCR enhancing additives tested, PK was statistically indistinguishable from extracted cohorts.

Conclusions
This study supports that sample type, dilution and volume, HT, and PCR enhancing additives are crucial for successful detection of PRRSV through dRT-rtPCR. However, further validation using additional specimen types and pathogens is required.

Notes:

P050 - Application of DNA aptamers against *M. tubercuosis* Complex-specific biomarkers for bovine tuberculosis detecion

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Session: Diagnostic testing

Objective
Bovine tuberculosis (TB) is one of the devastating diseases in the dairy industry globally. It is caused by *Mycobacterium bovis*. There is an urgent need for the development of rapid and early diagnostic tools for bovine TB in different animal species for its control and prevention. Our lab has recently validated pathogen-specific biomarkers (PSBs) during *M. bovis* infection in cattle, deer, and primates. Based on the successful identification of PBSs, discovering the DNA aptamers for these proteins using Systematic evolution of ligands by exponential enrichment (SELEX) will be highly beneficial for early diagnosis of TB in animal species. To enable facile testing in the field, we aim to develop a rapid test using DNA aptamers against two most common PSBs viz. MB2515C and pks5.

Methods
Systematic evolution of ligands by exponential enrichment (SELEX) is applied to select the aptamers against two mycobacterial specific proteins viz. MB2515C (a LuxR family transcription regulator) and pks5. The redundant aptamer sequences against these two proteins are identified and further characterized by gel shift assay and DNase I footprint assay followed by testing of sera from naturally infected and control animals (cattle, deer, elephants, and primates).

Results
We identified one redundant aptamer sequence against MB2515C and two sequences against pks5 after sixth round of SELEX. We will further characterize these aptamer candidates by electrophoretic mobility shift assay (EMSA) and DNase I footprint assay and finally validate them using naturally infected and healthy animals.

Conclusions
The identification, characterization and validation of pathogen specific DNA aptamers will help in the development of aptamer-based diagnostics for early detection of *M. bovis* and eventually help in the control of bovine TB in animals and humans.

Financial Support
U.S. Department of Agriculture

Notes:

P051 - Small but mighty: validation of a low-throughput nucleic acid extraction platform for NAHLN FAD diagnostic testing

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Session: Diagnostic testing

Objective
The Wisconsin Veterinary Diagnostic Laboratory (WVDL) is a NAHLN member supporting testing for the foreign animal disease (FAD) investigation program in animals. Testing of many viruses, such as Seneca Valley A virus (SVA) and Influenza A viruses (IAVs), are low-throughput but require rapid turnaround time. Current validated NAHLN workflows employ 96 well format high-throughput automatic nucleic acid platforms, or obsolete models of low-throughput platforms. Low-throughput automatic nucleic acid platforms allow for reduction of time and plastic consumables. Given this, and the competition for diagnostic testing resources during the SARS-CoV-2 pandemic, the need for validated alternative platforms for low-throughput nucleic acid extractions is clear.

Methods
WVDL will validate the the KingFisher (KF) Duo Prime, a low-throughput automatic nucleic acid platform for routine testing. The KF Duo Prime system allows for 1-12 samples per run. We completed side-by side extractions on these two platforms on a given set of samples and compared to the existing gold standard method of the KingFisher Flex 96 well automatic nucleic acid platform.

Results
After completing the side-by-side extractions of standard curve dilutions with high and low dilutions represented, as well as known positive and negative samples, we have determined the analytical sensitivity, analytical precision and Kappa coefficient of the equipment using NAHLN approved PCR assays. Overall, this study provides data to support the comparable performance of KF Duo Prime to the gold standard KF Flex 96 well platform.

Conclusions
After testing validation, WDVL will recommend the KF Duo Prime system for NAHLN consideration as an appropriate platform for daily FAD investigations to save member laboratories time and resources when completing low-throughput detection testing. The KF Duo Prime platform provides quality total nucleic acids that are suitable both for use in testing for other diseases, and by smaller laboratories with a low number of daily testing samples.

Financial Support
U.S. Centers for Disease Control and Prevention; U.S. Department of Agriculture, National Institute of Food and Agriculture; National Animal Health Laboratory Network; U.S. Department of Agriculture-Animal and Plant Health Inspection Service; Association of Public Health Laboratories

Notes:

P052 - Method comparison of VMRD 3B ELISA and Prionics PrioCHECK 3ABC ELISA for serosurveillance of FMD in the US

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Session: Diagnostic testing

Objective
Foot-and-mouth disease (FMD) poses an economic threat to the livestock industry in the United States. It is important to test for FMD with a competitive enzyme-linked immunosorbent assay (cELISA) that detects antibodies against non-structural proteins of FMDV due to potential vaccine use. The current study demonstrates a comparison between the PrioCHECK® ELISA assay and VMRD 3B assay.

Methods
Diverse serum sample sets from bovine, porcine and other cloven-hoofed animals were used to evaluate the analytical specificity and sensitivity, diagnostic specificity and sensitivity, and differentiation of infected from vaccinated animal (DIVA). VIAA (Virus Infection Associated Antigen) AGID was used as the reference test. The VMRD 3B ELISA Kit was used according to the manufacturer's instructions. The Prionics PrioCHECK® ELISA Kit was used according to the manufacturer's instructions.

Results
The analytical specificity results demonstrated 100% analytical specificity in both the VMRD and PrioCHECK® ELISA assays are specific for FMDV and do not cross react with sera collected from animals infected or immunized with other porcine and bovine viruses. The analytical sensitivity for the VMRD assay was two to four-fold higher and could detect antibodies specific for FMDV at lower concentrations and at earlier time points than the PrioCHECK assay. The diagnostic specificity of PrioCHECK® assay (99.7%) was higher compared to the VMRD assay (98.2%) using the kit insert cutoff values. The diagnostic sensitivity for the VMRD assay (99.8%) was higher compared to PrioCHECK® assay (98.9%). Both VMRD and PrioCheck® assays have DIVA capability based on the evaluation using limited sera from bovine and porcine vaccinated with NSP-purified inactivated FMDVs.

Conclusions
The VMRD 3B competitive ELISA kit (1.5-hour) demonstrated relatively higher analytical and diagnostic sensitivity, high analytical and diagnostic specificity with DIVA capability when compared to the currently used PrioCHECK® ELISA (24-hour) assay. The VMRD 3B ELISA assay is acceptable as a validated assay for serosurveillance of FMD in the United States.

Notes:

P053 - Evaluation of a multiplex real-time RT-PCR assay for the detection of African and classical swine fever viruses

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Session: Diagnostic testing

Objective
The detection of African swine fever virus (ASFV) and the clinically indistinguishable classical swine fever virus (CSFV) is of great importance in disease control. Virus specific singleplex real-time PCR assays currently used for surveillance in the United States were optimized for multiplex detection.

Methods
An ASFV/CSFV multiplex real-time RT-PCR assay was assessed for both analytical and diagnostic performance characteristics on two real-time PCR platforms. Optimization of the ASFV and CSFV assays included modification of ASFV primers, a change in CSFV reporter dye, and a change in CSFV primers and probe concentration.

Results
The analytical sensitivity between the multiplex and singleplex real-time PCR assays were comparable for two of three CSFV strains tested, with the observed difference when testing a Costa Rican strain. The analytical sensitivity differences between three ASFV strains were within one log or less of each (singleplex greater sensitivity in two testing instances, multiplex greater sensitivity in two testing instances). Intra- and inter-assay variability difference between singleplex and multiplex assays was determined based on standard deviation estimates, which were lower than 0.81 for both assays demonstrating low variability in results (ranges: CSF 0.18 - 0.35, ASF <0.01 - 0.81). There was 100% agreement between the multiplex and singleplex assays in ASFV diagnostic sensitivity testing using positive field samples (n=47: whole blood n=18, spleen n=17, and tonsil n=12). CSFV positive field sample testing (n=47: whole blood n=1, spleen n=5, and tonsil n=41) resulted in agreement between both assays, except for one tonsil with a >37 Ct value when detected. One hundred percent agreement between both assays was observed in diagnostic specificity testing.

Conclusions
These results support that the multiplexed real-time RT-PCR assay, including an exogenous internal control, is suitable for detection and differentiation of ASFV and CSFV for surveillance of disease-free zones and control in endemic regions.

Notes:

P054 - Injecting *F. necrophorum* into the circulation of pre-weaned calves failed as liver abscess challenge models

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Session: Disease Pathogenesis

Objective
The objective of this study was to develop and test experimental liver abscess induction models in pre-ruminant dairy calves through inoculation into the hepatic portal system or the peripheral circulation.

Methods
The study was divided into two phases, the hepatic portal circulation and the peripheral circulation model. In the hepatic portal system model, 20 calves were divided into four groups: CONPV, FUSOPV6, FUSOPV8, and FUSOPV10, receiving intraportal infusion of saline, 10^6, 10^8, and 10^{10} CFU of *F. necrophorum*, respectively, via ultrasound-guided percutaneous catheterization of the portal vein. In the peripheral circulation model, 18 calves were divided into four groups: CONIV, FUSOIV7, FUSOIV9, and FUSOIV11, receiving intrajugular infusion of saline, 10^7, 10^9, and 10^{11} CFU of *F. necrophorum*, respectively. During both phases, calves were ultrasounded daily to monitor progression of liver abscesses. Blood samples were collected on days 0,1,3,5,7, and 14 for hematology. Calves were euthanized 14 days after inoculation and examined for gross liver abscesses.

Results
None of the calves from either challenge model developed liver abscesses. However, there were significant changes in blood cell counts throughout both the challenges. During the peripheral circulation challenge, monocyte counts were greater for FUSOIV11 calves than CONIV, FUSOIV7, and FUSOIV9 on days 3 and 5 post-challenge ($P<0.01$). The neutrophil to lymphocyte ratio was greater for FUSOIV11 than CONIV ($P=0.03$) and FUSOIV9 ($P=0.04$) on day 7 post-challenge. During the hepatic portal circulation model, animals in the FUSOPV8 group had greater monocyte counts than CONPV ($P=0.04$) and FUSOPV6 ($P=0.04$). Additionally, FUSOPV6 calves had greater neutrophil counts than CONPV ($P=0.01$), FUSOPV6 ($P=0.04$) and FUSOPV10 ($P=0.02$) 5 days post-challenge.

Conclusions
Despite previous reporting, portal vein or intrajugular inoculation of *F. necrophorum* produced liver abscesses after 14 days. However, there were significant changes in blood cell counts that are associated with chronic or sub-acute infections.

Financial Support
Foundation for Food and Agricultural Research; Cactus Feeders

Notes:

P056 - Unrevealing the immune and metabolic changes associated with metritis in dairy cows

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Session: Disease Pathogenesis

Objective
The objective was to investigate the immune and metabolic changes associated with metritis in dairy cows

Methods
Cows with a red-brownish, watery, fetid vaginal discharge were diagnosed with metritis (MET). Holstein cows (n=128) had blood collected at -14, 0, 3, and 7 d relative to parturition (DRP) to characterize the phenotype and activation status of leukocytes. Flow cytometry was used to evaluate the number and proportion of blood leukocytes, and the median fluorescence intensity (MFI), and proportion of extracellular markers of cell adhesion and activation. Total cells, live cells (LiveDeadTM-), single cells, monocytes (CD172α+/CD14+), polymorphonuclears (PMN; CD172α+/CD14-/SSChigh), B-cells (CD21+/MHCII+), CD4+ T-cells (CD4+), CD8+ T-cells (CD8+), and γδ T-cells (γδTCR+) were evaluated. MHCII, CD62L, and CD11b were used as markers of cell activation. Prepartum (PP) body weight (ppBW), and prepartum body weight change (BWC) were evaluated. Plasma fatty acids (FA) were measured at -14, and 0 DRP. Data were analyzed by ANOVA for repeated measures.

Results
MET cows had greater ppBW, lost more weight PP, and had greater plasma FA at 0 DRP. During PP and at calving, MET cows showed lesser live cells. Overall, MET cows showed greater B-cell activation. Postpartum, MET cows showed lesser PMN counts with greater activation. Contrarily, MET cows showed greater monocyte counts with lesser antigen presentation and activation. Additionally, MET cows showed lesser postpartum T-cell activation.

Conclusions
We conclude that the greater PP adiposity in MET cows leads to systemic inflammation, denoted by greater B-cell activation, greater FA mobilization, metabolic stress, and greater cell damage. Chronic inflammation may also lead to postpartum immune tolerance, which is characterized by greater PMN activation and recruitment, but decreased monocytes and T-cells activation and recruitment. This dysfunctional immune response may lead to failure to prevent bacterial infection and metritis development.

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

Notes:

P057 - Influenza D virus pathogenesis and immune responses in calves previously exposed to bovine viral diarrhea virus

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Session: Disease Pathogenesis

Objective
Bovine Viral Diarrhea Virus (BVDV) infection leads to thymus atrophy and possibly long-term immune impairment. This study evaluates the Influenza D Virus (IDV) pathogenesis and immune responses in calves pre-exposed to BVDV.

Methods
BVDV-IDV-naïve calves were allocated into 4 groups of 5 animals. On day 0, animals in G1 and G3 were mock inoculated, while animals in G2 and G4 were inoculated with BVDV2. Necropsy was on day 13 for animals in G1 and G2. On day 21, animals in G3 and G4 were inoculated intranasally with IDV and necropsied on day 42. Nasal swabs and serum samples were collected for RT-qPCR and serology. Serum antibody levels were evaluated by virus neutralization (VN) and hemagglutination inhibition (HI). The thymus mass was evaluated relative to the kidneys' average mass. Lymphocyte proliferation and intracellular cytokine staining assays evaluated the cellular immune responses to BVDV and IDV in peripheral blood mononuclear cells (PBMC).

Results
Animals pre-exposed to BVDV shed IDV for an extended period and with increased virus concentration. The thymus mass in the BVDV acute infected animals (G2) was about 50% reduced compared to controls (G1). Notably, on day 42, 2 out of 5 calves in G4 (BVDV+IDV) demonstrated thymus depletion (about 60%), whereas 3 calves had apparently normal thymus mass. Alpha/beta T-cells, and especially CD8 T cells, exhibited lower proliferation to IDV recall stimulation in calves exposed to BVDV. Likewise, decreased T-cell proliferation and percentage of IFN-γ producing cells to IDV stimulation were noticeable in animals with persistent thymus atrophy. Conversely, antibody levels demonstrated no significant difference between groups.

Conclusions
BVDV infection may have prolonged suppressive effects on T-cells even after viral clearance, decreasing host responses to the subsequent IDV infection. BVDV-inducted thymus depletion may vary from transient to persistent. Persistent thymus atrophy was correlated with weak IFN-γ response and T-cell proliferation. Results suggest a correlation between persistent thymus atrophy and impaired T-cell immune response.

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

Notes:

P058 - Managing bovine leukemia virus by integrating surveillance of youngstock and whole herd scans

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Session: Disease Pathogenesis

Objective
Previous research indicates that bovine leukosis, caused by Bovine Leukemia Virus (BLV), primarily affects older cows. However, recent data suggest a portion of animals are already infected with BLV when entering their first lactation. This discovery prompted our study's aim to follow animals throughout life, from youngstock to producing cows, to determine when early-life BLV infections may be occurring.

Methods
Youngstock on five different Michigan dairy farms were enrolled in the study. Blood samples will be collected at three stages of life, (1) following birth (0-8 days of age), (2) before breeding protocols have been administered (11-13 months of age), and (3) after breeding (15-17 months of age). All samples will be assayed for BLV antibodies and BLV provirus. In addition to following youngstock, this study will investigate the effect that early-life infections have on the BLV prevalence in the milking herd of each farm. Whole milking herd BLV prevalence will be determined on each farm at the beginning, middle, and end of the study to assess prevalence as the youngstock enter the milking herds.

Results
Currently, blood samples from 254 calves between 0-8 days of age have been collected, of which, 45% had BLV antibodies and 1% had detectable levels of BLV provirus. In addition, BLV milking herd prevalence across the five farms had a mean of 31.8 ± 13.2%.

Conclusions
The detected BLV antibodies in youngstock at 0-8 days of age are passive antibodies and are inconclusive of a prior or current BLV infection. Little research has been conducted on the potential role that passive BLV antibodies possess. However, 1% of the animals had detectable levels of provirus, indicating BLV infection is possible in youngstock. Future samples collected at 11-13 months and 15-17 months of age will provide information of the timing of early-life infections. Determining when animals are becoming infected, and sharing the results with producers, will aid in prevention of future BLV infections.

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

Notes:

P059 - Impact of COVID-19 pandemic on the veterinary activities to control foot and mouth d*isease* in Armenia

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Session: Epidemiology

Objective
The main objective of control of transboundary diseases in Armenia is to create buffer zones to prevent entrance of exotic viral and bacterial diseases from neighboring countries. Implementation of systematic mass vaccination has helped to stabilize the situation for some diseases, like foot and mouth disease (FMD). However, regular monitoring is important, as circulating strains are subject to antigenic drift and globalization makes preventive measures and vaccines ineffective. This study aimed to compare serosurveillance results from 2019, 2020 and 2021 to understand if COVID-19 impacted the FMD situation in Armenia.

Methods
To estimate the effectiveness of vaccination campaigns and to determine the real situation of FMDV in the country we collected blood from a random selection (calculated by WinEpi software) of large (LR) and small ruminants (SR) across Armenia. A total of 3078 (2019), 3509 (2020) and 4384 (2021) LR and SR were tested. A Solid phase competitive ELISA was used to detect the level of antibodies to nonstructural proteins (NSP-Ab) of FMD in susceptible animals and the level of structural proteins (SP-Ab) in vaccinated animals.

Results
The NSP-Ab prevalence in cattle was higher in 2020 (3.6%) compared to 2.5% in 2019 and 3.5% in 2021. The same was true for SR: 2.8% (2019), 4.2% (2020) and 3.1% (2021). SP-Ab results for herd immunity showed a 24-42% increase from 2019 to 2021 with a decrease in 2020.

Conclusions
The NSP-Ab prevalence level was similar from 2019 to 2021 suggesting a stable FMD epidemic situation. The higher NSP-Ab in 2020 could be pandemic related as there were restrictions of activities on some control measures, and changes in the movement of animals and survey sampling. FMDV has circulated in country without evident clinical signs due to the annual vaccinations that are carried out. The decrease in SP-Ab may also be related to COVID-19 as restrictions in Armenia resulted in more passive visits with farmers and organization of vaccination. This information will be valuable to address in future pandemics to minimize the impact on infectious diseases in animals.

Notes:

P060 - *Leptospira* seroprevalence in companion animals in Tennessee

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Session: Epidemiology

Objective
Leptospirosis is a re-emerging zoonotic disease in humans and animals. It is estimated that there are more than 1 million new human cases worldwide per year, with almost 60,000 deaths. Leptospirosis can be life threatening in dogs and horses, and is becoming recognized as an emerging disease in cats. The purpose of this study was to investigate the *Leptospira* seroprevalence in companion animals in Tennessee.

Methods
We collected convenient serum samples from dogs, cats, and horses submitted to the UTCVM diagnostic laboratory. The samples were tested for leptospirosis by Microscopic Agglutination Test (MAT) against twelve *Leptospira* serovars.

Results
The overall *Leptospira* seroprevalence was 29.52% (111/376), 11.85% (16/135), and 47.73% (42/88) in dogs, cats, and horses, respectively. Highest seroprevalence in dogs was against serovar autumnalis (74.77%). In cats, the highest seroprevalence was against serovar bratislava (56.25%). In horses, the highest seroprevalence was also against serovar bratislava (43.18%). The titers ranged from 1:50 to 1:1600 in canines with the highest titers being against serovars autumnalis and bratislava. The titers ranged from 1:50 to 1:3200 in felines, with the highest titer being against the serovar hardjo. The titers ranged from 1:50 to 1:3200 in horses, with the highest titers also being against serovar hardjo. Evaluation of clinical urine samples submitted for diagnostic testing identified seven positive samples in this year. Our preliminary analysis by conventional PCR and sequencing of samples presumptively identified *Leptospira kirschneri* as the infecting species in dogs.

Conclusions
Our study concludes that these animals are commonly exposed to *Leptospira* and leptospirosis is not an uncommon disease.

Notes:

P061 - A field survey of larval development habitats of *Culicoides* midges in the Western US

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Session: Epidemiology

Objective
Culicoides midges are vectors of bluetongue virus (BTV), an arbovirus affecting wild and domestic ruminants. BTV distribution generally overlaps with vector range, so understanding the ecology of the vector is crucial for accurate predictions of virus distribution. *Culicoides* rely on moist substrate for oviposition and development, but an in-depth understanding of characteristics of suitable development habitat is lacking. In the northwestern US, the primary vector, *C. sonorensis*, is classically associated with domestic wastewater ponds, though there is some evidence that other substrates may also be important. We conducted a broad survey of immature habitat for *Culicoides* midges to better classify where vectors can develop.

Methods
We surveyed potential development habitat at 11 field sites in Northern Colorado: two dairies, two sheep farms, two research operations, two range sites, two natural spaces, and one equestrian site. At each site, 150 g of substrate was collected from the top few centimeters and the edges of soil-water interface at available moist microhabitats (e.g., natural ponds, puddles, wastewater ponds etc.). Samples were transported to the lab, kept at 82.4 °F on a 12 hr light-dark cycle, and monitored for adult emergence for 12 weeks.

Results
Emergence was observed from diverse microhabitats, including natural ponds, streambeds, and swampy substrate. Emergence occurred throughout the season at lower density and non-domestic sites, with high emergence on rangeland sites as well as on a bison refuge. Emergence from wastewater at high-density domestic sites began later than at more natural sites, with no consistent emergence until late July, when numbers increased sharply.

Conclusions
Naturally occurring development sites may play a more important role in maintaining vector populations than previously thought, particularly early in the transmission season, as adult *Culicoides* emergence occurred later on high-density domestic operations. Knowledge of these breeding grounds can help to understand how seasonal transmission is maintained in both wild and domestic landscapes.

Financial Support
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Notes:

P062 - Molecular epidemiological analysis of *Salmonella* Schwarzengrund in Japan

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Session: Epidemiology

Objective
Salmonella is recognized worldwide as an important foodborne and human zoonotic pathogen. In recent years, *S.* Schwarzengrund become the predominant serotype in human salmonellosis in Japan. Therefore, the purpose of this study was to explore the molecular epidemiological characteristics of *S.* Schwarzengrund isolates in this region.

Methods
A total of 29 *S.* Schwarzengrund isolates from chicken (3), chicken meat (3), chicken meat products (20) and human patient (3) were subjected to Multi-locus sequence typing (MLST) and pulsed-field gel electrophoresis (PFGE) analysis. Seven housekeeping genes (*aroC, dnaN, hemD, hisD, purE, sucA* and *thrA*) were amplified and sequenced for MLST analysis. The sequence data were submitted to the MLST database for the determination of allele and sequence type (ST) assignments. PFGE analysis was performed with *Xba* I digestion following the guideline of PulseNet International from CDC. The PFGE profiles were scanned and analyzed to clarify the diversity of those isolates.

Results
In MLST analysis, 29 *S.* Schwarzengrund isolates were typed into three groups, with ST241 being the most prevalent genotype (25 isolates), followed by ST96 (3 isolates) and untypable (1 isolate). All isolates from chicken meat or chicken meat products in 2020-2021 were typed into ST241, and those isolated from chicken before 2000 were typed into ST96. In PFGE analysis, two different PFGE patterns were obtained from 29 *S.* Schwarzengrund isolates. All isolates typed ST241 by MLST analysis were assigned to pattern 1, and all isolates typed ST96 were assigned to pattern 2.

Conclusions
These results indicate that ST241 is now the major genotype while ST96, which is now globally prevalent, was distributed among *S.* Schwarzengrund in Japan in the past. The MLST and PFGE analysis appeared to have comparable strain typing resolution, at least for this serotype.

Financial Support
Ministry of Health, Labour, and Welfare of Japan

Notes:

P064 - Q fever infection surveillance in Georgia

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Session: Epidemiology

Objective
Q fever is a disease caused by the bacteria *Coxiella burnetii*. *C. burnetii* is spread mainly by breathing contaminated air or eating or drinking contaminated food. Farm workers, especially those who work with animals, people who work in slaughterhouses and veterinarians are especially vulnerable to this disease. Other forms of transmission are rare but include tick bites and human to human transmission.
Coxiella burnetii (the agent of Q fever), are Category B Select Agents. Since 1930's, there have been sporadic reports of Q fever cases and outbreaks in Georgia. The overall goal of this study is One Health approach to study Q fever infections among humans and animals; The main objectives of this proposed study is to determine identity, distribution, and prevalence of diseases among domestic and peri-domestic animals.

Methods
The samples have been collected from different regions of the country from large and small ruminants. The sample size has been calculated by the epidemiology tool Win-Epi. http://www.winepi.net/uk/index.htm. The following samples have been collected: blood, serum, milk and swab for enzyme-linked immunosorbent assay (ELISA), Immunofluorescence Assays (IFA) and polymerase chain reaction (PCR) test. Totally, 1788 Samples were collected (1405 small ruminants, 383 large ruminants).1629 serum samples were tested by ELISA and IFA and 224 were positive. 159 milk and swab samples were tested on PCR and all has been negative.

Results
Based on testing results we propose that diseases are spread out in the country without any clinical signs. It means that the country should start active surveillance program to decrease cases of Q fever.

Conclusions
Based on testing results we propose that diseases are spread out in the country without any clinical signs. It means that the country should start active surveillance program to decrease cases of Q fever.

Notes:

P065 - Use of targeted NGS for pathogen surveillance in dogs from indigenous communities in Brazil

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Session: Epidemiology

Objective
Pathogen surveillance has been performed with next-generation sequencing (NGS) metagenomics because of its potential to detect any pathogen in a sample; however, it lacks the sensitivity needed for effective diagnostic use. Targeted NGS is the selective capture or amplification of nucleic acids of interest in a sample prior to sequencing, and if targeting pathogens, it can be used for clinical diagnostics because of the sensitivity and specificity of this method rival that of real-time PCR. Given the ability to include thousands of primers in a targeted method, it can be expanded for detecting pathogens for multiple syndromes or pathogens from multiple animal species, making it also ideal for surveillance.

Methods
We developed a targeted NGS panel for detecting 75 canine and feline pathogens using AmpliSeq custom-designed primers, automated library prep, and an Ion GeneStudio S5.

Results
This panel detected vector-borne pathogens from nucleic acids extracted from whole blood samples from 149 dogs from nine Indigenous communities in southeast and southern Brazil. This test method detected nine different vector-borne disease pathogens in these dogs. Co-infection with multiple vector-borne agents was standard in this population. Though vector-borne diseases were of specific interest, we were able to detect five additional unrelated pathogens of clinical significance (including canine parvovirus type 2b and canine distemper virus) because of the comprehensiveness of the panel, and sequences obtained from the samples with this method confirmed the results.

Conclusions
Targeted NGS is an effective method for surveillance use for canine populations with unknown infectious disease history.

Notes:

P067 - Detection of antibodies against *Ornithodoros moubata* saliva antigen in pigs slaughtered in central Uganda

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Session: Epidemiology

Objective
African swine fever (ASF) is an important disease of pigs in sub-Saharan Africa and is threatening the pig population and the agricultural economy of other continents. ASF virus is a DNA virus in the family *Asfarviridae* and can be transmitted from wild suis to domestic pigs through soft ticks of the *Ornithodoros* species. The role of *Ornithodoros moubata* in the transmission of the African swine fever virus to domestic pigs has been inadequately studied in Uganda. The objective of this study is to detect the presence of antibodies against *O. moubata* saliva antigen in pigs slaughtered in the Kampala metropolitan area to provide an understanding of their exposure to this vector.

Methods
A total of 1328 serum samples were collected from May 2021 through June 2022 from market pigs slaughtered at Wambizi, Lusanja, Budo, Katabi, Buwate, and Kyetume pig slaughterhouses. A stratified sampling with sample numbers weighted by annual slaughter numbers was used with systematic sampling of the pigs. Sera were tested for the presence of antibodies against *O. moubata* using an indirect rtTSGP1 ELISA developed at IRNASA, CSIC in Madrid, Spain and interpretation of results followed their guidelines. Summary statistics (frequency and proportion) of pigs with an exposure to the *O. moubata* tick was calculated across ASFV status and geographic origins, along with confidence intervals.

Results
To date, sera from 420 pigs was tested, and 44.5%, 36.4%,12.9% and 6.2% have negligible, medium, high, and very high probability of *O. moubata* presence among these domestic pigs, respectively. Further results on the probability of exposure based on a pig's ASFV status and geographic origin will be presented as well.

Conclusions
There is evidence of exposure to the *O. moubata* in domestic pigs sampled at peri-Kampala slaughterhouses, with 19.1% suggesting a high or very high presence. Upon completion of this analyses, it will be possible to discuss the relationship of tick present to the ASFV status of the pigs and their geographic origins.

Financial Support
U.S. Defense Threat Reduction Agency

Notes:

P068 - The pig supply chain in African swine fever hotspots in Uganda

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Session: Epidemiology

Objective
Uganda is a major pork-producing country in East Africa, but African swine fever virus has had a catastrophic impact on the pig industry in Uganda. ASFV is the cause of a highly fatal, hemorrhagic disease of pigs that contributes to poverty, malnourishment, and food insecurity thereby limiting the development of the industry. The study intends to describe the pig supply chain of live swine across and through districts that are impacted by African swine fever.

Methods
The survey was conducted among pig farmers using a semi-structured questionnaire in districts identified as ASF hotspots. Responses were recorded using Kobo software and will be triangulated using an observation checklist and subject matter expert survey. The farmer questionnaire was translated from English to two local languages, namely; Luganda and Lusoga, and all the three languages were used to collect data. Districts affected by ASFV were identified based on pathologic signs of pigs sampled from peri-Kampala slaughterhouses as part of a larger project. The identified hotspots include: Wakiso, Masaka, Mpigi, Luwero, and Kamuli districts.

Results
Ninety nine (99) farmer questionnaires were successfully administered in the five ASF hotspot districts from June to July 2022. Pigs (sows, boars, weaned) are purchased locally and across district lines and sold to traders, butchers, and or other farmers. Further diagrams and discussions of pig movements for each district and pig type will be presented.

Conclusions
Pigs are mainly sourced and sold within the ASF hotspot districts, maintaining a local cycle of infection, which may limit the spread of ASF than if movement was widespread. Movement to other districts does pose a disease threat if pigs are infected. We will further discuss the overall value chain and challenges to control based on the complete evaluation of the data.

Financial Support
U.S. Department of Defense

Notes:

P069 - Geographic and temporal distribution of African swine fever virus in slaughterhouse samples from Kampala, Uganda

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Session: Epidemiology

Objective
African swine fever virus (ASFV) is a contagious disease of domestic and wild swine. The disease can have very high morbidity and mortality rates that cause enormous economic losses and severely impacts food security and trade. Previous studies have looked at ASFV prevalence in Uganda but none has been recently published with monthly sampling for a calendar year. This study sought to document the year-round distribution of ASFV in swine slaughtered around Kampala

Methods
A total of 1313 pigs were systematically sampled from six slaughterhouses (Wambizi, Lusanja, Budo, Buwate, Entebbe and Mukono) around Kampala following a stratified sampling plan, from May 2021 through June 2022. DNA was extracted from sampled blood using Qiagen DNeasy kits and amplified using a real-time PCR assay previously described by Zsak et al. (2005). Prevalence rates by slaughterhouse, month, season, and region were compared using an omnibus chi-squared test at a significance level of 0.05

Results
The annual ASFV prevalence was 18.5%, with the highest rates in June, July and December 43.8%, 24.7%, and 22.6% respectively. April, March, and August had the lowest prevalence of 6.0%, 6.5%, and 6.7% respectively. Budo (31.9%) and Wambizi (19.2%) slaughterhouses had the highest prevalence while Buwate (10.8%) and Mukono (13.6%) had the lowest. Samples collected in the dry season had a higher prevalence (24.3%) than the rainy season (14.3%). Prevalence by region was such that the Northern had the lowest rate (11.8%), then the Eastern (17.3%), Central (18.9%) and the Western (35.0%). There was a statistically significant association between ASFV prevalence and month ($p<0.001$), season ($p<0.001$), and slaughterhouse ($p<0.001$)

Conclusions
ASFV is regularly detected in pigs slaughtered in urban and peri-urban Kampala throughout the year, with higher positivity rates observed during the dry season. An evaluation of regions where pigs originated from reveals that slaughtered pigs were obtained from across the country, and all regions had ASFV positive pigs in every month of the year

Financial Support
U.S. Defense Threat Reduction Agency

Notes:

P070 - Detection of african swine fever virus antibodies in pigs slaughtered from abattoirs in central Uganda

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Session: Epidemiology

Objective
African swine fever virus (ASFV) is a DNA arbovirus in the family *Asfarviridae* a highly fatal and hemorrhagic disease of pigs with mortality approaching 80-100%, although severity differs by genotype. ASFV constrains the pig industry, causing poverty and food insecurity. A surveillance system that includes antibody detection is needed to detect areas of recent infection. This study analyzed serum from pigs slaughtered at abattoirs in central Uganda to ascertain the seroprevalence, which informs on strain virulence and the need for active surveillance.

Methods
There were 1327 pigs systematically sampled based on a stratified sampling method at six abattoirs around Kampala from May 2021 to June 2022. Blood samples were collected and clinical and pathological signs for each pig were recorded. The Ingenasa ASF indirect ELISA was used to detect antibodies against ASFV in serum. Seroprevalence and 95% confidence intervals were calculated.

Results
There were 4 (0.3%, 95% CI: 0.09%, 0.8%) pigs with antibodies against ASFV. One pig had skin hemorrhages around the ears, legs and flanks, a markedly enlarged and darkened spleen, as well as enlarged and diffusely hemorrhagic gastro-hepatic and renal lymph nodes. The other three pigs were emaciated. Confirmatory test results using an immunoblot assay are pending.

Conclusions
There was a very low ASFV seroprevalence among pigs slaughtered at the six abattoirs around Kampala between 2021 and 2022. This indicates that very few pigs were infected with ASFV and survived, although confirmation is needed of these positive results. The low seroprevalence may suggest that ASFV strains circulating in Ugandan pigs are virulent, killing the pigs before the humoral immune response is mounted, or that most pigs are sold off at first indication of disease in an area. This selloff would limit the opportunity for antibody development and survival.

Financial Support
U.S. Defense Threat Reduction Agency

Notes:

P072 - Confounding in epidemiological studies of residential exposure to animal feeding operations and human health

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Session: Epidemiology

Objective
Multiple studies have been conducted to determine the role of AFOs in the health of nearby communities. Efforts to synthesize the available evidence have not reached a conclusion because the studies on this topic are primarily observational, making results more susceptible to systematic bias and mixed findings. Our aim was to systematically review primary studies to comprehensively assess the impact of confounding on the body of work.

Methods
A systematic review was conducted to identify relevant observational studies on this topic. Exposure-outcome effect sizes were extracted. We evaluated if the authors intended to make causal inferences as indicated by employing multivariable models. We then assessed the reported a priori approach for variable selection such as a directed acyclic graph (DAGs) and the use of variable retention approaches in models such as a change in estimation. Second, for studies that evaluated lower respiratory disease we compared the causal structure proposed by the authors with a DAG of the association between bronchitis and AFO exposure proposed by the Environmental Protection Agency (EPA).

Results
7 case-control, 15 cross-sectional and 11 cohort studies were identified. None of the studies identified justified the choice of a set of variables as confounders, none used DAGs to illustrate a causal structure, multivariate methods were the unique used to control confounding and the lack of discussion for the selection of confounding variables could lead to the presence of overadjustment, residual confusion, unnecessary adjustment, and collinearity. Many studies controlled variables that would not be considered necessary and mediator variables based on the EPA DAG.

Conclusions
Confounding is a major factor that can prevent drawing causal conclusions about an observed association. Authors should justify the rationale for the selection and identification of confounding factors using tools such as DAGs. Such an approach would enable end users to properly assess the causal thinking behind the model and assess this approach properly.

Financial Support
National Pork Board

Notes:

P073 - Can cross-sectional studies estimate the incidence of health events? Analysis in a systematic review

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Session: Epidemiology

Objective
The ability to estimate measures of effect representing causal parameters depends upon the study design and the validity of assumptions about the underlying population studied. Randomized controlled trials are the ideal design to study the occurrence of new cases but observational studies such as population-based cohort studies are also an option. Conditional on meeting structural assumptions about the population, the effect measure reported in cross-sectional studies might also be interpreted as incidence density ratio (IDR). An area where cross-sectional studies are often employed is in assessing the association between living near animal feeding operations (AFOs) and community member health. Our goal was to evaluate the effect measure reported by authors of cross-sectional studies on this topic and to assess the ability of this group of studies to potentially report causal parameters.

Methods
We identified cross-sectional observational studies as part of a systematic review conducted to determine the effect AFOs on nearby communities. Exposure-outcome effect sizes were extracted, and thereafter we evaluated if the authors discussed the assumptions about the underlying population. In parallel, we evaluated the assumptions to establish our opinion of the interpretation of the reported effect size.

Results
Fifteen studies were identified, from which 153 effect sizes were extracted. For 44% of the effect sizes extracted, the effect measure obtained by the authors potentially could have been interpreted as IDR. No author group discussed the population assumptions required to make causal inferences.

Conclusions
Studies reporting the impact of AFOs on nearby communities have not been discussing important epidemiological assumptions necessary to interpret the measure of effect as IDR. Given the important percentage of exposure-outcome effect sizes that might be interpreted as providing estimates of IDR, authors should discuss the assumptions and help readers understand their study's contribution to a causal relationship in the body of work.

Financial Support
National Pork Board

Notes:

P074 - Comparison of prevalence of shiga toxin and enteropathogenic *E.coli* in lambs born at different seasons of the year

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Session: Epidemiology

Objective
Shiga toxigenic *E.coli* (STEC) and enteropathogenic *E.coli* (EPEC) are important human and animal pathogens. The shedding patterns of these bacteria are known to be affected by many factors, including stress, the season of the year, among others. Instituting management protocols that reduce the prevalence and risk of transmission of these pathogens requires an understanding of the shedding patterns in host animals. The objective of this study was to compare the shedding patterns of these *E.coli* pathotypes in growing lambs born at different seasons of the year.

Methods
Two groups of lambs born March/April and July/August respectively were enrolled in a longitudinal study at two weeks of birth. Fecal samples were collected from each lamb at two weeks of age, day of weaning, one and seven days after weaning. Subsequently lambs were sampled at one month, two months and six months after weaning. Fecal samples were enriched in tryptose soy broth and total DNA extracted from each. Detection of the STEC and EPEC was determined molecularly by using primers targeting four primary *E.coli* genes (Shiga toxins 1 and 2, intimin and hemolysin). Prevalence of the genes among lambs was done by comparison of proportions using Chi-test.

Results
Both STEC and EPEC were detected in lambs except at the day of weaning when no EPEC were detected. Proportion of lambs testing positive for at least one *E.coli* virulence gene was higher in lambs born in July/August compared to March/April at seven days and one month after weaning. For both groups, the highest number of lambs testing positive for at least one virulence gene was detected one week after weaning. Proportion of lambs positive for *stx2, eae, hly* genes at 2 weeks of age, *eae and hly* genes at one week and, *stx1, stx2, eae and hly* genes at one month after weaning was significantly in July/August lambs than March/April born lambs.

Conclusions
The results indicate shedding of pathogenic *E.coli* in lambs may be affected by the season of year among other factors. More studies to unravel specific factors within the seasons that affect shedding are needed.

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

Notes:

P075 - *Salmonella* Kentucky ST152 and ST198 lineages are metabolically distinct

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Session: Epidemiology

Objective
Salmonella Kentucky is an emerging foodborne pathogen comprised of two major genetic lineages, ST198 and ST152. ST152 is prevalent in US poultry and sporadically associated with the human disease while ST198 is globally prevalent in and commonly associated with human disease. Here, we hypothesized that ST198 and ST152 lineages have evolved to become metabolically distinct. The objective of this study was to identify specific metabolic differences between *S.* Kentucky ST152 and ST198 lineages.

Methods
Metabolic differences between the 7 *S.* Kentucky strains (ST198 n=3; ST152 n=4) isolated from US poultry and human sources were identified using the Biolog™ Phenotype Microarray (PM, Biolog, USA). Strains were tested at avian physiologic temperature (42C) using a total of ten, 96-well PM plates constituting eight metabolic panels (PM1 to PM8), one osmotic/ionic response panel (PM9), and one pH response panel (PM10). To identify metabolic differences for a total of 950 metabolites, the mean respiratory activity (RA) units difference threshold of >50 with a $P \leq 0.01$ (Student t-test) for any metabolite or condition was considered as a significant difference between ST152 and St198.

Results
The RA of ST198 strains was significantly higher in the presence of 38 (4%) out of 950 metabolic conditions tested which included 28 carbon and nitrogen sources and 10 osmotic and pH response conditions. In contrast, the RA of ST152 strains was significantly higher in the presence of 6 (0.63%) osmotic and pH response conditions. ST198 strains utilized several carbon and nitrogen sources more efficiently when compared with the ST152 strains. Moreover, ST198 strains showed higher RA in the presence of high osmolarity and low pH conditions.

Conclusions
ST198 strains are metabolically distinct and more efficient than ST152. It is likely that ST198 strains are metabolically better adapted to the human host, thus more commonly associated with infection. The metabolic phenotypes may also serve as an epidemiologic indicator for differential detection of ST198 and ST152 lineages.

Notes:

P076 - Effects of body condition score at dry-off on postpartum production and health

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Session: General health and physiology

Objective
Dramatic changes in body condition score (BCS) during the first 3 weeks of lactation has been demonstrated to lead to depressed immune function and reproductive health. BCS loss during the early postpartal period are associated with increased incidence of metabolic disease, depressed immune function, and poor reproductive outcomes, therefore our goal was to determine the effects of BCS on postpartum health, reproduction and production.

Methods
Beginning 75 days prior to dry-off multiparous cows blocked by lactation and BCS and were randomly assigned to one of two diets, low-energy (LE; NE_L=1.57 Mcal/kg; n=44) and high-energy (HE; NE_L=1.82 Mcal/kg; n=45) until dry-off at which point all cows were placed on a common dry-cow diet, and after parturition, a common lactating cow diet. Milk production and feed intake were recorded daily. Milk, feed samples, BCS and backfat ultrasound analysis were analyzed three times weekly. Blood samples collected from -21 through 21 DIM and will be analyzed for multiple biomarkers. Immune function analysis will be measured by neutrophil oxidative burst and phagocytosis on days 0, 2, and 7 postpartum. Effects of BCS at dry-off on reproduction will be measured by endometrial cytobrush at 50 DIM and assessment of embryo quality on 67 DIM. In conclusion, our data will reveal the effects of BCS at dry-off on the metabolism, immune function, reproductive outcomes, and production of cows in their subsequent lactations.

Results
At dry-off HE cows had a BCS of 3.72 compared to 3.28 in LE cows ($P<0.001$). Postpartum, dry matter intake was increased in LE cows from 2-40 days in milk (DIM) compared to HE cows ($P < 0.05$). Daily milk production was also increased in LE cows compared to HE cows from 7-42 DIM ($P = 0.05$). Analysis of other samples are currently underway.

Conclusions
In conclusion, our data will reveal the effects of BCS at dry-off on the metabolism, immune function, reproductive outcomes, and production of cows in their subsequent lactations. Management of BCS during the late-lactation period may be critical for future health of lactating animals.

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

Notes:

P077 - Survey of bulk tank milk quality, udder health and hygiene on organic dairies in Vermont by facility type

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Session: General health and physiology

Objective
A cross-sectional observational study on organic dairies had the objective of identifying whether bulk tank milk quality, udder health and hygiene outcomes were associated with facility type, and whether bedded pack systems are a viable option for winter housing in VT. We aimed to collect bulk tank milk samples, udder hygiene scores, and complete a survey on mastitis risk and bedding management on 40 farms, in order to compare the two most common winter housing systems in the state (freestalls, tiestalls) with those using a bedded pack.

Methods
The survey was completed on 21 farms (5 bedded packs, 6 freestalls, 10 tiestalls) before interruption due to the pandemic. DHIA information captured included avg. linear score (LS; unweighted and weighted by production), % cows with any intramammary infection (IMI; LS ≥4.0), % cows with new IMI (LS <4.0 to ≥4.0), and % cows with chronic IMI (≥4.0 last 2 tests).

Results
There were no significant differences between bulk tank udder health measures, culture data, and hygiene scores between facility types. As sample sizes were limited, a multivariable model to describe outcomes by facility type was abandoned in favor of univariate linear regression to identify associations between management factors and outcomes for all farms combined. Farms with deeper bedding showed a tendency ($p \leq 0.20$) toward a lower bulk tank SCC, lower % new IMI, lower % any IMI, lower weighted and unweighted average LS, and improved hygiene metrics. Farms with lower mean udder hygiene scores tended towards having lower % chronic IMI, lower % any IMI, and lower weighted and unweighted average LS. Increased bedding depth measures tended to be associated with improved udder hygiene metrics.

Conclusions
Although statistical power was limited, the current study provided insight on factors affecting bulk tank milk quality, udder health and hygiene measures on organic dairy farms in Vermont. Additionally, outcomes for bedded packs were comparable to more commonly used winter housing systems, and are therefore a viable option for pasture-based herds interested in a loose-housing system in VT.

Financial Support
U.S. Department of Agriculture

Notes:

P078 - Effect of altering pre- and postpartum protein supply on intake and skeletal muscle of dairy cows

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Objective
The rationale of this study was to determine the effect of increasing the metabolizable protein (MP) supply during the pre- and post-partum period on dry matter intake (DMI) and measurements of muscle mass change. We hypothesized that increasing MP supply would alter feed intake and conservation of lean muscle mass in the peripartum period of dairy cows.

Methods
Close-up diets fed 28 d before expected calving were formulated to contain either low (L) or high (H) protein (87 vs 114 g of MP/kg of dry matter (DM)), respectively, to target an estimated 1,188 and 1,560 g daily MP intake. At calving, fresh diets fed until 21 DIM were formulated to contain either low (L) or high (H) MP (107 and 133 g of MP/kg DM), respectively, to target an estimated 2,152 or 2,684 g daily MP intake. Multiparous Holstein cows (n=96) were randomly assigned to 1 of 4 treatments of a combination of these pre- and postpartum diets: 1) low-low (LL), 2) low-high (LH), 3) high-low (HL), or 4) high-high (HH). Daily DMI was recorded from -28 to 42 d relative to calving and the longissimus dorsi muscle diameter (LDM) was measured by ultrasound at -28, -14, -7, 7, 21, and 40 d relative to calving. Data were analyzed as mixed effects repeated measures (PROC MIXED, SAS v. 9.4) with Bonferroni adjustment of multiple pairwise comparisons.

Results
Cows in the H close-up diet had greater DMI (14.0 vs. 13.5 kg/d; P=0.05). Postpartum DMI was greater in LH compared to LL in wk 1 to 3 (21.7 vs. 20.1 kg/d; P=0.03) and greater than LL (24.2 vs. 22.5 kg/d; P=0.02) and HL (24.2 vs. 22.6 kg/d; P=0.03) in wk 1 to 6, respectively. A treatment by time interaction was identified for postpartum LDM such that all treatments decreased (P <0.01) in diameter from 7 to 21 and 21 to 40 DIM, except in LH diameter was maintained between 21 and 40 DIM (P >0.99).

Conclusions
These data suggest that feeding LH results in greater postpartum DMI and minimizes the decrease in muscle mass at 40 DIM. Strategic use of higher MP diets in the peripartum period may therefore be beneficial to mitigate the metabolic stress of the transition period.

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

Notes:

P079 - The association of prepartum rumination time and subclinical hypocalcemia in the first 3 days postpartum

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Session: General health and physiology

Objective
A common management practice to address subclinical hypocalcemia (SCH) is to blanket treat cows with calcium (Ca) boluses at calving, including cows that would otherwise be normocalcemic after calving and those that would naturally adjust to the Ca demands of their new lactation. Changes in prepartum behaviors have the potential to be used as early indicators of cows at risk of SCH development after calving and to improve management techniques for SCH. Our main objective is to look at the difference in the rate of change in total daily rumination time from -3 days prepartum to calving (TDRΔ) for cows that had SCH at D0 or D3 relative to calving.

Methods
A case-control study was conducted on 50 Holstein dairy cows, enrolled 3 weeks prior to the expected calving date and followed until 21 days in milk (DIM). Blood samples were collected at D0 and D3 relative to calving from the coccygeal vessels to measure the total blood plasma Ca concentration. Data for total daily rumination was recorded continuously using an ear-tag accelerometer and reported as the total number of min/d spent ruminating. Dry matter intake (DMI) and milk yield data was obtained from farm records. Data was analyzed using a linear model with SCH status (i.e., normocalcemic cows, SCH-, or cows with SCH, SCH+) as a fixed effect and baseline rumination, baseline DMI, breed, lactation, and milk yield and DMI in the first 6 DIM were offered to the model as potential confounders. The outcome, TDRΔ, was calculated based on the slope of change from -3 to 0 days relative to calving.

Results
The TDRΔ was not significantly different between SCH+ cows (-33 min/d, 95%CI: -44.8, -21.5) and SCH- cows (-42 min/d, 95%CI: -64.8, -20.0) at D0 (P = 0.46). Similarly, no differences were found in the TDRΔ when comparing SCH+ cows (-33 mind/d, 95%CI: -43.9, -22.7) and SCH- cows (-34 mind/d, 95%CI: -48.4, -19.3) at D3 (P = 0.75)

Conclusions
The TDRΔ was not different for cows with different SCH status at D0 or at D3. Our results suggest that the rate of change in prepartum TDR might not be an effective estimator of SCH postpartum

Notes:

P080 - Survey of breeding ewe management practices and udder health in California sheep

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Session: General health and physiology

Objective
Information is lacking on the prevalence, risk factors, and economical impact of mastitis in ewes in California. California is the second largest sheep producing state in the United States and mastitis is a health and welfare concern for breeding ewes and nursing lambs. Recent reports of the sheep industry indicated mastitis as a top reason for culling breeding ewes, antibiotic use on operations, and as a herd disease of high economic impact.

Methods
An online survey was constructed in Qualtrics XM which consisted of 48 questions pertaining herd demographics, breeding, pregnancy, and lambing management, udder conformation considerations, mastitis management, and lamb care. Fifty-seven completed surveys were obtained from April to July 2022. Descriptive statistical analysis was performed in Excel; Chi square tests and Multiple Correspondence Analysis (MCA) were performed in R.

Results
Most respondents were from the Northern (37%, 21/57) and Sierra regions (26%, 15/57) of California. Herd size varied with 51% (29/57) of respondents having herds <100 sheep and 49% (28/57) having herds with ≥100 sheep. Few operations tested for Ovine Progressive Pneumonia (14%, 8/57). Most respondents (55%, 31/57) indicated that they use udder conformation as a selection criterion for keeping ewes or choosing replacements. Most respondents (80%, 45/56) reported having ≤5% of ewes with udder abnormalities per lactation. Mastitis treatment included injectable antibiotics (44/57; 77%) most commonly, followed by intramammary (23/57; 40%) or oral (2/57; 4%) antimicrobials. The MCA included 11 variables and suggested that ≤5% of orphan lambs, more intensive management practices (<500 sheep, moving ewes prior to lambing, use of lambing jugs, and early weaning), and treatment of mastitis with antibiotics were associated with <5% of ewe udder abnormalities per lactation.

Conclusions
Our results help characterize demographics, management practices, and producer reported udder health abnormalities in California ewes. These results indicate risk factors that should be examined in future research on mastitis in ewes.

Notes:

P081 - Neonatal immune function and epigenetic modification following in-utero infection with bovine viral diarrhea virus

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Session: Immunology

Objective
In pregnant, susceptible cattle exposed to bovine viral diarrhea virus (BVDV), fetal infection is common. Neonatal calves infected in-utero suffer from decreased growth rates and dysfunctional immune responses increasing their susceptibility to calf hood infections such as pneumonia. The immune mechanisms of increased neonatal morbidity and decreased productive efficiency following in-utero viral exposure is unknown. The overall objective is to identify the functional and molecular immune cell profiles along with epigenetic mechanisms associated with in-utero viral infection. The central hypothesis is that fetal infection with BVDV will result in a dysfunctional innate and adaptive immune response in the neonatal period.

Methods
Hypothesis testing will be achieved through two aims, (1) determining immune cell function and describing transcription and (2) epigenetic markers of genes critical for mediating inflammatory response. Research methodology will be performed on calves born to pregnant heifers allocated to the following groups: 1) Control group (n=8): pregnant heifers receiving sham inoculation at gestational day 75 and 200, 2) Persistent Infection (PI) group (n=8): pregnant heifers inoculated with BVDV at gestational day 75, 3) Transient Infection (TI) group (n=8): pregnant heifers inoculated with BVDV at gestation day 200.

Results
We expect to identify significant differences in immune response observed as differential gene expression between treatment groups within different immune cell types. A subset of these differences will be directly associated with epigenetic changes thus revealing loci that are associated with in utero BVDV infection.

Conclusions
Gaining knowledge in this area will lead to a better understanding of the causes of immune dysfunction following fetal BVDV infection. Understanding these mechanisms will allow development of strategies to prevent neonatal morbidity and mortality helping to maintain healthy agricultural animals to ensure a safe and adequate food supply.

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

Notes:

P082 - Characterization and effects of bacterial modified live vaccination on natural killer cell populations in cattle

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Session: Immunology

Objective
Cell Mediated Immunity (CMI) is an important component of the immune response associated with protection against *Brucella abortus* and *Mycobacterium bovis* infection, the causative agents of bovine brucellosis and bovine tuberculosis (bTB), respectively. *B. abortus* strain RB51 is the commercially available vaccine for use in cattle against brucellosis, and it has been shown to induce IFN-γ-mediated $CD4^+$ T cell responses. Similarly, the bacillus Calmette-Guerin (BCG) vaccine, used in humans against tuberculosis, is also known for inducing IFN-γ-mediated $CD4^+$ T cells responses. Despite our understanding of the effects of these vaccines on the adaptive immune response, less is known regarding their interaction with and effects on innate immunity. Natural killer (NK) cells are known to produce IFN-γ in response to viral infections, but less in known regarding their role in response to bacteria. Therefore, we sought to understand the effects of RB51 and BCG vaccination on NK cells in cattle.

Methods
Peripheral Blood Mononuclear Cells (PBMC) from cattle vaccinated with either RB51, BCG or both RB51 and BCG, were utilized to evaluate circulating NK cell frequency, NK cell subsets, and NK cell IFN-γ production in response to various stimulation conditions via flow cytometry.

Results
Our data demonstrated some unique characteristics of NK cells in response to vaccination with bacterial modified live vaccines. While both vaccines elicit a similar and predicted CD4+ T cell response, the NK response appreciated for each vaccine antigen was unique and distinct for both antigens and vaccines. The data may suggest that the NK response may be more antigen driven than antigen specific.

Conclusions
This study shed light on the bovine NK cell response following vaccination with two bacterial modified live vaccines, RB51 and BCG.

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

Notes:

P083 - Intramammary liposome-TLR agonist dose titration: effect on differential somatic cell count

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Session: Immunology

Objective
Antibiotics (AB) are commonly used for the treatment of mastitis in dairy farms contributing to the emergence of AB resistant bacteria. Therefore, identifying alternative treatments capable of upregulating the immune system will contribute to the control mastitis and enhance AB stewardship on dairy farms. Thus our objective with this pilot project is to determine the lowest effective dose of a liposome-TLR agonist (LTC) immune stimulant that induces a significant increase in somatic cell count (SCC) and differential somatic cell count (DSCC), and not induce clinical signs of udder inflammation.

Methods
Eight mid-lactation (120 ± 3 days in milk) dairy cows, without clinical mastitis and SCC <200,000 cells/mL will be assigned to two 4x4 Latin Squares with experimental periods of 7d separated by a 28-day interval. On the first day of each experiment period, prior to treatment infusion, a milk sample from each quarter will be collected. After sample collection, the left hind quarter will receive the administration of treatment. Cows in the control group will receive 10 mL of LTC diluent IMM using a syringe and teat cannula, cows in high dose group will receive 10 mL of a solution containing 0.25 mL of LTC, cows in the medium dose will receive a solution containing 0.1 mL of LTC, and cows in the low dose will receive a solution containing 0.05 mL of LTC. In addition to the milk sample collected prior to treatment administration, samples will be collected aseptically every 12h relative to treatment administration during the following 7 days for the determination of SCC and DSCC.

Results
With this experiment we expect to define what is the lower LTC dose capable of inducing an increase in the mammary gland immune response without inducing clinical signs of udder inflammation.

Conclusions
The results of this experiment will be essential for the development of future experiments investigating the effects of intramammary LTC administration on prevention of mastitis.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture (2021-67015-34558)

Notes:

P084 - Development of a gene expression assay for intestinal mucosal immunity in hybrid striped bass

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Session: Immunology

Objective
The aquaculture industry is constantly challenged by pathogenic microorganisms infecting food-production fish, like the hybrid striped bass (*Morone saxatilis* x *M. chrysops*). The intestinal mucosal is a significant barrier defending the fish against pathogens, yet this organ's immune function is understudied. Immune proteins, like cytokines, in the intestinal mucosal, are indicators to measure immune response in fish, such as pro-inflammatory tumor necrosis factor (TNFα), interleukin 1 beta (IL1β), and anti-inflammatory transforming growth factor-β (TGFβ). To maximize survival, we are developing an updated molecular assay to determine the expression of mRNAs encoding immune-related proteins in the intestinal mucosal. This assay will serve as a tool to measure the expression of immune response genes in the fish gut and provide a better understanding of intestinal mucosal immunity.

Methods
Ribonucleic acid (RNA) was extracted from the hybrid striped bass intestinal mucosal layer. We tested primers for IL-1β, TNF-α, TGF-β, and the reference gene elongation factor alpha for striped bass. Gene expression was measured with quantitative real-time PCR, utilizing the quantification cycle (Cq) values to compare mRNA from experimental hybrid striped bass in future nutrition and husbandry studies.

Results
The sequences for these primers are as follows - Forward EF1α: CTTGACGGACACGTTCTTGA; Reverse EF1α: GTGGAGACCGGTGTCCTGAA; Forward IL1β: CAGACTGGCTTTGTCCACTG; Reverse IL1β: AGTCCTGCTGATTTGGATCTACC; Forward TNFα -AACGATGGTGAAGAGGAAAG; Reverse TNFα: CCTATGGAGTCTGAGTAGCG; Forward TGFβ: ATGGTTAAGAAAAAGCGCATTGAA; Reverse TGFβ: TCCGGCTCAGGCTCTTTG. The relative Cq displays that the glycine diet upregulated IL1β and TNFα, while TGFβ was downregulated.

Conclusions
This data will be helpful for future nutrition studies indicating the expression of mRNAs for genes involved in innate and adaptive immune responses in the intestinal mucosa. This knowledge will also help to fill up the deficient knowledge on the immune function of the fish gut epithelial cells.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture (2021-67015-34)

Notes:

P086 - RNA- Sequencing characterization of differentially expressed genes in *Mycobacterium paratuberculosis fur* mutant under iron stress

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Session: Immunology

Objective
Mycobacterium avium subsp. *paratuberculosis* (MAP) causes Johne's disease in cattle that is associated with significant impact on agricultural economy and animal health. Unlike other mycobacteria, MAP has special nutrient requirements. For optimal growth in laboratory media, MAP requires supplementation of a siderophore (mycobactin), and takes eight to sixteen weeks to produce colonies in culture, a major hurdle in timely diagnosis. MAP carries a fur like gene on its genome which is absent from other mycobacteria. Ferric Uptake Regulator (Fur) is well studied in other bacteria and has been shown to be involved in global regulation of iron homeostasis. However, very little is known about this novel regulator in MAP. As a key virulence determinant, iron regulation in MAP and its role in pathogen survival and infection are important areas of research that would lead to advances in ability to improve in-vitro culturing methods. Here, we hypothesize that MAP3773c controls a subset of genes and pathways required for optimal environmental iron sensing and in-vivo survival. Thus, understanding intra-mycobacterial iron homeostasis and extracellular iron sensing mechanisms are expected to provide strong scientific foundations to improve diagnostics and define its in-vivo survival mechanisms.

Methods
A wildtype strain (K-10) and an in-frame MAP3773c deletion mutant derived from K-10 were exposed to iron restricted conditions for 5, 35, 65, and 95 minutes. MAP3773c deletion mutant was created successfully through homologous recombination. Total RNA was extracted, and quality and integrity of RNA was determined before RNA-Seq was performed.

Results
Data analysis showed 40 genes to be differentially regulated between wildtype and mutant over different time points under iron stress. These genes are involved in cell wall synthesis, respiration, metabolism and transcriptional regulation.

Conclusions
We were able to show different expression patterns at different time post iron restriction between wild type and mutant strain. The findings will be validated in other iron restricted replicates using qRT-PCR.

Financial Support
U.S. Department of Agriculture

Notes:

P087 - Identification and modulation of oxidative stress in dairy cows for a successful transition into lactation

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Session: Immunology

Objective
A key factor responsible for the development of transition cow health disorders is oxidative stress (OS). A major obstacle in mitigating the detrimental impact of OS on transition cow health is the lack of an accurate way to assess the amount of oxidative injury that is associated with subsequent diseases. In human medicine, plasma isoprostane (isoP) concentrations are considered the gold standard for assessing OS associated with disease risk. In veterinary medicine, however, we lack critical thresholds for OS biomarkers. Therefore, the *central hypothesis* of this project is that plasma isoP during late gestation are an accurate biomarker to predict clinical disease in early lactation.

Methods
We will investigate the diagnostic ability of novel biomarkers of OS, their role in disease pathogenesis, and nutritional-based strategies to reduce disease occurrence during the transition period. In order to investigate how isoP contribute to early lactation diseases, a prospective cohort study will be conducted to establish cow-level critical thresholds for prepartum plasma isoP concentrations that can be used to predict periparturient diseases. Subsequently, we will investigate the bioactivity of selected isoP on immune responses of cattle. For this, we will perform in vitro dose-response studies to assess the effect of isoP on cell survival and functionality under conditions of OS using bovine endothelial cells, macrophages, and neutrophils as our targets. Lastly, we will evaluate, first in vitro and then in vivo, the effectiveness of various micronutrient supplementation strategies on modulating isoP production and its effects on immune cell function and disease risk.

Results
This is a new award and no results have been generated to date.

Conclusions
The results from this proposal will be used to improve the health status of dairy cows during the critical stage of transition from gestation to lactation, leading to a reduction in dairy cow morbidity through targeted nutritional manipulations.

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

Notes:

P088 - The WC1 multigenic array in the immune response to zoonotic pathogens in agricultural species

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Session: Immunology

Objective
Gamma delta T cells are a crucial component of the immune response to a number of increasingly relevant and largely zoonotic pathogens to which efficacious vaccination is lacking. In ruminants and swine, gamma delta T cells represent a major population of peripheral blood and epithelial tissue-resident lymphocytes. Upon activation, gamma delta T cells illicit a variety of effector functions and play an indispensable role of orchestrating the downstream immune response. These characteristics make gamma delta T cells a promising candidate for recruitment by vaccination. WC1 is expressed as a multigenic array on gamma delta T cells in ruminants. In cattle there are 13 unique WC1 genes (WC1-1 to WC1-13), each comprised of 6-11 SRCR domains that selectively bind unprocessed antigen in a manner that resembles a pattern recognition receptor (PRRs). WC1 functions as a hybrid PRR and co-receptor for the gamma delta TCR. We hypothesized that a swine WC1 multigenic array has co-evolved with pathogens and thus sought to characterize its diversity and ligand-binding potential.

Methods
We used 5' and 3' RACE and RT-PCR to isolate full-length cDNA clones, and the MAKER annotation pipeline to annotate *Sscrofa11.1.* Binding pull-down assays were carried out with recombinant WC1 SRCR protein and fixed bacteria.

Results
We isolated cDNA and genomic evidence for a porcine WC1 multigenic array consisting of 9 genes (WC1-1 to WC1-9), each encoding 6 SRCR domains with unique pathogen binding potential. We annotated *Sscrofa11.1* for sequence derived from full-length cDNA transcripts representing the 9 porcine WC1 genes. We mapped 7 of the 9 genes, leaving two (WC1-4 and WC1-8) unplaced in the current assembly. We defined three subpopulations of porcine gamma delta T cells based on expression of WC1 and CD2, and characterized WC1 antibody reactivity. Finally, we confirmed that porcine WC1 SRCR domains are capable of directly binding whole fixed bacteria including *Leptospira spp* and *Mycobacterium bovis*.

Conclusions
Porcine WC1 exists as a multigenic array, is expressed on gamma delta T cells and binds to pathogens.

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

Notes:

P089 - Swine immune reagents development for understating the immune responses and for biomedical research

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Session: Immunology

Objective
The Objective of the USDA-NIFA Swine Immune Toolkit Initiative has been to generate priority immune reagents based on inputs from veterinary immunology researchers worldwide, and pipeline them for marketing.

Methods
We express soluble proteins in yeast systems and then produce panels of monoclonal antibodies (mAbs) using collaborations with commercial partners for protein expression and mAb production.

Results
We generated several panels of mAbs reactive to porcine IL-6, IL-13, IFN-γ, IL-17A, IL-28B, CXCL10, and BAFF and screened their reactivity in multiple immune assays. Reactivity tests of labeled α-IL-6 and α-IL-13 mAbs for intracellular staining of porcine immune cells using flow cytometry assays are in progress. Our results have confirmed the reactivity of porcine IL-17A, IFN-γ and CXCL10 mAbs. A sensitive sandwich ELISA is now available for IL-17A, IL-13 and CXCL10; other targets are being screened for best mAb pairs for ELISA. We developed IL-5 and IL-21 mAbs and determined their reactivity with orthologous proteins and further characterization is in progress. For each target, our goal is to provide the veterinary community with new commercial reagents and standardized assay techniques for their research efforts.

Conclusions
Tools and reagents generated by this project will undoubtedly advance our understanding of swine immune responses to disease, vaccine and biomedical research efforts.

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

Notes:

P090 - Characterization of anti-porcine CXCL10 monoclonal antibodies

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Session: Immunology

Objective
C-X-C motif chemokine ligand 10 (CXCL10) facilitates chemoattraction of important immune cells to tissues. As part of the USDA-NIFA Swine Immune Toolkit Initiative, our goal is to provide the veterinary community with new commercial immune reagents and standardized assays for future research efforts.

Methods
For the chemokine target CXCL10, we used yeast expressed, recombinant porcine CXCL10 (rPoCXCL10) to generate a panel of aCXCL10 mAbs. Each of the aPoCXCL10 mAbs was assessed by ELISA using cross-inhibition analyses of biotinylated mAbs, and direct binding to orthologous yeast expressed CXCL10 proteins.

Results
We assigned six distinct epitope groups for the 9 generated aCXCL10 mAbs. Subsequently, we screened AF647-tagged aCXCL10 mAbs for intracellular staining of pig immune cells using different stimulation conditions. Of the 9 mAbs only 2 detected intracellular CXCL10 expression in PMA/ionomycin or rPoIFNg-stimulated porcine cells. Further, cell characterization assays verified CXCL10+ cells as CD3-CD4-CD172+, with occasional CD4+ subsets. Overall, we have determined that aCXCL10-1.4 mAb is the best mAb clone to use in analysis of intracellular signaling to detect interactions that regulate cell migration. A sandwich ELISA was also developed to quantitate CXCL10 protein expression; it verified reactivity with native porcine CXCL10. Immunohistochemistry analysis for binding of these anti-CXCL10 mAbs on formalin fixed pig lymph nodes and spleen tissues is in progress.

Conclusions
CXCL10 mAb will be useful for evaluating swine infectious disease immunity and vaccine responses. New reagents identified by the Swine Immune Toolkit Initiative will undoubtedly advance future swine research efforts.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture, Agriculture and Food Research Initiative grant # 2019-67015-29815

Notes:

P091 - Expression of human interferon lambda-4 in adenovirus and verification of its cross-species activity in mouse

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Session: Immunology

Objective
Interferon-lambda lambda (IFN-λ), classified as type III interferon, is a representative cytokine that plays an important role in innate immunity along with type I interferon. IFN-λ can elicit anti-viral states by inducing peculiar sets of interferon-stimulated genes (ISGs).

Methods
In this study, an adenoviral vector expression system with tetracycline-operator system was used to express human IFN-λ4 in cells and mice. This recombinant adenovirus was amplified in human 293 cells and purified using chromatography-base system.

Results
Host cell DNA and host cell proteins were removed through purification process and purified recombinant adenovirus particles were identified by transmission electron microscopy. Transduction of this recombinant adenovirus could produce human IFN-λ4 and induce ISGs in mouse-derived cells (CMT-93) as well as human-derived cells (A549). It did not affect cell viabilities when determined by using CCK-8 assay. Based on those results, cytotoxicity of human IFN-λ4 and formation of replication-competent adenovirus were excluded. Establishment of antiviral state in BALB/c mouse was assessed following intranasal administration with the recombinant adenovirus. Expressions of OAS1, ISG15, and Mx1 mRNAs and their proteins were identified in the lower respiratory tract.

Conclusions
These results imply that human IFN-λ4 could induce production of ISG proteins in mouse. Therefore, recombinant adenovirus expressing human IFN-λ4 developed in this study could be applied to a mouse viral infection model before clinical application of it to humans.

Financial Support
Korea Health Technology R&D Project, Korea Health Industry Development Institute (KHIDI), Ministry of Health & Welfare, Republic of Korea (grant number: HI21C2566)

Notes:

P092 - NRF2 agonists modulate RSV-induced pro-inflammatory cytokine expression in respiratory tract epithelial cells

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Session: Immunology

Objective
Bovine respiratory disease (BRD) is a highly prevalent disease in the cattle industry that remains a leading cause of morbidity and mortality with BRSV being a major etiological agent. Widespread use of vaccines and antimicrobials has led to drug-resistant pathogens, leading to the proposal of using immunomodulation strategies to reduce BRD severity and prevalence. The NRF2 pathway and one of its key products, itaconate, has a crucial role in the downregulation of inflammatory and the upregulation of antioxidant responses in respiratory viral infection, highlighting its potential as an immunomodulation target. Here we explored the effects of synthetic NRF2 agonists, 4-octyl-itaconate(4-OI) and dimethyl fumarate (DMF), in modulating immune response to bovine and human RSV infection in respiratory tract epithelial cells as a non-antibiotic strategy to prevent RSV infection.

Methods
Bovine turbinate cells (BTs) and human lung epithelial cells (BEAS-2b) were stimulated with either 4-OI (100,200µM) or DMF (50, 100µM), then infected with BRSV (BTs) or hRSV (BEAS-2b), respectively. RNA was isolated from cells at 36h (hRSV) or 72 h (bRSV) post infection, and transcripts of pro-inflammatory cytokines, chemokines and antiviral mediators were determined by RT-PCR.

Results
Our results indicate that DMF and 4-OI treatment inhibits transcription of IL-6, IL-1B, CCL5, IFN-B ($p< 0.0001$) and CXCL8 ($p<0.001$) on BTs cells after BRSV infection at all doses tested. DMF treatment also upregulated transcripts of antioxidant enzyme Nqo1 ($p<0.0001$). In BEAS-2b cells, we observed downregulation of transcripts for TNF and IRF1 ($p<0.0001$) on all DMF and 4-OI treated cells. We also noted downregulation of transcripts for CCL5 ($p<0.001$) and IFN-b ($p<0.01$) on cells treated with 100 µM of DMF.

Conclusions
These results suggest DMF and 4-OI reduce the inflammatory and antiviral response to RSV in both human and bovine respiratory tract epithelial cells, and thus future in vitro and in vivo studies are warranted in order to explore NRF2 agonists as immunomodulators to prevent severe RSV infection and BRD.

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

Notes:

P093 - Characterization of the neutrophil response to SARS-CoV-2 infection in a feline animal model

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Session: Immunology

Objective
Neutrophilia and the release of neutrophil extracellular traps (NETs) correlate with worsened clinical outcomes in SARS-CoV-2 infected people. The delicate balance between beneficial inflammation and neutrophil-derived host tissue damage highlights a significant area of study for therapy. While natural infection is evident in felines with distinct clinical signs, previous studies developed a translational feline model analogous to acute COVID-19 in humans. We hypothesize that excessive neutrophil recruitment and the release of NETs worsen pulmonary damage in the domestic cat.

Methods
Domestic cats (n=12) were inoculated with SARS-CoV-2 (B.1.617.2) or vehicle (n=6). Plasma (0-days post-infection (dpi), 4 dpi, 8 dpi, and 12 dpi) and bronchoalveolar lavage (BAL) samples (4dpi and 12dpi) were collected to evaluate neutrophil recruitment and NET-specific markers. Myeloperoxidase (MPO)-DNA ELISA was utilized to identify MPO-DNA complexes. Immunofluorescence assays (IFA) on lung tissue samples localized histone (H3), MPO, and neutrophil elastase.

Results
Increased NET production is evident via the detection of MPO-DNA complexes in plasma and BAL samples from SARS-CoV-2 infected cats. A significant increase of MPO-DNA complexes was detected at 4 dpi and 8 pi in the plasma of infected when compared to uninfected cats ($p<0.05$). Alterations in NETs detection in infected cats were observed between 0 dpi and 8 dpi, 4 dpi and 12 dpi, and 8 dpi and 12 dpi ($p<0.05$). MPO-DNA complexes were elevated in BAL samples of infected cats at 4 dpi and 12 dpi when compared to the uninfected cats ($p<0.05$). Also, there was a marked representation of all three IFA markers in the lung tissues of infected cats.

Conclusions
These results support continued evaluation of the host neutrophil response during feline SARS-CoV-2 infection as a translational model for COVID-19. Further studies aim to clarify the mechanistic drivers of this neutrophil response, thereby understanding the relationship between innate immune dysfunction and COVID-19 disease progression. This model offers the potential to develop immunomodulatory control strategies for COVID-19.

Financial Support
Oklahoma State University

Notes:

P094 - Enteral *Rhodococcus equi* at birth induces trained immunity and protects foals against intrabronchial challenge

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Session: Immunology

Objective
Rhodococcus equi is an intracellular pathogen that causes severe pneumonia in foals and immunocompromised people. Similar to other neonates, the immature adaptive immune system of foals renders them especially dependent on innate immunity. Our objective was to determine whether enteral live, virulent *R. equi* at birth induces trained immunity and protects foals against intrabronchial challenge at age 28 days.

Methods
Foals were gavaged with saline (controls; n=6) or virulent *R. equi* (principals; n=5) at ages 2 and 4 days, and intrabronchially infected with *R. equi* at age 28 days. Blood was collected at ages 2 and 28 days for isolation of neutrophils and monocytes. RNA-sequencing (RNA-Seq) of neutrophils was performed, and used the differentially-expressed genes (DEG) to selected target genes for chromatin immunoprecipitation (ChIP)-PCR and identification of regions enriched with H3K4me3 or H3K27me3 in neutrophils and monocytes of foals. Data analysis was performed in R with significance set at $P<0.05$ and included Fisher's exact test (clinical data), FastQC and Cutadapt (RNA-Seq library quality), HISAT2 (mapping to equine genome), and DESeq2 (DEG RNA-Seq between groups).

Results
Gavage with *R. equi* at ages 2 and 4 days significantly ($P<0.05$) reduced the incidence of pneumonia (0%; 0/5) compared to controls (67%; 4/6). We observed ~1100 DEG in neutrophils of control and principal foals using RNA-Seq, which included trained immunity-related genes. In both neutrophils and monocytes, we detected regions enriched with H3K4me3 or H3K27me3 on IL-1a, IL-1RN, IL-8, IL-32, and TNF-a, among other genes. ChIP-PCR data analysis is pending, but will be available at the conference.

Conclusions
To our knowledge, this is the first study to demonstrate that enteral administration of live bacteria (more specifically, *R. equi*) to newborn foals induces changes in gene expression of circulating neutrophils and potentially induces epigenetic modifications in neutrophils and monocytes, indicating that trained immunity may be a mechanism protecting foals against pneumonia caused by intrabronchial challenge with *R. equi*.

Financial Support
Grayson Jockey Club Research Foundation; Link Equine Research Endowment and Department of Large Animal Clinical Sciences; Equine Research Award by Boehringer Ingelheim Animal Health

Notes:

P095 - Elucidation of gut signaling pathways involved in cattle enteric antimicrobial defense

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Session: Immunology

Objective
Enterobacteriaceae are one of the leading causes of gastrointestinal diseases as it pertains to foodborne illness in the world. Food animals are often the sources of these contaminants to humans, as is the case with cattle and Enterohemorrhagic *E. coli* (EHEC). Understanding the inherent mechanisms of the cattle gut antimicrobial (AM) defense is key to developing methods for eliminating these contaminants in the antibiotic-free era. We previously demonstrated the link of the mTOR pathway, a central regulator of metabolism and physiology, with the intestine AM peptide activity in chickens. The aim of this study is to elucidate its involvement in cattle enteric AM defense.

Methods
Fresh cattle colonic explants were incubated in appropriate media with and without mTOR activator and inhibitor, respectively, for 6 hours at 37°C and 5% CO_2. Explant supernatants were collected, centrifuged, and stored at -80°C. For the bactericidal assay, supernatants were incubated with a lab *E. coli* isolate (1:1) at 37°C for one hour, and bacterial counts were determined by plating.

Results
Addition of an mTOR inhibitor significantly reduced the level of *E. coli,* whereas addition of an mTOR activator had the opposite effect, increasing bacterial levels beyond that of the no-treatment control. The treatments in the media alone had no effect on the level of *E. coli,* indicating the effect was host mediated.

Conclusions
Overall, our results show that inhibition of mTOR pathway could increase cattle colonic AM defense against *Enterobacteriaceae.* Further investigation of strategies targeting the mTOR pathway may allow for a better way to manage bacterial infection in cattle without conferring antibiotic resistance.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture, Hatch Project IOW0 5700

Notes:

P096 - Development of a co-culture model to evaluate hepatocyte-natural killer cell interaction in neonatal calves

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Session: Immunology

Objective
The liver is a metabolic organ but it also has a role in immune development. The antigens and metabolites processed by the liver can educate a developing immune system, yet little is known about its role in circulating immune cell training. Natural killer (NK) cells are in the center of innate and adaptive immunity, functioning non-specifically but with adaptive-like cytotoxic effector functions. NK cells have important implications for viral and bacterial infections in calves. However, there is disputing evidence regarding NK cell functionality in pre-ruminant immune development. Current approaches evaluate NK-cells with a single-cell perspective which is an informative, yet simplified, approach. The objective of this model is to evaluate neonate NK-cell expression, communication, and hallmark effector functions in a hepatic-microenvironment context. This model can be further modified evaluating other organ-dependent pathways of NK cell education and self-regulation for improved animal health.

Methods
Isolated primary enriched hepatocytes (PEH) were acclimated to a transmembrane-well system before culturing with isolated PBMCs. Before neonatal application, non-parturient cows were used for validation. For this, cow PBMCs were co-cultured with PEH. Cell viability and apoptosis for different media were assessed in replicates for each cow. Subsequently, NK cells were assessed for functionality changes.

Results
We have observed media-dependent cell viability and will complete apoptosis, and gene and surface marker expression analyses. We expect that co-culturing PEH and PBMCs will not induce apoptosis, or significantly change gene and surface marker expression with consistent viability to PBMC-only cultures. NK cell effector functions will be assessed through activation, degranulation, cytokine release, and mediated-cytotoxic killing of infected cells.

Conclusions
Upon completion of development, this co-culture model can be used to elucidate mechanisms of hepatocyte-NK cell communication across disciplines to better understand animal health.

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

Notes:

P097 - Immunological evaluation of peptides derived from the *Babesia bovis* RAP-1 and RRA vaccine candidate antigens

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Session: Immunology

Objective
Bovine babesiosis is an acute tick-borne disease of worldwide impact caused by *Babesia bovis*. This disease can be controlled using pharmacotherapy, ixodicides, and live attenuated vaccines. However, these strategies have several drawbacks engendering demand for alternative approaches. Unfortunately, attempts to develop vaccines based on immunodominant parasite antigens have yet to be successful. Here we evaluated the antigenicity of peptides derived from conserved regions in the subdominant N-terminus region of rhoptry associated protein 1 (RAP-1 NT) and the RAP-1-related antigen (RRA). These two antigens are highly conserved among *Babesia* species, suggesting a functional role for parasite survival, and share a strain and species conserved 14-amino-acid motif in their NT-region. We hypothesize that the region containing the conserved RAP-1/RRA motif is poorly immunogenic compared to previously defined antigenic repeated areas of RAP-1 and, if administrated with the molecular adjuvant flagellin FliC, can be a component of an effective subunit vaccine against acute babesiosis.

Methods
We generated synthetic peptides representing the RAP-1 and RRA conserved 14-mer motifs and a 23-mer peptide representing a repeat in the CT region of RAP-1 and tested their antigenicity in ELISA with sera from *B. bovis* infected and protected cattle.

Results
Serological analysis demonstrated that sera from cattle experimentally infected with *B. bovis* contain antibodies to both, the RRA NT 14-mer peptide and to RAP-1 CT 23-mer peptide. However, there is a 10-fold higher antigen-antibody reaction to the RAP-1 CT peptide, when compared to the 14-mer RRA NT peptide.

Conclusions
Altogether, the results are consistent with the hypothesis that conserved regions in the RRA and RAP-1 proteins contain subdominant epitopes. These regions will be used to test whether these subdominant antigens, when administered with the molecular adjuvant FliC, can elicit protective immune responses against *B. bovis*.

Financial Support
U.S. Department of Agriculture

Notes:

P098 - Comparison of calf morbidity, mortality, and future performance across categories of passive immunity

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Session: Immunology

Objective
Four categories of transfer of passive immunity (TPI) were recently proposed instead of the dichotomous pass/fail classification. However, the risks of preweaned morbidity and mortality and future performance among these TPI categories have not been compared to date. Thus, the objective of this retrospective cohort study was to compare dairy calf morbidity, mortality, growth until weaning, and reproductive efficiency until first calving among the categories of poor (<5.1g/dL total protein), fair (5.1 - 5.7 g/dL), good (5.8 - 6.1 g/dL), and excellent (>6.2 g/dL) TPI.

Methods
Records from 4,336 dairy calves randomly selected for weekly serum total protein determination were analyzed. For each calf, data regarding disease status, growth, and reproductive parameters were obtained from the farm's software database. Associations of TPI categories with disease events (diarrhea and/or pneumonia), reproduction indices (age at first insemination, successful insemination, and calving; and number of inseminations), first lactation milk yield and average daily gain (ADG) at weaning were evaluated by survival analysis and mixed models.

Results
Compared to calves with excellent TPI, calves in the inferior TPI categories showed increased risk of diarrhea. However, the risk of pneumonia differed only between the calves in the poor and excellent TPI groups. The preweaned mortality risk was also higher in calves with poor TPI compared to excellent TPI. However, mortality risks were not statistically different between calves with fair or good TPI and those with excellent TPI. Similarly, calves with poor TPI had lower risks of reaching first insemination, successful insemination, or first calving, respectively. However, there were no differences in ADG, number of inseminations, or first lactation milk production across TPI groups.

Conclusions
Our results confirm the positive effects of optimal TPI in calves' health and post-weaning reproduction. The 4 TPI categories can assist in decreasing the incidence of diseases that occur in the first weeks of life but have more limited impact on future health and performance.

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

Notes:

P099 - Histo-blood group antigen-expressing bacteria can serve as decoy epitopes for rotaviruses of groups A, B and C

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Session: Immunology

Objective
In order to initiate and maintain a productive infection, rotavirus (RV) has to pass through the mucus layer and reach the apical surface of host intestinal epithelial cell (IEC). The major organic part of mucus consists of mucins with glycans including histo-blood group antigens (HBGAs). Several bacteria were shown to express mucin-like molecules (MLMs) including HBGA. While the ability of mucin glycans to protect against RV infection is a well-known fact, the role of MLMs in RV infection is obscure. Our recent study demonstrated that several bacteria (*Escherichia coli G-58, Bifidobacterium adolescentis, Bacteroides thetaiotaomicron, Streptococcus bovis,Clostridium clostridioforme*) expressed HBGAs (HBGA+) while *Lactobacillus brevis* and *Bifidobacterium longum* were HBGA-negative (HBGA). The main goal of this study was to evaluate the protective effects of HBGA+ vs. HBGA- bacteria against RVA, RVB and RVC infection *in vivo*.

Methods
Germ-free piglets were colonized with either HBGA+ or HBGA- bacteria cocktail (10^5CFU/ml each) and infected with 10^6 FFU/ml of either RVA (human RVA, Wa G1P[8] and porcine RVA, OSU G5P[7], RV0084 G9P[13], Gottfried G4P[6]), porcine RVB (Ohio, non-typed) and porcine RVC (Cowden G1P[1]; RV0104 G3P[18]; RV0143 G6P[5]). Rectal swabs were collected daily (for 11 days post-infection, dpi) for RV quantification and diarrhea severity scoring.

Results
Piglets colonized with HBGA+ bacteria had delayed onset of diarrhea, reduced diarrhea duration and cumulative fecal score after inoculation with the most RVs used in this study. Further, these piglets had decreased the 1st (1-3 dpi) (RVA G9P[13] and G5P[7]) or 2nd (5-7 dpi) (RVA G4P[6], RVB and RVC G3P[18]) peak of diarrhea. However, the effect of the colonization on viral shedding was genotype-specific. Colonization with HBGA+ bacteria led to significantly reduced shedding compared to HBGA- group on dpi 1-3 (for G9P[13], G4P[6]and RVC G1P[1] or on dpi 6 (for RVA G1P[8] and G5P[7]).

Conclusions
Our data suggested that HBGA+ bacteria may serve as decoy epitopes for RV binding thus alleviating RV infection and disease.

Financial Support
International Development Research Centre

Notes:

P100 - Short transportation did not impact circulating cortisol levels or leukocyte abundance in cattle

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Session: Immunology

Objective
Cattle are the primary reservoirs of Shiga toxin-producing *E. coli,* a foodborne pathogen, and stress events such as transportation can increase fecal shedding of STEC, presenting an increased contamination risk of the carcass and/or environment. Our objective was to understand the impact of transportation stress on host parameters to help elucidate the interplay between the host mucosal immune system, nervous system, and intestinal microbiome.

Methods
Market weight heifers were maintained on pasture and half (n=12) were loaded onto a trailer and transported for 2 hours (approximately 100 miles) to mimic transportation stress before slaughter. The remaining non-transported animals (n=12) were held in a field barn for a similar amount of time. Blood was collected from transported cows at 0 (before transportation), and all cattle at 0.5, 5, 24, and 72 hours post-transport/mock transport. Serum cortisol levels were measured by ELISA and an 8-color flow cytometry panel was developed to enumerate various whole blood cell populations.

Results
Serum cortisol levels are a common measure of stress; however, no significant differences were detected in transported cattle compared to non-transported controls at any time point measured. Similarly, no differences in circulating granulocytes, neutrophils, gamma delta T-cells, B-cells, alpha beta T-cell, or monocytes were detected. Additionally, mean fluorescence intensity and the number of cells expressing the cell trafficking and homing molecule, L-selection (CD62L), were measured for each population. Expression of CD62L fluctuated slightly over time, but no significant differences between treatment groups were observed.

Conclusions
Serum cortisol levels and alterations in whole blood cell populations may not serve as markers for stress in cattle as a result of transportation. Additional parameters such as serum cytokine levels are being assessed as markers for transportation stress in cattle.

Financial Support
U.S. Department of Agriculture, Agriculture and Research Services CRIS project 5030-32000-225-000D

Notes:

P101 - Expression of short-chain fatty acid receptors and transporters in the porcine gastrointestinal tract

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Session: Immunology

Objective
Short-chain fatty acids (SCFAs) are microbiota-derived metabolites associated with increased intestinal barrier integrity and enhanced mucosal immunity. SCFAs signal via G protein-coupled receptors (GPCRs) expressed on the cell surface and/or inside the cell after transport into the cell via monocarboxylate (MCT) or Na-coupled monocarboxylate transporters (SMCT). Despite many studies investigating the impact of SCFAs on pig health and production, the distribution and localization of SCFA receptors and transporters in the pig gastrointestinal (GI) tract is minimally characterized.

Methods
To identify whether SCFA receptors and transporters are present in the pig GI tract, single-cell RNA sequencing (scRNA-seq) was utilized to recover cell type-specific gene expression from intestinal tissues. Spatial transcriptomics was performed to validate expression patterns within specific regions of the small intestine and RT-qPCR was used to evaluate gene expression of bulk cell fractions.

Results
Under basal conditions, SCFA receptors *FFAR2*, *FFAR3*, and *HCAR2*, which encode for GPR43, GPR41, and GPR109a, respectively, were minimally expressed throughout the porcine GI tract. scRNA-seq revealed *FFAR2* was highly expressed in *NEUROD1*^{hi} enteroendocrine cells. SCFA transporter *SLC16A1* (MCT1) was highly expressed throughout the GI tract. The two SMCTs had different cell type-specific gene expression patterns. *SLC5A8* (SMCT1) was highly expressed in crypt cells while *SLC5A12* (SMCT2) had higher expression in enterocytes. Spatial transcriptomics confirmed the cell type-specific expression patterns, demonstrating localization of *SLC5A8* expression in crypts while *SLC5A12* was predominantly expressed in villi.

Conclusions
In conclusion, SCFA receptors and transporters are expressed throughout the GI tract under basal conditions. Non-antibiotic strategies to improve pig intestinal health include modulation of SCFAs, and a clear understanding of the types of cells and locations throughout the GI tract where SCFA receptors and transporters are present is important for understanding how SCFAs modulate intestinal health.

Financial Support
U.S. Department of Agriculture, Agriculture and Research Services CRIS project 5030-32000-225-000D; U.S. Department of Agriculture, Agriculture and Research Services SCINet project #0500-0093-001-00-D

Notes:

P102 - Isotonic protein solution modulates intestinal barrier integrity and innate immunity in PEDV-infected piglets

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Session: Immunology

Objective
Coronavirus enteric infections pose a great menace to human and animal health. Porcine epidemic diarrhea virus (PEDV) causes contagious disease accompanied by disturbance of intestinal barrier integrity.Isotonic protein solution (IPS) has a unique content and could protect intestinal functions. The study aimed to examine the relative contribution of IPS to support innate immunity and barrier integrity of the intestine in PEDV-infected piglets.

Methods
Twenty 14-day-old piglets were inoculated with PEDV in a dose of 1000 virions per animal. All infected piglets were divided into control (PEDV group) and IPS-exposed (PEDV+IPS) groups. IPS-exposed piglets were euthanized on 5, 14, and 21 PID, and intestine tissue was sampled to assess intestinal morphology, interferon-α (IFN-α), fibronectin, and E-cadherin as the markers of gut barrier function.

Results
All infected pigs showed severe watery diarrhea and/or vomiting and severe atrophic enteritis. The diarrhea symptoms were observed in the PEDV group on the 2nd PID while it starts in PEDV+IPS on 3th PID. PEDV antigens were detected in the intestine tissue by both PCR. The content of E-cadherin in the PEDV group was statistically reduced in comparison to the PEDV+IPS group ($P<0,01$). The content of fibronectin was similarly less in the PEDV group ($P<0,05$). Moreover, there was observed an increase in the intact fibronectin fragmentation in the PEDV group compared with the PEDV+IPS group ($P<0,01$). The upregulation of IFN-α expression was shown in the PEDV+IPS group compared with the PEDV group. Taken together, our results suggest a protective effect of IPS concerning epidemic diarrhea symptoms. The first mechanism of protection may be mediated by the support of adherens junctions and extracellular matrix tightness.

Conclusions
The present results provide evidence that IPS is a prospective feed additive to support intestinal barrier integrity and resistance to PEDV infection in piglets.

Notes:

P103 - Investigating the time of establishment of the uterine microbiome in dairy cattle

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Session: Omics

Objective
The objective of this project is to investigate the time of the establishment of the uterine microbiome in dairy cattle.

Methods
Holstein heifers were euthanized 4.6 ± 2.3 hours after birth (n=14) and at 62.9 ± 1.5 days of age (n=14) via non-penetrating captive bolt and exsanguination. The uterus, vagina, and vulva were aseptically excised and tissue samples were cultured in varied media and atmospheric conditions. Swabs of the uterine serosa were taken as negative controls. Positive cultures were speciated by 16S rRNA gene sequencing. qPCR targeting a universal bacterial 16S rRNA gene sequence was performed to identify and quantify bacterial DNA in the sampled tissues. The V4 hypervariable region of the 16S rRNA gene was amplified by PCR for metagenomic sequencing on the MiSeq platform. Two-way mixed ANOVA was used to compare bacterial copy number differences between the groups.

Results
Bacterial growth did not occur in 27 of 28 cultured uterine samples. Only 1 Day-60 uterine sample and 1 Day-60 vaginal sample were culture-positive for bacterial growth of *Staphylococcus simulans* and *Corynebacterium glutamicum* respectively. All vulvar samples were culture positive from which 316 isolates were identified, predominantly composed of Firmicutes, Fusobacteria, Bacteroidetes, and Actinobacteria. The abundance of bacterial DNA was greater in the vulva (4.35±0.51, $P<0.01$) and vagina (4.21±0.13, $P=0.04$) than in the uterus (3.91±0.33). There were no differences in copy number of 16S rRNA per mg of tissue for any site between Day-0 and Day-60 heifers. PCR products of V4 region for metagenomic sequencing of the uterus were minimal with few visible bands.

Conclusions
Our results demonstrate scant presence of bacterial DNA in the bovine uterus and vagina immediately after birth with low microbial viability at 60 days of age. Species-level metagenomic analyses of the heifer reproductive tract microbiome are necessary to guide the culture conditions used in order to determine bacterial viability with more certainty. Furthermore, the bovine uterine microbiome is not established until after 60 days of life.

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

Notes:

P104 - MAGResp - a database of respiratory metagenome-assembled genomes (MAGs) for cattle and swine

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Session: Omics

Objective
Respiratory health and disease has a major financial impact on commercial swine and cattle production systems. Shotgun metagenomic sequencing can help improve our understanding of the microbial composition of the respiratory tract, and metagenome assembled genomes (MAGs) may allow for more specific taxonomic classification compared to other metagenomic analysis methods. MAGResp will be a database of swine and cattle respiratory MAGs generated from open-source shotgun metagenomic datasets. The goals of this project were to identify candidate datasets for MAG assembly and to generate MAGs for at least one of those datasets.

Methods
Datasets were identified using PubMed with structured keyword search phrases. We excluded studies that did not study swine or cattle, utilize shotgun metagenomic data, sample the respiratory tract, or have publicly available sequence data. Raw datasets were downloaded, filtered for low-quality and host (Bos taurus or Sus scrofa) reads, and MAG assembly was performed using previously described methods [Chen et al. 2021].

Results
The above search terms yielded 32 unique studies, only four of which could be analyzed after applying exclusion criteria. From these, two of the raw datasets were not available for download, and a third was not sequenced to a great enough depth to generate MAGs. The remaining dataset consisted of samples collected from cattle with respiratory disease, and 23 MAGs were generated. Identified taxa included *Mannheimia haemolytica*, *Mycoplasmopsis bovis*, and *Trueperella pyogenes*. Host-associated reads accounted for an average of 98% of reads across all samples, which limited the number of MAGs generated from these data.

Conclusions
While our literature search was probably not comprehensive, it highlights the current lack of studies that utilize shotgun metagenomic data to assess the microbial composition of the swine and cattle respiratory tracts. As a result, there simply aren't enough publically available respiratory shotgun metagenomic datasets to create a comprehensive database of respiratory MAGs for these important livestock species.

Financial Support
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Notes:

P105 - Transcriptomic analysis of peripheral blood mononuclear cells (PBMCs) of horses during equine herpesvirus 1 and 4

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Session: Omics

Objective
Equine Herpesvirus 1 and 4 are the cause of acute respiratory disease in horses around the world. In addition, EHV-1 causes secondary disease manifestations such as abortion in pregnant mares and Equine Herpesvirus Myeloencephalopathy (EHM) in older horses. Particularly EHV-1 but not EHV-4 establishes a cell-associated viremia after respiratory infection in PBMCs. This event promotes viral transfer to the endothelia of secondary sites of infection such as the CNS and pregnant uterus. Despite the difference in pathogenesis between EHV-1 and EHV-4, they share sequence homology of 55% and up to 96%. The aim of this study is to identify differential gene expression between EHV-4 and EHV-1 that will help elucidate viral factors involved in EHM pathogenesis.
We hypothesized that infection of EHV-1 will show greater downregulation of differentially expressed genes in relation to immune regulation than EHV-4 infection.

Methods
We isolated PBMCs of horses infected with EHV-1 and horses infected with EHV-4. We used RNA sequencing to establish a gene expression analysis between groups. Principal component analysis (PCA) and Gene Ontology (GO) were performed to identify gene clusters and key pathways that are up/downregulated during EHV-1/4 infection.

Results
Our preliminary results suggest an overrepresentation of genes involved in host immune modulation during EHV-1 viremia that are not present in EHV-4. Further analyses will show the functional pathways involved.

Conclusions
Our findings contribute to the identification of viral factors that play a role in EHM and contribute to our existing knowledge of PBMC-mediated viremia.

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

Notes:

P106 - Design of an RNA bait set to enrich *M. bovis* genomic DNA in samples of the bovine respiratory tract

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Session: Omics

Objective
Mycoplasma bovis is a Mollicutes bacterium that has been linked to respiratory disease in beef cattle. Currently, detection of *M. bovis* in cattle can be performed via bacteriological culture and PCR, but these methods do not allow a detailed investigation of the number and identity of *M. bovis* strains present. Targeted enrichment is an approach that uses complementary RNA baits to increase the relative proportion of target DNA in a biological sample. The objective of this study is to design an RNA bait set to enrich *M. bovis* genomic DNA in samples from the bovine respiratory tract.

Methods
577 representative genomes of *M. bovis* were downloaded from NCBI GenBank. The Syotti algorithm was used to design RNA bait molecules that align to the DNA of the *M. bovis* strains. The baits were 120 bases in length and allowed 20, 30, or 40 mismatched base pairs, to enable them to bind to the genomic DNA of multiple, unknown *M. bovis* strains. Kraken2 was then used to identify RNA baits that align to DNA from organisms other than *M. bovis*, and the nonspecific baits were removed from the bait set. We also plan to manually enrich with baits that bind to elements of the *M. bovis* genome relevant to pathogenesis and identification, such as variable number tandem repeats (VNTRs), variable surface proteins (Vsps), and insertion sequence (IS) elements.

Results
We created 3 bait sets, one for each value of mismatches tolerated. The Syotti results were as follows: 20 mismatches resulted in 119,452 baits, 30 mismatches in 101,918 baits, and 40 mismatches in 84,310 individual baits. There were no gaps in coverage for any of the bait sets. The bait sets were classified with Kraken2, resulting in: 67,032 (56.12 %) baits classified as *M. bovis* at 20 mismatches; 55,630 (54.58 %) baits at 30 mismatches; and 43,462 (51.55 %) baits at 40 mismatches.

Conclusions
In conclusion, we produced three bait sets that completely cover the *M. bovis* genomes. Each bait set was made with a different tolerance for mismatching, which will permit them to bind to DNA fragments from multiple strains of *M. bovis* to different degrees.

Notes:

P107 - Short versus long reads for microbiome profiling of the respiratory tract and environment of feedlot cattle

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Session: Omics

Objective
Illumina (short reads, SR) and Nanopore (long reads, LR) are two common sequencing platforms used for profiling the microbiome. However, the agreement level between these workflows for samples from the upper respiratory tract (URT) and environment of feedlot cattle is unknown. The objective of this study was to compare the agreement and discriminatory power of the two workflows (SR vs LR) in the profiling of microbial communities in the URT and environment of feedlot cattle.

Methods
Samples from the URT (37 nasal swabs) of feedlot cattle and environment (13 hanging ropes) were collected. DNA was extracted from all samples using the PowerFecal kit to estimate microbial communities applying the two different approaches. The SR approach consisted of the sequencing of the 16S rRNA gene V3-V4 region using NovaSeq 2X250bp platform. DADA2 and Greengenes database were used to estimate the amplicon sequence variants and classification of the SR. The LR approach consisted of the sequencing of full-length 16S rRNA gene on a MinION Mk1C device. Centrifuge was employed to classify the LR using the NCBI nt database. Read counts were aggregated at the genus level and the beta diversity of the samples were compared between the approaches.

Results
Our findings showed that the microbiome composition from SR was more dissimilar among samples than that from LR, suggesting a greater degree of discrimination. Furthermore, the choice of approach explained 56.7% and 15.7% of the variance in the Aitchison and Jaccard dissimilarity distances of the microbiome composition, respectively ($P<0.001$). The dispersion of Jaccard dissimilarity distances did not differ by approach ($P=0.829$). By contrast, Aitchison dissimilarity distances were more dispersed in SR than in LR (average distance to centroid: 21.93 in SR vs. 16.07 in LR, $P<0.001$).

Conclusions
SR showed greater discrimination when classifying microbial communities from the URT and environment of feedlot cattle than LR. However, further studies leveraging larger sample sizes and other databases for SR, such as SILVA, are warranted to draw a more robust inference.

Financial Support
Texas A&M University

Notes:

P110 - CircoMatch™: An online tool for prediction of PCV2 vaccine efficacy based on T cell Epitope Content Comparison

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Session: Omics

Objective
CircoMatch is an online tool that compares the putative T cell epitope content shared between commercial Porcine Circovirus type 2 (PCV2) vaccines and field isolates to identify which vaccine may confer broader cross-reactive immune response to field isolates based on T cell epitope relatedness.

Methods
CircoMatch receives as input one or multiple capsid protein sequences of field isolates and uses PigMatrix to identify putative SLA class I and II T cell epitopes. The Epitope Content Comparison (EpiCC) algorithm assesses the relatedness of T cell epitopes contained in capsid proteins between field isolates and 4 commercial vaccines (3 based on PCV2a, and one a PCV2a and PCV2b bivalent).

Results
CircoMatch generates an EpiCC score for each vaccine-field isolate comparison and a corresponding assessment of T cell epitope coverage. Higher EpiCC scores represent greater relatedness and produce higher T cell epitope coverage. The CircoMatch report includes scales and radar plots for comparison of EpiCC scores calculated for each field isolate-vaccine pair. The EpiCC score scale provides a simple yet effective visual comparison that can be used to rank vaccines for an individual field isolate. The radar plot shows EpiCC scores for each vaccine-field isolate comparison, including reference isolates from different genotypes (PCV2a, b and d), and regions (America, Asia and Europe). EpiCC scores can also be compared to benchmark scores calculated based on comparisons between the vaccines and 746 field isolates from 2017 to 2021.

Conclusions
CircoMatch has been developed to help veterinarians and producers select the best-matched commercial vaccine for immunization against circulating PCV2 isolates and to support surveillance to identify variants that may represent a potential threat. Clinical data received as part of the submission may help to refine EpiCC predictions and understand the relationship between shared epitope content and clinical outcomes.

Financial Support
Zoetis

Notes:

P111 - US-UK Collab: Influence of vaccines, host genetics, and mutation rates on the evolution of infectious diseases

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Session: Omics

Objective
Imperfect vaccines or host genetic resistance may alter the balance of selection between pathogen transmission and virulence by allowing more divergent but still virulent strains to be transmitted at reduced cost. Our objectives are 1) determine the influence of imperfect vaccines and host genetics on viral transmission and evolution; 2) validate viral genome polymorphisms associated with increased virulence; 3) build models to develop strategies to control the ecology, evolution and economic burden of Marek's disease (MD); and 4) disseminate information on Marek's disease virus (MDV) and infectious bronchitis virus (IBV), and the impact of vaccination to the public using various tools.

Methods
Efforts on Objective 1 are provided. We use a shedder-sentinel challenge model to naturally passage MDV through 10 successive groups. Each group consists of 10 birds kept in an individual isolator and replicated 3-6x. Viral replication and transmission are assessed by sampling shedder (donor) birds that transmit infectious virions prior to, at, and following co-housing with the contact (recipient) birds. Birds infected in Passage 1 transmit virus to recipients in Passage 2, and so on.

Results
Three experiments have been completed. Two experiments compared unvaccinated to vaccinated donors, with one comparing donors vaccinated with diluted dosage, a common industry practice. The third experiment compared susceptible vs. resistance donors. A high MD incidence has been maintained through passage of the virus through unvaccinated and susceptible chickens, and low incidence maintained in both vaccinated groups as well as resistant chickens. We have yet to observe an increase in virulence based on clinical disease associated within each group.

Conclusions
MDV is being transmitted through serial passage in all groups of chickens, however, without clinically observable changes in virulence. We are currently analyzing samples from each passage for virus load in feathers to detect changes that may be associated with evolved traits. Our next step is to compare results using commercial donor birds.

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

Notes:

P112 - Spatio-temporal clusters and molecular characterization of *O. rhinotracheale* and *P. multocida* in turkeys

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Session: Omics

Objective
Respiratory infections caused by *Ornithobacterium rhinotrachealis* (ORT) and *Pasteurella multocida* (PM) bacteria cause economic losses and welfare issues for the U.S. poultry industry. Despite increased focus on these pathogens, molecular characterization and spatio-temporal analyses of these bacteria isolated from confirmed field cases are largely lacking. The objective of this retrospective observational study was to characterize isolates and investigate the existence of spatio-temporal clusters of ORT and PM for confirmed outbreaks in commercial turkey sites in the U.S Midwest.

Methods
Isolates from confirmed ORT and PM outbreaks (ORT = 103, and PM = 69) between 2013-2021 were obtained and characterized using a novel Core-Genome Multilocus Sequencing Typing (cgMLST) Scheme specifically designed for ORT and PM. A cluster analysis at the monthly level was conducted using SaTScan™ software using a retrospective space-time permutation model. Allelic differences between well-defined core genes of individual isolates were determined by strain characterization, and Spearman rank's correlation was used to detect any association between allelic differences and spatial distance between sampling locations for each pathogen separately.

Results
No spatio-temporal clusters were detected for ORT ($P>0.05$). For PM, one cluster was detected between May to July 2018 with six observed cases within a 260 km radius (expected 0.57 cases, $P<0.01$); and a second cluster with 5 cases for February 2019 to February 2021 within a 9 km radius (expected 0.91 cases, $P<0.01$). A weak negative correlation between allele differences and spatial distances was observed for ORT ($r=-0.04$, $n=4032$, $P=0.01$) while a weak positive correlation was observed for PM ($r=0.11$, $n=2756$, $P<0.01$). Five and 13 genetic groups were found for ORT and PM, respectively.

Conclusions
This study revealed two regional spatio-temporal clusters for PM between 2018 and 2021. Results also provided new insight regarding the relationship between genotypic plasticity and spatial distance between regional bacterial subtypes for both pathogens.

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

Notes:

P113 - Evolution of replicons in *Staphylococcus aureus* large plasmids

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Session: Omics

Objective
Plasmids are important vectors in spreading various virulence elements via horizontal gene transfer, which lead to the evolution of their host genome. Plasmid genome consists of conservative regions that remain quite stable for long evolutionary periods. Thus, to analyze their dynamic and evolution in different ecological niches, these core genes can be valuable markers. Replication initiation genes (*rep*) are the core part of plasmids replication and proliferation, and recently, a sequence-based typing method has been proposed for enterococci and Gram- positive plasmids based on the homology of *rep* genes. The objectives of this work were to study the evolution of replicons in *Staphylococcus aureus* large plasmids and to determine if plasmids fusion is involved in forming those large plasmids.

Methods
The distribution and evolution of 331 plasmid sequences of *Staphylococcus aureus* retrieved from the NCBI database were analyzed in order to understand their origin and evolution using 114 *rep* sequences belong to 26 *rep* family and 23 unique *rep*-family of Gram-positive bacterial plasmids.

Results
A total of 25 *rep*-families were identified with 51% of plasmids carried more than one *rep* sequence and almost all multi-*rep* plasmids were larger plasmids. This indicates that plasmids fusion among *S. aureus* strains is a common event and the genome of large plasmids are more dynamic. Some combinations between *rep*-family subtypes were prevalent among plasmids, which means plasmids fusion is not random and it might be relevant to plasmids incompatibility. The phylogenetic analysis of the *rep*-families nucleotide sequences showed several well-defined subtypes representing true phylogenetic groups. Some subtypes belong to the same *rep* family can be carried on the same plasmid, but they have different evolutionary history.

Conclusions
In conclusion, plasmids fusion among *S. aureus* strains to form larger plasmids is a common event. It also appears that the genomes of large plasmids are more dynamic.

Notes:

P114 - Proportion of Brahman genotype influenced blood leukocyte profiles of calves in a multibreed Angus-Brahman herd

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Session: Omics

Objective
Determine the effect of breed composition on characteristics of peripheral blood leukocytes.

Methods
Eighty-six calves from multibreed Angus-brahman herd with linearly varying breed composition from 100% Brahman to 100% Angus were used for the investigation. Calves were categorized in 1 of 5 breed groups (BG) based on breed composition (i.e., BG1 = 80 – 100 % Angus, BG5 = 80 – 100 % Brahman). Calves were vaccinated with Bovi-Shield GOLD at 3 months of age with a booster at 4 months of age. Blood samples were collected at 0, 1 and 2 months relative to the primary vaccination. Immune cells were evaluated for size, granularity, viability and protein markers (CD4, CD8, γδTCR, CD21, CD11b, CD62L, and CD14) using flow cytometry. Repeated measures data were analyzed for the effect of BG, month, and interaction of BG using mixed models. Contrasts for the linear effect of BG (BG 1 to 5) and regression analysis using breed proportion as a continuous variable also were performed.

Results
Of the 27 immune cell variables that were analyzed, 18 revealed a linear ($P < 0.05$) relationship with BG. The linear associations were such that BG5 had more lymphocytes (BG1 = 2,364 vs. BG5 = 3,079 ± 204 cells/µL, $P = 0.006$) and neutrophils (BG1= 778 vs. BG5 = 1,456 ± 75 cells/µL, $P < 0.001$) compared with BG1. Within the lymphocyte population BG5 had more CD8 (BG1 = 281 vs. BG5 = 434 ± 30 cells/µL, $P < 0.001$) and γδTCR-positive lymphocytes (BG1 = 471 vs. BG5 = 656 ± 69 cells/µL, $P = 0.0251$), but fewer CD4-positive lymphocytes (BG1 = 424 vs. BG5 = 248 ± 42 cells/µL, $P < 0.001$) compared with BG1. The concentrations of B cells (BG1 = 843 vs. BG5 = 947 ± 99 cells/µL, $P = 0.42$) and monocytes (BG1 = 315 vs. BG5 = 349 ± 20 cells/µL, $P = 0.12$) did not differ among BGs, however, monocytes from BG1 expressed more CD14 (BG1 = 5,924 vs. BG5 = 3,618 ± 371, $P < 0.001$) compared with monocytes from BG5.

Conclusions
Breed composition affected multiple immune cell populations, notably concentrations of CD4 and CD8 T cells and neutrophils in blood.

Notes:

P115 - Vaginal microbiota dynamics associated with parity and gestational age but not with reproductive outcomes in pig

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Session: Omics

Objective
A healthy vaginal microbiota of the pregnant sow is critical to the health of both the sow and offspring. Microbiota imbalances could result in post-farrowing complications such as prolapse, urogenital and womb infections. Farrowing performance is a major economic concern in swine production. Here, we aimed to examine the dynamic changes in the vaginal microbiota throughout gestation and identify whether these changes are associated with parity and reproductive outcomes

Methods
Vaginal swabs were collected at three different times (last three weeks of gestation, farrowing week and two weeks post-farrowing) from 126 pigs (representing one block of our larger experiment) including 78 primiparous and 48 multiparous sows. Sows were identified as high or low performance sow based on the number of pigs weaned. Using 16S rRNA amplicon community profiling, richness and relative abundance were assessed. A linear discriminant analysis with effect size (LEfSe) was used to elucidate the differently abundant taxa on each time point

Results
Sows had significantly different observed taxa during gestation compared with farrowing ($p=0.02$) and post-farrowing ($p=0.017$) period. Primiparous group had higher richness compared to multiparous group based on Shannon diversity index ($p = 0.00$). Using NMDS, the farrowing group was clustered between the gestation and post-farrowing groups. Further, PERMANOVA analyses supported the significant differences between the gestation, farrowing and post-farrowing groups ($p= 0.01$) as well as between the primiparous and multiparous group ($p=0.001$). LEfSe analysis identified 20 taxa at gestation, 16 at farrowing and 17 at post-farrowing that were differently abundant between primiparous and multiparous group

Conclusions
Our study highlights that parity and gestational stage should be accounted for in future studies on sow's reproductive outcomes. Although an effect on reproductive success was not evident in this subset, this work involved multiple experimental units of sows and the full dataset may yet identify associations between vaginal microbiota and reproductive success in sows

Financial Support
Ontario Pork

Notes:

P116 - Effect of Holstein cattle genotype on *Brucella abortus* challenge response

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Session: Omics

Objective
Brucellosis is typically caused by infection with *Brucella* spp. through contact with infected animals or consumption of milk/milk products. Genetic selection for milk production traits in U.S. Holsteins has additionally impacted immunity genes. Characterization of genes associated with resistance vs. susceptibility to *Brucella* are important for the development of improved vaccines and diagnostic strategies. The objective of this study was to compare the transcriptomic response of PMBCs following *in vitro* challenge with the commercial brucellosis vaccine, RB51, between contemporary Holsteins and those unselected for milk production traits.

Methods
Total RNA was extracted from PBMCs after 24 hr with or without stimulation with RB51. Library preparation and sequencing were performed at the Iowa State Genomics Center, and 100 bp paired end reads were generated utilizing the Illumina Hiseq6000. After quality control, trimming, and aligning differential gene expression (DEG) was performed using DeSeq2 based on the model genotype + stimulation +E. The ClueGO plugin of Cytoscape was used to identify and cluster significantly enriched GO terms and KEGG pathways for significant DEGs.

Results
412 genes were significantly (FDR $P < 0.05$, log fold change > |1|) differentially expressed. Upregulated genes (higher expression in the selected vs. unselected cattle), were enriched for 19 terms and pathways, forming 7 distinct clusters, including alanine, aspartate, and glutamate metabolism, indicating a cellular stress response. Downregulated genes (higher expression in the unselected vs. selected cattle), were enriched for a greater number of terms and pathways (37) which formed 13 distinct clusters. These clusters include a number of diverse immune responses, including natural killer cell-mediated immunity, interferon-gamma production, negative regulation of interleukin-10 production, and cytokine receptor activity.

Conclusions
These results suggest that selection for milk production traits has affected the Holstein immune response to RB51.

Notes:

P117 - Protein targets for preventative measures and diagnostics of *Mycoplasma bovis* from North American bison and cattle

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Session: Omics

Objective
Mycoplasma bovis is an economically important pathogen of cattle and bison. In cattle, *Mycoplasma* is a production disease causing mastitis, arthritis, and pneumonia. Bison have a different clinical presentation, with pneumonia and polyarthritis leading to high case fatality rates. Because *Mycoplasma* species lack a cell wall and have adapted to intracellular invasion and replication within host cells, many antibiotic treatments are ineffective. In this study we used existing sequencing data of North American *M. bovis* strains isolated from cattle and bison to compare the sequence diversity of proteins known or suspected to be outer membrane, extracellular, or immunogenic.

Methods
Unassembled sequencing reads from one hundred and fifteen *M. bovis* strains were downloaded from the NCBI Sequence Read Archive and assembled into contigs with Spades. Additionally, twenty-five complete *M. bovis* genomes were downloaded from Genbank. The complete genomes and assembled contigs were annotated with DFAST and core and pan genome analysis was performed using the bioinformatic software package EDGAR. Predicted or known membrane, extracellular, or immunogenic proteins of *M. bovis* were identified from the literature and compared at the community level. Epitope binding efficiencies of these proteins to bovine MHC Class I were predicted with NetMHCpan.

Results
The majority of the proteins examined in this study are highly conserved at the sequence level, including at the sites of predicted epitope binding with bovine MHC Class I. Protein variants were not found to be host species specific. Despite the high sequence conservation, proteins with differences in predicted epitope sequences and binding strength with bovine MHC Class I were identified.

Conclusions
The high conservation of protein sequences across the population of North American *M. bovis* samples and the lack of host specificity among protein variants and their predicted epitope sequences provide opportunities for the development of preventive mitigation measures and new diagnostic methods.

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

Notes:

P119 - Tulathromycin-induced gene expression evaluated in high-risk beef cattle through whole blood transcriptomics

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Session: Omics

Objective
Bovine respiratory disease (BRD) is a multifactorial disease complex influenced by host metabolism and immunity, offending pathogenic influence, and the overt environment condition. Because its often undifferentiated in cattle at arrival, metaphylaxis, specifically tulathromycin, is often administered to control rates of BRD in high risk populations. However, tulathromycin's impact on host genomic mechanisms is poorly understood. Therefore, this study examined tulathromycin's influence on bovine gene expression by comparing high-risk cattle without clinical BRD via whole blood transcriptomics.

Methods
Eighty-four commercial steers (average: 239 kg; s.d.= 16 kg) were randomly enrolled into two treatment groups for 70 days (META, n=42; NOMETA, n=42); META cattle received a one-time subcutaneous injection of tulathromycin on day 0 at label dosing. Jugular blood samples from all cattle were collected into Tempus RNA blood tubes at days 0, 7, 14, and 21. Samples for RNA-Seq were randomly selected from seven META and seven NOMETA cattle (n=56 samples). Isolated mRNA from samples were sequenced (NovaSeq 6000, 150bp PE; ~40M reads/sample) and bioinformatically processed via a bovine genome reference-guided HISAT2/StringTie2 pipeline. Differentially expressed genes (DEGs) were identified with the R packages edgeR and glmmSeq (FDR<0.1). Functional enrichment analysis of DEGs was performed with KOBAS API (FDR<0.05).

Results
Treatment groups expressed no DEGs at day 0, but DEGs were identified within the META group over time, associated with inflammatory modulation, cellular clearance, and immune modulation. Distinguishment of DEGs between META and NOMETA was most prominent between days 7 and 14.

Conclusions
Gene expression analysis of cattle free of BRD illuminated the inflammatory and immunological modulation effects of tulathromycin on the cattle transcriptome. These findings better our understanding of host immune modulation by metaphylaxis, and identify novel host mechanisms to be leveraged in future infectious disease therapy research.

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

Notes:

P120 - Effect of vaccination and marketing strategies on gene expression patterns in beef cattle via time course RNA-Seq

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Session: Omics

Objective
Modified-live viral (MLV) vaccination and direct purchasing are strategies used to control bovine respiratory disease. However, the influence these strategies and their interactions have on host immune function, cellular metabolism, and inflammation are not well understood. The objective of this study was to evaluate the impact of marketing strategy and MLV vaccination on host gene expression of cattle over time between initial vaccination through arrival to a backgrounding facility via whole blood transcriptomics.

Methods
Jugular whole blood was collected from twelve randomly selected healthy beef steers allocated across four groups, based on MLV vaccination (VAC) or not (NOVAC), and direct shipment from Mississippi to a Texas backgrounding operation (DIR) or shipped to a commercial auction market and order-buyer for three days prior to Texas backgrounding (AUC). Five time points were utilized (T1-T5) in both Mississippi and Texas: at initial MLV vaccination (T1; ~99 days of age), seven days post-vaccination (T2; ~106 days of age), revaccination (T3; ~175 days of age), weaning (T4; ~222 days of age), and arrival to Texas backgrounding operation (T5; ~226 days of age). Isolated mRNA from blood was sequenced (NovaSeq 6000; ~35M reads/sample), and data were processed through ARS-UCD1.2 reference-guided assembly (HISAT2/Stringtie). Differentially expressed genes (DEGs) and changes in expression patterns were analyzed with edgeR and Trendy, respectively (FDR<0.05). Functional enrichment was performed with WebGestalt (FDR<0.05).

Results
Across all four groups, 3,637 unique DEGs were identified. All AUC cattle demonstrated an increase in type I interferon (IFN) gene expression at T5; NOVAC-AUC cattle expressed a wider number of type I IFNs compared to VAC-AUC. Specialized proresolving mediator production (SPM) increased in all cattle T1-T4, however decreased at T5 in NOVAC-AUC.

Conclusions
We demonstrate that marketing and vaccination strategies influence gene expression related to inflammatory resolution, type I interferon production, and immune functionality.

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

Notes:

P122 - Shotgun metagenomics for pathogen detection & surveillance of antimicrobial resistance in shelter dog population

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Session: Omics

Objective
This study aimed to employ shotgun metagenomics as a screening tool to detect canine and potentially zoonotic pathogens and to determine the antimicrobial resistome in the shelter dog population.

Methods
The genomic DNA extracted from fecal samples from 58 healthy dogs from a total of 10 shelters from the Cumberland Gap Region in Kentucky, Tennessee, and Virginia were sequenced with the ZymoBIOMICS shotgun sequencing pipeline, and the data was analyzed using ZymoBIOMICS bioinformatics analysis pipeline.

Results
The percentage prevalence of pathogens detected in the fecal samples of shelter dogs was as follows:
Known or potentially zoonotic pathogens: Protozoa [T*oxoplasma gondii* (12%), *Balamuthia mandrillaris* (7%)]; Nematodes [*Trichuris trichuria* (13.8%)]; Viruses [Human poliovirus 1 Mahoney (10.3%)]; Bacteria [*Salmonella enterica* (1.7%), *Clostridioides difficile* (15.5%), *botulinum* (10%), and *perfringens* (19%), *Campylobacter jejuni* (20.7%), and *upsaliensis* (75%)]; Canine pathogens: Viruses [Bocaparvovirus (1.7%), Parvovirus (5.2%)]; Bacteria [*Bordetella bronchiseptica* (1.7%)]; Opportunistic pathogens (canine and humans): Yeast [*Candida parapsilosis* (1.72%), *Malassezia globose* (3.5%), *M. restricta* (8.6%), *M. sympodialis* (1.7%)]; mold [Saksenaea oblongispora (3.5%)] and Bacteria [*Pasteurella multocida* (1.7%)]; Pathogens of significance to other animals: Bacteria [*Campylobacter fetus* (1.7%), *Chlamydia abortus* (27.6%), *Streptococcus agalactiae* (3.4%), *pyogenes* (1.7%), and *suis* (5.2%)]; Resistome: Antimicrobial resistance genes (ARGs) encoding resistance to a total of 14 antibiotic groups/classes were detected. Fecal samples from each dog were positive for ARGs encoding 6 to 10 antimicrobial classes. ARGs encoding 7 (50%) out of 14 antibiotic classes were detected in each shelter. ARGs encoding 4 antimicrobial classes were detected in each dog.

Conclusions
Carriage of canine and potentially zoonotic pathogens and the ARGs in shelter dogs poses a significant risk to canine and public health including shelter workers and potential adopters.

Notes:

P123 - Efficacy of Bacillus thuringiensis crystal protein X against monogastric and ruminant gastrointestinal nematodes

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Session: Parasitology

Objective
This study analyzed the antiparasitic efficacy of: 1) Bacillus thuringiensis (Bt) crystal (Cry) protein X in vitro against equine cyathostomins (EC); 2) a single dose treatment of BtCry protein X in vivo against, A) Haemonchus contortus (HC) in ruminants (sheep), and B) Ancylostoma ceylanicum (AC) and Heligmosomoides polygyrus (HP) in monogastric (rodents).

Methods
Objective 1.) A lysate of Bt CryX was used in vitro in EC egg to larval assay at 0.1, 1, 10 and 100 ng/ml. Objective 2) An Inactivated Bacillus with a Cytosolic Crystal (IBaCC) paraprobiotic form of the Bt CryX was utilized in this experiment. Objective 2A: Dorset lambs were infected with 10,000 HC infective larvae. Once the infection was patent, lambs were stratified by fecal egg count (FEC) and sequentially assigned into one of three treatment groups (n=5). Lambs were orally administered either Bt CryX IBaCC at 30 or 15 mg/kg BW or untreated control (water). FEC were measured daily until lambs were euthanized at day seven and total worm burdens were quantified and sexed from the recovered abomasums. Objective 2B: Mice and hamsters infected with HP and AC, respectively, were gavaged with a single dose of Bt CryX IBaCC at 50 (mice) and 20 mg/kg (hamsters). Worm burdens and FEC were determined after 5 days.

Results
Objective 1: There was an 84% reduction in egg to larval development in EC at 0.1 and 1 ng/ml Bt CryX lysate and 100% reduction at 10 ng/ml and greater. Objective 2A: There was an 85% and 97% reduction in FEC and a 69% and 93% reduction in worm burden for lambs in the 15 mg/kg BW and 30 mg/kg BW Bt CryX IBaCC treatment groups, respectively. Objective 2B: There was a significant reduction in intestinal parasite burdens and fecal egg counts of HP and AC when mice and hamsters, respectively, were gavaged with Bt CryX IBaCC.

Conclusions
These studies provided evidence of anthelmintic efficacy of Bt Cry X against nematodes of monogastric and ruminant animals. Further studies to optimize and identify new novel anti-parasitic Cry proteins is warranted.

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

Notes:

P124 - Reverse vaccinology approaches targeting the invasive Asian longhorned tick, *Haemaphysalis longicornis*

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Session: Parasitology

Objective
Haemaphysalis longicornis (the Asian longhorned tick) is a vector of multiple animal and human pathogens and a major invasive pest of commercial livestock. Native to East Asia, this tick has established parthenogenetic populations in New Zealand, Australia, multiple Pacific islands, and as of 2017 the Northeastern United States. Host-seeking *H. longicornis* ticks in VA have since tested positive for *Theileria orientalis* Ikeda genotype, and thus effects on U.S livestock industry could be devastating should *H. longicornis* establish near commercial ranchlands. Acaricides have shown varying success in controlling tick populations and carry risks e.g., resistance. Here we present our USDA-NIFA AFRI funded work that utilizes current genome data to produce and trial reverse-vaccine candidates effective against this rapidly expanding ectoparasite.

Methods
Sequencing and assembly of a high-quality, contiguous *H. longicornis* genome from North American ticks using PacBio Sequel II single-molecule sequencing is underway. Genome assembly, gene / protein prediction, and ortholog clustering with other tick proteomes will take place prior to identification of potential vaccine candidate proteins via heuristic epitope predictive tools and characteristics of known antigens. Recombinant proteins are synthesized using e.g., a pPICZ expression vector and screened for immunogenicity in cattle via stall trials to assess tick morbidity and/or mortality.

Results
We present here results obtained thus far during the first year of our project, inclusive of a reference *H. longicornis* genome and tissue-specific gene expression to localize putative antigen candidates. We expect to finalize our candidate list and synthesize recombinants during Year 2 for stall trials during Year 3.

Conclusions
Results of this work will form the basis for current and future disease prevention and control technologies that lessen reliance on acaricides in the face of a new and rapidly expanding tick invasion.

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

Notes:

P125 - Antigen-specific cytokine production in cattle received repeated drug-truncated infections by *Ostertagia ostertagi*

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Session: Parasitology

Objective
Ostertagia ostertagi, a pathogenic gastrointestinal parasite, causes significant losses to cattle industry. There is no vaccine available against this nematode because no protective antigens (Ag) have been identified and mechanism of protection is poorly understood. We developed a repeated, drug-truncated infection (rDTI) procedure that elicits worm-specific immunity and confers protection. This study determined Ag-specific cytokines produced by peripheral blood mononuclear cells (PBMC) of *O. ostertagi*-immune cattle.

Methods
Steers (4-5 months old, n=5) were treated with or without rDTI. In brief, each round of DTI had 34 days, involving infections with 5,000 *Ostertagia* larvae on days 0-4 followed by anthelmintic treatment on day 14 and a 21-day resting period. Treated animals received 5 rounds of rDTI. Controls (n = 5) received tap water and same anthelmintic treatment. Animals were challenge-infected with 50,000 *Ostertagia* larvae 30 days after the last rDTI. Sera were collected weekly for 4 weeks post challenge. PBMC were isolated between 30-60 days post challenge, stimulated by worm extract (OoAg) or excretory/secretory product (AdES) for 8 days and supernatants were collected for testing. Cytokine levels were determined by bovine cytokine arrays.

Results
Challenge infection resulted in non-rDTI animals exhibiting upregulated serum chemokine (C-C motif) ligand 2 (CCL2), interleukin-4 (IL-4), and IL-15, but downregulated chemokine (C-X-C motif) ligand 10 (CXCL10). In contrast, rDTI animals displayed elevated CCL2 and acidic fibroblast growth factor in sera, consistent with tissue repair. OoAg/AsES elicited PBMC proliferation and a Th2 phenotype ex vivo, characterized by elevated IL-4, IL-13, CD40 ligand, CCL5, CXCL9, and CXCL10. Interestingly, IL-2 was not induced in PBMC of rDTI cattle.

Conclusions
A Th2 cytokine profile is consistent with anti-worm immunity. A lack of IL-2 suggests potential T cell exhaustion. Overall, this study shows rDTI-mediated protection correlates with Th2 cytokine responses. Ongoing research is to identify parasite Ag eliciting key Th2 cytokines.

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

Notes:

P126 - Effects of yeast postbiotic supplementation on the lung transcriptome of calves with a viral-bacterial coinfection

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Session: Preventive medicine

Objective
Bovine respiratory disease (BRD) causes morbidity and mortality in cattle of all ages. Supplementing with postbiotic products from *Saccharomyces cerevisiae* fermentation (SCFP) has been reported to improve growth and provide metabolic support required for immune activation in calves. The objective of this study was to determine effects of SCFPsupplementation on the transcriptional response to co-infection with bovine respiratory syncytial virus (BRSV) and *Pasteurella multocida* (*PM*) in the lung using RNAseq.

Methods
Twenty-eight calves were enrolled and assigned to two treatment groups; control vs. SCFP-treated (1g/d SmartCare®, milk and 5g/d NutriTek®, starter grains). Calves were infected with ~10^4 $TCID_{50}$ BRSV, followed 6 days later by intratracheal inoculation with ~10^{10} CFU of *PM* (strain P1062). Calves were euthanized on day 10 post-viral infection. Antemortem and postmortem bronchoalveolar lavage (BAL) and lesion (LL) and non-lesioned (NLL) lung tissue samples were collected at necropsy for RNA extraction and sequencing.

Results
Sequence reads were aligned to the bovine reference genome (UMD3.1) and EdgeR used for differential gene expression (DEGs) analysis. There were no differences in pathogen burden between treatments, but SCFP calves experienced milder pathology as shown by ultrasonography. Transcriptional responses in lung tissues and BAL samples from SCFP calves were different compared to the controls. There were 537 DEGs identified in both tissue samples and only 73 genes were shared between the two tissues. Of the top DEGs predicted, the top enriched pathways in SCFP-treated lungs were associated with decreased expression of inflammatory genes and increased expression of plasminogen, supporting effective lung repair. SCFP calves also had higher expression of genes involved in vitamin D processes and immune system development in pre-infection BAL samples.

Conclusions
These data suggest SCFP supplementation modulates immune function in the lungs, leading to increased resistance to BRD, and provides insight into potential for SCFP products to serve as alternatives to antimicrobials.

Financial Support
Iowa State University; Diamond V Mills Inc.; Cargill

Notes:

P127 - A novel probiotic mixture to support the health of calves challenged with *Clostridium perfringens* type A

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Session: Preventive medicine

Objective
This study evaluated the effects of a novel probiotic mixture on the health score of newborn beef calves challenged with *Clostridium perfringens* type A

Methods
Twenty (n = 20) healthy 1-day-old beef calves (initial body weight = 41.2 ± 1.03 kg) were assigned to one of two experimental groups: (1) Control: no probiotic supplementation (CON; n = 10), and (2) Probiotic: 6.0×10^9 colony forming units (CFU)/head per day of a probiotic mixture containing *Lactobacillus animalis*, *Propionibacterium freudenreichii*, *Bacillus licheniformis*, and *B. subtilis* (PRO; n = 10; BOVAMINE DEFEND® Plus, Chr. Hansen, Milwaukee, USA). The experimental period lasted 21 days. Seven days after the beginning of the trial and PRO supplementation, all calves were orally dosed with 1.0×10^8 CFU of *Clostridium perfringens* type A strain S-107 (ATCC 13124). All animals were observed daily for health status and observations for clinical signs of disease associated with *C. perfringens* infection including the following for health (general impression and appearance) and diarrhea score. General impression and appearance were scored from 0 to 4, where 0 = good impression and clean backside, tail, and legs and 4 = calf is moribund or dead. Diarrhea score was assessed from 0 to 3 score, where 0 = normal feces and 3 = severe diarrhea, with watery feces. All data were analyzed as the number of days calves presented an abnormal health score and the sum of the aforementioned scores.

Results
Supplementation with the novel PRO mixture increased the number of days in which calves were scored 0 (or normal) for general impression, appearance, and diarrhea ($P < 0.04$). Therefore, calves fed PRO also had a lower score for general impression, appearance, and diarrhea ($P < 0.04$) vs. CON.

Conclusions
In summary, supplementation of a probiotic containing *L. animalis*, *P. freudenreichii*, *B. licheniformis*, and *B. subtilis* improved health and diarrhea scores of newborn beef calves following a *C. perfringens* type A challenge, demonstrating the efficacy of this mixture in alleviating potential negative effects of *C. perfringens* type A infection.

Notes:

P128 - Characterization of the duration of immunity of *Brucella abortus* strain RB51 vaccination in cattle

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Session: Vaccinology

Objective
The objective of the study was to characterize the efficacy of the RB51 vaccine in protecting cattle against experimental challenge at 4, 5, and 6 years after vaccination.

Methods
Fifty-two, Hereford heifers were obtained from brucellosis-free herds and randomly assigned to vaccination with *Brucella abortus* strain RB51 (RB51, n=32) or control (n=20) treatments. Vaccinates received 10^{10} CFU of a commercial lyophilized RB51 vaccine. Immunologic responses were characterized after vaccination. A subgroup of control and vaccinated pregnant cattle were experimentally challenged at 4, 5, and 6 years after vaccination with 10^7 CFU of *B. abortus* strain 2308 att 170 to 180 days gestation.

Results
Vaccinates demonstrated significantly greater ($P<0.05$) antibody, interferon-gamma, and proliferative responses to RB51 antigens after inoculation as compared to controls. After experimental challenge, 6 of 14 (43%) control animals aborted at a higher rate ($P<0.05$) when compared to RB51 vaccinates in years 4 and 5, but not year 6 (0, 10, and 50%, respectively). When comparing recovery of *Brucella* from all tissues except head lymph nodes draining the site of challenge, RB51 vaccinates had reduced infection rates ($P<0.05$) after experimental challenge at 4 years (14%), but not at 5 or 6 years (78 and 67%, respectively) when compared to non-vaccinated cattle (93%).

Conclusions
Our data suggest that calfhood vaccination with RB51 does not induce lifelong immunity and suggests implementation of booster vaccination by 4 to 5 years of age should be utilized in endemic areas to maintain high levels of protection.

Notes:

P129 - Decreased *Brucella abortus* 2308-specific humoral responses following RB51 vaccination of cattle

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Session: Vaccinology

Objective
Bovine brucellosis is caused by infection with *Brucella abortus* and is primarily associated with fetal losses or infertility in cattle. In order to control brucellosis in domestic livestock, regulatory programs assess disease prevalence and/or exposure primarily through serologic assays. *Brucella abortus* strain RB51 (RB51) is the commercial vaccine against bovine brucellosis, and it is used in the United States to vaccinate cattle. Vaccination with RB51 prevents abortion, but does not necessarily prevent infection. In this study, we set out to characterize the dynamics and magnitude of the humoral response to virulent *B. abortus* in the context of RB51 vaccination.

Methods
Using serum collected from naïve, RB51-vaccinated, and RB51-vaccinated and boosted animals, we assessed the humoral response following challenge with virulent *B. abortus* 2308 via enzyme-linked immunosorbent assay (ELISA) and the fluorescence polarization assay (FPA). Additionally, we assessed bacterial colonization in various tissues at necropsy, to determine *B. abortus* 2308 dissemination.

Results
The data presented here indicate that vaccination of cattle with RB51 decreases the magnitude of the humoral response to *B. abortus* 2308 infection as detected by ELISA and FPA. Single and boosted RB51 vaccination provided protection against challenge, as compared to non-vaccinated animals. *B. abortus* 2308 was recovered from approximately 45% of single vaccinates, 16% of RB51 boosted, and 80% of naïve animals. Not unexpectedly, serological responses in culture-positive vaccinates were higher as compared to culture-negative vaccinates.

Conclusions
The information presented here demonstrates that, as expected, RB51 vaccination affords protection against virulent *B. abortus* challenge. However, this protection does decrease the magnitude of the humoral response, which is critical for surveillance and diagnostics. This information should be taken into account in the context of regulatory purposes, as some animals may appear negative on current serological tests, but may still harbor bacteria.

Notes:

P130 - Assessing CD4+ T cell memory responses to vaccination with RB51 in cattle

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Session: Vaccinology

Objective
Bovine brucellosis is caused by infection with the intracellular bacteria *Brucella abortus*. This disease can cause major economic losses to cattle producers by inducing abortions. In the United States, *Brucella abortus* strain RB51 (RB51) is the modified live vaccine used in cattle against bovine brucellosis. Proliferative and IFN-γ responses to RB51 vaccination have been previously described. The goal of this study was to further characterize the expression of CD4+ T cell memory phenotypes in response to vaccination with RB51.

Methods
Peripheral blood mononuclear cells (PBMC) from RB51-vaccinated cattle were used to evaluate the frequency of circulating CD4+ T cells, CD4+ T cell proliferation, IFN-γ production, and CD4+ T cell memory phenotypes following an antigen-specific, *in vitro* recall response via flow cytometry.

Results
The data presented here indicates that RB51 vaccination induces a memory response driven by CD4+ T cells. Various RB51-specific memory phenotypes were identified.

Conclusions
The results of this study further describe the CD4+ T cell response to the current RB51 vaccination strategy implemented by cattle producers. These results serve as a standard for future development of an improved bovine brucellosis vaccine.

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

Notes:

P132 - Surveillance of Newcastle disease virus vaccination in the Republic of Armenia

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Session: Vaccinology

Objective
Newcastle disease (ND) is a highly contagious viral disease which is able to infect over 200 species of birds. Due to its important socioeconomic impact, ND has been categorized in the Republic of Armenia as an especially dangerous disease. However, over the last 2 years, the government does not cover the expense of prophylactic vaccination, and vaccinations, which are not obligatory, are carried out exclusively at the expense of farmers. The aim of this study was to examine domestic birds in Armenia for the presence of NDV antibodies to analyze the vaccination coverage, and to assess the probable risks.

Methods
Laboratory testing was conducted in the Reference Laboratory of Especially Dangerous Pathogens (RLEDP). We utilized the ELISA kit by ID vet (ID screen Newcastle disease Indirect conventional Vaccines). In total, 920 blood samples were randomly selected and investigated from small farms in all 10 marzes. All farmers mentioned they used the vaccine against NDV (H strain) between 3-7 months prior to sample collection.

Results
NDV-specific antibodies were found in 61% (561) of the tested samples. The values varied between 35-70% when compared across marzes.

Conclusions
Based on these results, 39% of birds have a higher chance of becoming infected. Potential reasons for the low vaccination coverage include the possibility that birds were not vaccinated as stated by the farmer, the timing post vaccination, or the conditions of storage, transportation, and use of the vaccine were compromised. We highly recommend that the government reinstates vaccination through governmental budget. This will ensure a consistent and comprehensive vaccination process which will contribute to the transparency and traceability of the process and prevent the spread of disease.

Notes:

P134 - Post-vaccination monitoring of Newcastle Disease at Spitak Poultry Plant of the Republic of Armenia in 2021

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Session: Vaccinology

Objective
Newcastle disease (ND) is a highly contagious viral disease of various species of poultry, wild and domestic caged birds, with high rates of mortality. Due to the COVID-19 pandemic in 2020, veterinarians were unable to visit and vaccinate all farms for ND which led to the removal of the vaccine from the state program for vaccination of farm animals in the Republic of Armenia in 2021.
The aim of the study was to monitor post-vaccination immunity in a private poultry farm to assess the risk of outbreak and spread of the disease after removing the ND vaccine from the list of measures for 2021.

Methods
We vaccinated 150 layer chickens against Newcastle disease with the following vaccination schedule: ND K inactive vaccine (injection) and Clone30 live vaccine (spray) day 1, La Sota live vaccine (spray) at day 9, and the La Sota live vaccine (spray) again at day 19. Blood samples were collected 14 days after the last vaccination to determine the level of antibodies by indirect ELISA.

Results
The results of the study showed that 14 days after vaccination, 80% of the vaccinated birds had antibody titers of 1:256 - 1:512, 30 days after vaccination, 70% of the birds had antibody titers of 1:512 - 1:1024, and 90 days after vaccination, 70% of the birds had antibody titers of 1:64 - 1:128.

Conclusions
The study results showed that the tested vaccination scheme provides a sufficient titer of antibodies. However, the titers gradually decrease over time dropping below protective levels (≤ 1:128) after 90 days suggesting the need for re-vaccination. This indicates that the removal of the Newcastle disease vaccination of birds from the list of the state vaccination program in 2021 may be a serious risk for future outbreaks and spread of the disease in the Republic of Armenia.

Notes:

P135 - Immunogenicity characterization of a multivalent vaccine for porcine post-weaning diarrhea

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Session: Vaccinology

Objective
Enterotoxigenic *Escherichia coli* (ETEC) strains producing F4 (K88) or F18 fimbriae, enterotoxins including heat-labile toxin (LT), heat-stable toxins (STb, STa), and Shiga toxin 2e (Stx2e) are the primary cause of porcine post-weaning diarrhea (PWD). PWD remains to be an important swine disease and causes significant economic losses to swine producers worldwide. A vaccine that protects against adherence of F4 (K88) and F18 fimbriae and enterotoxicity of ETEC toxins would be effective against PWD.

Methods
Aiming to develop a broadly protective vaccine against PWD, we applied a novel epitope- and structure-based vaccinology platform multiepitope-fusion -antigen (MEFA) to construct a polyvalent protein immunogen named PWD MEFA. This PWD MEFA protein immunogen uses an LT toxoid monomer (one A subunit mutant fused to one B subunit as a single peptide) as a backbone and presents functional epitopes of F4, F18, LT, STa, STb and Stx2e. In this study, we reversed the monomeric PWD MEFA to an AB_5 holotoxin-structure with insertion of the native cistron structure between the A and the B subunits, expressed the holotoxin-structured PWD MEFA in a vaccine strain for a modified live PWD vaccine candidate, and applied an heterologous immunization schedule – a primary with the monomer PWD MEFA protein intramuscularly and a booster with the modified live PWD vaccine candidate orally.

Results
The immunized piglets developed antibody responses to F4 and F18 as well as to LT, STa, STb and Stx2e. When challenged with a F18 ETEC strain, the control pigs developed diarrhea, and the immunized pigs did not and had a significant reduction of bacterial colonization of small intestines.

Conclusions
Results indicated that this PWD vaccine candidate is broadly immunogenic. Additional challenge studies with other F18 ETEC strains as well as with F4 ETEC strains will help to better evaluate vaccine efficacy against PWD.

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

Notes:

P136 - Polyanhydride nanoparticles induce innate activation of bovine epithelial cells and alveolar macrophages *in vitro*

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Session: Vaccinology

Objective
Bovine respiratory disease (BRD) is a leading cause of morbidity and economic losses in the cattle industry in the USA, despite the widespread use of antimicrobials and vaccines. Concerns about antimicrobial resistance have led to increased efforts on immunomodulation strategies to improve disease resistance in food animals. To date, there are no effective immunomodulation strategies to prevent BRD. Polyanhydride nanoparticles have intrinsic immunostimulant properties on myeloid cells and enhance adaptive responses when used as vaccine platforms. Here, we explore the potential of TLR-loaded polyanhydride nanoparticles (NPs) to activate epithelial and innate cells from the bovine respiratory tract

Methods
Bovine turbinate cells (BTs) and alveolar macrophages (AMs) were stimulated with 21 different nanoparticles or unloaded (empty) nanoparticles at 200 μg/mL. After 18h, RNA was isolated from cells, and transcriptional responses in key mucosal innate immunity targets were determined by RT-PCR

Results
Overall, NP-stimulated AMs show increased Il-1b and TNF transcription in comparison to unstimulated AMs, as well as robust upregulation of antibacterial mediators such as CXCL8 and iNOS. On the other hand, BTs response to NP stimulation is characterized by enhanced secretion of IL-6, CCL2, CCL5, and ISG15. Noteworthy, the addition of CL413 and MPLA agonists increases immunostimulatory capacity of NPs when compared to unloaded NP controls. To confirm these results, we are currently determining the effect of these nanoparticles on BTs and AMs at the protein level

Conclusions
Our results indicate that NPs can activate BTs and AMs, leading to different transcriptional profiles in response to different TLR cargos. There preliminary results suggest that polyanhydride NPs can be loaded with TLR-agonists to enhance the immune status in the respiratory mucosa and have the potential to be further explored in their ability to prevent respiratory infections in cattle by modulating cells at the frontline of defense

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

Notes:

P137 - Update on the impact of management decisions on BRD morbidity, mortality, and performance in beef calves

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Session: Vaccinology

Objective
1) Examine the effect of vaccination twice during preweaning on inflammatory mediators, preweaning performance, and BRD morbidity and mortality during backgrounding; 2) quantify the impact of marketing decisions on inflammatory mediators, BRD morbidity, mortality, and performance in weaned beef calves sent directly to backgrounding or sent via an auction market and order buyer; and 3) evaluate associations between pen- and yard-level management factors and health outcomes during the feedlot phase of production.

Methods
Objectives 1 and 2: In a 3-year randomized control trial with a split-plot design, 84 male calves per year will be vaccinated at ~90 and ~180 days of age with a 5-way modified-live respiratory vaccine or not during preweaning then marketed directly to a backgrounding facility or marketed via an auction market prior to transport to a backgrounding facility for 45 days. Blood was collected for analysis of inflammatory mediators, and performance, BRD morbidity, and mortality were recorded throughout.
Objective 3: An existing relational feedlot database was used to explore the impact of pen- and yard-level factors (stocking density, shared resources between pens, animal flow) on BRD morbidity and mortality using generalized linear mixed effect models ($p<0.05$).

Results
Objectives 1 and 2: Year 1: During backgrounding, 39.5% morbidity, 18.8% retreatment, and 1.2% mortality rates were recorded; average daily gain (ADG) was 1.11 kg ± 0.45. Blood inflammatory mediator analysis is underway. Year 2: During backgrounding, 10.7% morbidity, 0.0% retreatment, and 0.0% mortality rates were recorded; ADG was 1.24 kg ± 0.31. Full statistical analysis will be performed following the collection of Year 3 data.
Objective 3: Results of objective three have been finalized and published.

Conclusions
Objective 3 of this study is complete, with continuation of live animal data collection for Objectives 1 and 2 occurring in 2022-2023.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture; Agriculture and Food Research Initiative Competitive Grant No. 2019-67015-29845

Notes:

P138 - Mucosal vaccination with a nanoparticle and STING agonist combination adjuvant elicits cross-reactivity in pigs

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Session: Vaccinology

Objective
Adjuvants enhance immune responses to inactivated and subunit vaccines, but novel adjuvants for mucosal delivery must be developed. In this project, the immune response in pigs to an H1N2 split virus vaccine adjuvanted with a phytoglycogen-based nanoparticle (Nano-11) and a STING agonist (ADU-S100) was examined. The ability of the vaccines to induce antibody-secreting cells (ASCs) in the bone marrow, representing long-lived plasma cells, was investigated.

Methods
Inactivated detergent-split H1N2-OH10 influenza viral antigens were mixed with Nano-11 ± ADU-S100. ADU-S100 adsorption on Nano-11 was studied by UPLC/MS and particle size and Z-potential by dynamic light scattering. Vaccines were administered intranasally to pigs, followed by a booster dose and a viral challenge two weeks later. Swine serum, nasal swabs, lung lysate, and BAL fluid were tested for H1N1-OH7, H1N2-OH10, and H3N2-OH4-specific IgG and IgA. The ELISpot test was used to count H1N2 and H1N1-specific ASCs in vaccinated pig bone marrow.

Results
Nano-11 effectively adsorbed negatively charged ADU-S100 and H1N2 split antigens while maintaining a positively charged surface. Nano-11 ± ADU-S100 increased serum antigen-specific IgG production compared to the mock group. Nasal swabs from Nano-11 ± ADU-S100 vaccinated pigs had more antigen-specific IgA than mock-vaccinated animals. NanoS100-immunized pigs had higher antigen-specific IgG and IgA in their lung lysates. Comparatively, there was a considerable increase in antigen-specific IgA in the BAL-fluid of pigs vaccinated with NanoS100 versus Nano-11 alone. Nano-11 ± ADU-S100 increased H1N2 and H1N1 ASCs in the bone marrow. Nano-11 increased the number of H1N1-specific IgA ASCs; however, combining Nano-11 with ADU-S100 further increased the number of H1N1-specific IgA ASCs.

Conclusions
Intranasal injection of a swine split influenza vaccine adjuvanted with Nano-11 ± ADU-S100 increased vaccine-mediated immunogenicity and induced long-lived plasma cells in the bone marrow. These data support Nano-11 and NanoS100 as vaccine adjuvants.

Notes:

P139 - Micronutrient supplementation effects on redox balance and intranasal vaccine response in dairy calves pre-weaning

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Session: Vaccinology

Objective
Calves experience oxidative stress, decreasing lymphocyte function which might affect calves' response to vaccination. Supplementing micronutrients parenterally at birth has the potential to counteract the over production of pro-oxidants, ameliorating oxidative stress. The objective of this study is to determine the effectiveness of injectable micronutrient supplementation (IMS) at birth on redox balance and intranasal vaccine responsiveness in dairy calves from birth to weaning.

Methods
A total of 120 Holstein heifers from 2 farms (60/farm) were enrolled at birth and randomly allocated into one of four treatment groups: 1) control (saline), 2) IMS A (Zn, Cu, Se, Mn), 3) IMS B (Vit E, Se), and 4) IMS C (Vit A, D, E). Weight and hip height were recorded, and blood and nasal secretion samples were collected 2h after administration of colostrum. Calves then received the treatments and an intranasal vaccine against Bovine Rhinotracheitis, Parainfluenza 3, and Respiratory Syncytial Virus. Samples of blood and nasal secretions as well as health scores were collected weekly until weaning. Serum and nasal samples were flash frozen in liquid nitrogen then stored at -80°C pending analysis. Redox status was analyzed in serum as the ratio of reactive oxygen and nitrogen species to antioxidant potential. Micronutrient concentrations will be quantified in serum. Nasal anti-BHV1 and anti-BRSV IgA concentrations will be determined as proxy to intranasal vaccine response. All outcomes will be analyzed using mixed models with repeated measures, including the pre-treatment values as covariates in the models.

Results
We are currently finalizing the data collection and analysis and will update the abstract accordingly before the presentation.

Conclusions
Our results will determine the extent to which the different commercially available antioxidant supplements cause a biological change in calves' redox status and intranasal vaccine response. Moreover, analyzing the trends of the different micronutrients in serum over time will allow us to make evidence-based recommendations regarding re-supplementation.

Financial Support
Michigan Alliance for Animal Agriculture

Notes:

P140 - Assessment of an enterobactin conjugate vaccine in layers to protect offspring from colibacillosis

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Session: Vaccinology

Objective
Colibacillosis, caused by avian pathogenic *Escherichia coli* (APEC), is an important infectious disease in poultry. Newly hatched chicks display high mortality with colibacillosis. The novel enterobactin (Ent) conjugate vaccine is a promising vaccine candidate for controlling APEC. In this study, we aimed to determine if vaccination of layers with the Ent conjugate vaccine would protect hatchlings against colibacillosis through vertical transfer of protective antibodies.

Methods
Twelve breeder hens at 20 weeks of age were evenly assigned into 2 groups with one rooster (rode island red) being introduced to each group for fertilization. The layers in the treatment group were subcutaneously immunized with the Ent conjugate vaccine (100 μg per bird) three times with a 2-weeks interval while the other group served as non-immunization control. One week after the last vaccination, the eggs collected from each group were used for hatching. The newly hatched chicks (~30 birds per group) were housed separately. In each group, the chicks were intratracheally challenged with APEC O78 strain (10^7 CFU per bird) at 5 days of age. At two days post the challenge, all chicks were euthanized to assess lesions and APEC load in major organs. The egg yolks and blood samples (collected from both hens and hatchlings) were subjected to ELISA analysis.

Results
Vaccination of the Ent conjugate vaccine significantly increased (16 fold) serum anti-Ent IgY titer when compared to the control group. The Ent-specific InY titers were in egg yolk and hatchlings treatment group were also significantly increased 32-fold and 8 fold, respectively, when compared to the control. However, the APEC challenge only led to very mild lesions in liver, lung, spleen, heart, and air sac in both groups with no significant difference observed with respect to both lesion scores and APEC load.

Conclusions
Vaccination of layers with the Ent conjugate vaccine could lead to successful vertical transfer of anti-Ent IgY from the vaccinated hen to her offspring. The APEC challenge model needs to be optimized in the future.

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

Notes:

P141 - Vaccine candidates to sheep-associated malignant catarrhal fever: safety and efficacy in a laboratory model

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Session: Vaccinology

Objective
Sheep-associated malignant catarrhal fever (SA-MCF) is an often-fatal syndrome of ungulates caused by ovine herpesvirus-2 (OvHV-2). The virus is adapted to sheep, which serve as carriers, but can cause disease when transmitted to non-adapted species, including cattle, bison, and deer. SA-MCF is particularly important to the bison industry due to the high susceptibility of bison to the disease, with devastating outbreaks occurring when bison and sheep are kept in proximity. A vaccine to protect disease susceptible species is necessary to avoid animal losses and the negative impact of the disease on agriculture. The goal of this study was to evaluate the safety and protection efficacy three chimeric viruses that serve as vectors to OvHV-2 glycoprotein B (gB), which is known to induce protective immune responses.

Methods
Three non-pathogenic chimeric viruses, based on bovine herpesvirus 4 and a recombinant alcelaphine herpesvirus-1-ORF73-null, that express OvHV-2 gB were tested in immunization/challenge experiments using a laboratory rabbit model. Seven animals per group were immunized with the vaccine candidates using various vaccination regimes and delivery routes and then challenged with a lethal dose of OvHV-2.

Results
All vaccine candidates were deemed safe, as no local or systemic clinical signs were observed in any trial. Anti-OvHV-2 gB antibodies were detected in all vaccinated animals. Following challenge, protection rates in immunized animals ranged from 28.5 to 71.4%.

Conclusions
The safety and high protection efficacy of some vaccine candidates in the laboratory model were very promising and prompt us to perform vaccine trials in more relevant target species, including cattle and bison.

Financial Support
U.S. Department of Agriculture

Notes:

P142 - Recombinant vaccines against H1N1 swine influenza virus circulating in Brazil: immunization in a murine model

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Session: Vaccinology

Objective
In Brazil, at least four lineages of Swine Influenza Virus (SIV) circulate in pig population: H1N1 from the 2009 pandemic origin, H3N2 and H1N1 and H1N2 from human seasonal lineages (huH1N1 and huH1N2, respectively). Studies related to the occurrence of SIV in several Brazilian pig farms shows an increasing of huH1 lineages. The aim of this study was to construct inactivated recombinant vaccines against swine huH1N1 virus and test the immunogenicity in murine model.

Methods
The virus was constructed by reverse genetics using plasmids encoding the HA and NA sequences from a wild huH1N1 virus, isolated from an infected pig, and plasmids encoding the other segments of the A/PR8/34(H1N1) virus. The amplified recombinant, with 128 HA units, was inactivated and an oil in water (OW) and gel polymer (GP) adjuvants were used to formulate vaccines. Vaccination was performed by intramuscular route in 2 doses with 3 weeks interval. Mice of the C57Bl6/lineage were randomly divided into 8 groups as following: Group 1: OW vaccine; Group 2: PBS plus OW adjuvant; Group 3: GP vaccine; Group 4: PBS plus GP adjuvant; Group 5: live virus by intranasal route; Group 6: intranasal PBS; Group 7 and 8 did not receive any treatment. Except for Group 8, three weeks post last vaccination animals were challenged intranasally with a wild huH1N1 virus and observed during 14 days for weight changes. Serum samples were collected before immunization and after the first and second dose and neutralizing antibodies were measured.

Results
After the second dose, the vaccinated groups seroconverted with protective titers. Following challenge, the vaccinated groups had no significant weight loss compared to their control groups.

Conclusions
The results have shown that the WIV vaccines induced neutralizing antibodies by the IM route following 2 doses and have provided protection in a murine model. Reverse genetics approach can be used to produce new and updated swine influenza vaccines which is promising to apply for the control of SIV in Brazil.

Financial Support
Minas Gerais Research Funding Foundation (Fapemig); Brazilian National Council for Scientific and Technological Development (CNPq); Brazilian Coordination for the Improvement of Higher Education Personnel

Notes:

P143 - T cell Epitope Content Comparison of PCV2 strains from 2017-2021 confirms global relevance of a bivalent vaccine

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Session: Vaccinology

Objective
PCV2 is a globally important pathogen of swine with a high capacity for genetic change, potentially including evolution of strains less susceptible to vaccine-induced immunity. In silico tools can be used to predict the T cell epitope content of the PCV2 capsid protein in vaccines and field strains, allowing calculation of Epitope Content Comparison (EpiCC) scores reflecting the number of T cell epitopes held in common. Previous work showed that a bivalent PCV2a/PCV2b vaccine gave greater T cell epitope coverage (higher EpiCC scores) than either PCV2a or PCV2b as a monovalent, with the more complete match suggesting that vaccine efficacy may be preserved or enhanced. This study extends the above, using refined methodology to compare the putative T-cell epitope content of 4 PCV2 vaccines (3 based on PCV2a, and one a PCV2a/PCV2b bivalent) to a larger and more contemporary global sample of field strains.

Methods
PCV2 ORF2 nucleotide sequences (n=746) from diagnostic submissions dating 2017-2021 were analyzed. These comprised PCV2a (129), PCV2b (109), and PCV2d (508), and originated from Asia (185), Europe (269), North America (235), and South America (57). DNA sequences were translated to amino acid sequences, screened for class I and II T cell epitopes, and using the EpiCC algorithm, compared to those of 4 vaccines.

Results
EpiCC scores for the bivalent vaccine were significantly higher than for the monovalents for all genotypes in all regions, showing the global relevance of the bivalent approach. Of most practical relevance, given that most commercial vaccines are based on PCV2a, the addition of PCV2b increased T cell epitope coverage by 33% and 21% for PCV2b and PCV2d respectively.

Conclusions
The addition of genotype PCV2b to PCV2a in a single vaccine enhanced T cell epitope coverage against a, b and d field isolates, reflecting the value of an additional strain in countering genetic diversity within as well as between genotypes. The impact was consistent across regions and suggests a bivalent vaccine may result in enhanced and longer-term efficacy in the face of continuing virus evolution.

Notes:

P144 - Evaluating two live-attenuated vaccines against outbreak-associated *Salmonella enterica* serovar Reading in turkeys

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Session: Vaccinology

Objective
A recent foodborne outbreak of *Salmonella enterica* serovar Reading revealed the need for effective control of this serovar in turkey production. Vaccinations can reduce *Salmonella* in poultry, but a better understanding of vaccine efficacy against outbreak-associated *S.* Reading and the impact on turkeys is needed. This study assessed the ability of two live-attenuated *Salmonella* vaccines, the commercial AviPro® Megan® Egg vaccine and an internally developed cross-protective BBS 866 DIVA vaccine, to reduce *S.* Reading colonization in turkeys when given via two vaccination protocols.

Methods
In this study, male turkey poults were divided into six groups (Mock/Mock, Mock/Reading, BBS 866 O/O, BBS 866 A/W, AviPro O/O, and AviPro A/W). Birds were primary and booster vaccinated with 10^9 colony forming units (CFU) of the appropriate vaccine or an equivalent volume of PBS. Vaccinations were given by oral gavage (O/O) or by aerosol then drinking water (A/W) at 1 day and 3 weeks of age. At 7 weeks of age, poults were challenged with 10^9 CFU of outbreak-associated *S.* Reading (SX 446), except Mock/Mock received PBS. Cecal contents, cecal tonsil, cloaca, and spleen were collected at 2, 7, 14, and 21 days-post-inoculation (dpi) for *Salmonella* enumeration and enrichment (n = 12-16 birds/group/dpi), and pairwise contrasts of colonization (FDR < 0.05) were performed.

Results
Compared to the Mock/Reading group, all vaccinated groups had significant 1-3 $\log_{10}$ CFU/g reductions in cecal tonsil colonization at 7 and 14 dpi and cecal contents at 14 dpi. At 7 dpi, only oral gavage was significant in cecal contents, while three vaccinated groups decreased splenic *Salmonella* load. Four significant contrasts also suggested that oral gavage may be more effective than aerosol/water vaccination.

Conclusions
Vaccination with BBS 866 or AviPro® Megan® Egg significantly reduced colonization by *S.* Reading in turkeys, indicating that these vaccines are cross-protective and could be a potential intervention strategy against this serovar, thereby providing consumers a safer food supply.

Financial Support
U.S. Department of Agriculture, Agriculture and Research Services; U.S. Poultry and Egg Association

Notes:

P145 - Development of a broadly protective vaccine against swine influenza A virus based on the matrix 2 envelope protein

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Session: Vaccinology

Objective
Swine influenza A virus (swIAV) is one of the top pathogens affecting pigs in all phases of pork production. The protection provided to swine by commercial whole inactivated virus (WIV) influenza vaccines against contemporary swIAV is limited, due in part to the constantly increasing antigenic diversity of the virus hemagglutinin (HA). The substantial diversity of the HA among co-circulating swIAV in swine herds poses a significant challenge for effective vaccine development. Notably, the matrix 2 (M2) protein of swIAV is a highly conserved protein present in the virus envelope. More than 98% of IAV strains circulating in U.S. swine herds share the same M2 isoform. M2 is a 97 amino acid residues long tetrameric type III membrane protein that acts as a viroporin, consisting of an intracellular C-terminal domain (positions 47 to 97), a transmembrane domain (positions 24 to 46), and an extracellular N-terminal domain (positions 1 to 23). The ectodomain of M2 (M2e) has been pursued for many years as candidate antigen for a potential universal influenza vaccine for humans.

Methods
In this study, we examined the immunogenicity and protective efficacy of a novel M2 protein-based swIAV vaccine in weaner pigs. This vaccine consists of recombinant full-length M2 protein, which is displayed in soluble nanoscale membrane assemblies called nanodiscs (M2:NDs) in its natural transmembrane configuration.

Results
We determined that intramuscular immunization with M2:NDs elicited the production of antibodies capable of recognizing swIAV virions as well the generation of virus-specific interferon-gamma-producing cells. Further, protective immunity against swIAV challenge was also generated as indicated by a reduction of viral load in the lungs of swine challenged intratracheally with H3N2 swIAV.

Conclusions
These studies demonstrate that the M2 protein incorporated into the NDs are immunogenic and can provide protective immunity against swIAV. Future work will aim to improve the formulation of the M2:ND vaccine to increase its immunogenicity and the level protective efficacy afforded by this novel vaccine.

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

Notes:

P146 - Mannose-chitosan nanoparticle coated influenza vaccine potentiates cross-protective cellular immunity in pigs

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Session: Vaccinology

Objective
Swine influenza A virus (SwIV) is a major threat to the swine industry, and vaccination is the most viable strategy to control SwIV infections. Poor induction of mucosal and cellular immunity in the respiratory tract by current intramuscular SwIV vaccines is responsible for limited cross protection. Our objective is to improve the mucosal and cellular immunity induced by whole inactivated SwIV entrapped mannose-chitosan nanoparticle (mChit-SwIV-NP) vaccine by including a STING (stimulator of interferon gene) adjuvant.

Methods
We developed mChit-SwIV-NP vaccine containing whole inactivated SwIV H1N2 and STING adjuvant ADU-S100, either encapsulated (mChit-SwIV+S100-eNP) or surface adsorbed (mChit-SwIV+S100-sNP). Influenza free nursery pigs were vaccinated intranasally twice at 3-week intervals and challenged with the pandemic 2009 H1N1 virus. Nasal swabs collected at day post challenge (DPC) 2, 4 and 6 (necropsy day) were analyzed for infectious virus load. Peripheral blood mononuclear cells (PBMCs), bronchoalveolar lavage fluid (BAL) cells, and tracheobronchial lymph nodes mononuclear cells (TBLN MNCs) isolated at DPC 6 were evaluated for virus specific activated lymphocyte subsets by flow cytometry and T-cell proliferation.

Results
The infectious virus load was reduced by around one $\log_{10}$ in the nasal passage of both mChit-SwIV+S100-eNP and mChit-SwIV+S100-sNP vaccinates, with the latter performed relatively better. Immunologically, the frequency of activated $IFN\gamma^+$ and $IL\text{-}17A^+$ cytotoxic T lymphocytes and T-helper/memory cells in PBMCs and TBLN MNCs were increased in mChit-SwIV+S100-sNP vaccinates better than mChit-SwIV+S100-eNP group. While in TBLN MNCs, virus specific increase in lymphocytes stimulation index was observed in mChit-SwIV-eNP vaccinates.

Conclusions
In summary, the electrostatic surface conjugation of preformed mannose-chitosan nanoparticles with SwIV antigen and STING adjuvant stimulated the cross-protective specific mucosal and cellular immune responses in intranasal vaccinated pigs better than their cohort, a potential vaccine candidate to mitigate swine influenza in pigs.

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

Notes:

P147 - Exploring the role of zinc in improving neonatal dairy calf vaccine responsiveness

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Session: Vaccinology

Objective
The oxidative stress (OS) experienced by dairy calves during the preweaning period compromises their immune function, limiting their ability to respond to vaccines. Micronutrients such as Zn, which is chaperoned by metallothionein (MT), are able to combat OS and could support immune function. Our proposal seeks to elucidate the effect that Zn has on improving calf lymphocyte functions relevant to vaccine responsiveness by ameliorating OS through increased MT.

Methods
Zn will be supplemented to PBMCs isolated from dairy calves to enhance MT production and siRNA will be utilized to inhibit MT in vitro. MT will be quantified using gene and protein expression. Cells will be oxidatively challenged and activated. OS will be assessed by measuring reactive oxygen species and isoprostane production. Both humoral (antibody production) and cell mediated adaptive (T cell activation and proliferation, immunophenotyping, and cytokine production) immunity will be evaluated in vitro. In vivo, dairy calves will be supplemented with Zn in milk replacer and their response to vaccination assessed via humoral (titers) and cell mediated adaptive responses for the primary and secondary responses. Oxidative status of the calves will also be monitored.

Results
We anticipate that MT knockdown will result in reduced lymphocyte function due to an inability to cope with OS. We also expect that Zn supplementation will increase MT and rescue the OS-affected lymphocyte functions needed for vaccine responsiveness. We will determine the Zn concentration necessary for improving lymphocyte functions in vitro. Subsequently, we will use this concentration as the target blood concentration for our in vivo trial. We anticipate that dietary Zn supplementation will improve calves' response to vaccination.

Conclusions
We will characterize the role of Zn and MT in enhancing lymphocyte functions at a time when calves experience OS. This research is the first step in establishing preweaning Zn supplementation recommendations for dairy producers.

Financial Support
U.S. Department of Agriculture

Notes:

P148 - A platform-based vaccine approach to emerging minor species diseases

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Session: Vaccinology

Objective
Minor-species animals, including wild animals, can serve as important reservoirs of both animal and zoonotic diseases. While many of these diseases are vaccine-preventable, the development, licensing, and implementation of vaccines against minor species can be difficult and expensive. As a result, relatively few vaccines are specifically available for the prevention of disease in these species. Platform based vaccines, licensed under new "prescription-platform" regulations from the USDA are a potential solution to this problem.

Methods
We have reported tthe rapid development and commercial availability of a vaccine against Rabbit Hemorrhagic Disease Virus 2. Since the prior report, we have completed over a year of field deployment indicating a high safety and performance profile. In addition, a similar platform-based approach was used to develop a vaccine to prevent Epizootic Hemorrhagic Disease (EHD) of white-tailed deer and other cervids. Given the difficulties associated with direct challenge experiments, this vaccine has been available under ongoing field evaluation studies, in areas of significant disease spread, for several years.

Results
Furthermore, recent data indicates high titers of EHD-specific antibodies can be produced not only in vaccinated deer, but passive immunity appears to be passed to fawns. This is critical, due to the fact that EHD season correlates very strongly to the late suckling/early weaning period of fawns. These results indicate a level of protection likely available to the fawns during this early stage, and further studies may be used to determine optimal protocols for vaccination of these animals.

Conclusions
These vaccines were available to be used in these minor species due to the unique regulatory and developmental tools available through Platform-based vaccine products, and can be leveraged to produce vaccines for other animal species with very limited market sizes. They present options for development and availability of vaccines that otherwise would represent non-commercial opportunities for minor species.

Notes:

P149 - ZFP36 Ring Finger Protein Like 1 significantly suppresses human coronavirus replication

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Session: Virology

Objective
There is strong evidence that severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a betacoronavirus can spill over from humans to animals such as mink, dogs, lions, tigers, and domestic cats which showed SARS-CoV-2 like symptoms. Additionally, free-living white-tailed deer in the United States also revealed the presence of SARS-CoV-2 antibodies in them. In the current study, we used another member of human betacoronavirus, strain OC43, and measured the effect of zinc finger protein 36L1 (ZFP36L1) on virus replication with a future goal that ZFP36L1 could be used as a potential intervening target for SARS-CoV-2 replication. ZFP36L1 is a member protein of CCCH type Zinc finger protein family. It is well characterized as an RNA-binding protein that controls cellular mRNA turnover by degrading its poly A tail.

Methods
We overexpressed or knockdown ZFP36L1 in HCT-8 cells and infected them with HCoV-OC43, and measured time course virus production.

Results
Results showed that HCoV-OC43 replication was significantly reduced with overexpression of ZFP36L1 while knockdown of ZFP36L1 significantly enhanced the virus replication as compared with wild-type HCoV-OC43 infected cells ($p<0.05$). Knocking down ZFP36L1 facilitated the infectious virus production as early as 48 hours p.i. while wild-type or ZFP36L1 overexpressed cells start producing infectious virus at 72 hours p.i. Virus titer at 96 hours p.i. in ZFP36L1knockdown cells (5.85±0.01 log 10/ml) were significantly higher than virus titer in wildtype cells (5.42±0.10 log10/ml) or ZFP36L1 overexpressed cells (4.32±0.00 log 10/ml) ($p<0.05$). The comparison of virus titer in wild-type and ZFP36L1overexpressing cells also showed that ZFP36L1 overexpressing significantly suppressed virus titer at 96 hours p.i as compared to wild-type cells.

Conclusions
Overall, the current study revealed that ZFP36L1overexpression suppressed the HCoV-OC43 replication. Future studies are needed for its potential application in mitigating the HCoV-OC43 mediated adverse effects in the host and the role of ZFP36L1 in another human coronavirus replication.

Notes:

P150 - Characterization of age-dependent effects of influenza A virus and PRRSV infection on porcine macrophages

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Session: Virology

Objective
Macrophages have central roles in the innate immune response and inflammation. In the lung, macrophages are located beneath the respiratory epithelium and are part of the first-line defences. In the case of a swine Influenza A Virus (swIAV) or Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) infection, they can also be an early infection target. Among swine, young pigs are most affected by respiratory diseases and work in humans revealed that macrophages derived from newborns exhibit impairments in many functions, including chemotaxis, phagocytosis and TLR function. Thus, in this project, we aimed to determine the influence of age on infection dynamics of swIAV and PRRSV on porcine macrophages.

Methods
Porcine alveolar macrophages or monocyte-derived macrophages (MDMs) were used to evaluate differences between swIAV (H1N1) and PRRSV (VR2385). Multiple time points were included in the experimental setup. Viability and infection rate as well as cell surface markers were analyzed by flow cytometry. The number of infectious virus particles was quantified by using the median tissue culture infectious dose (TCID50) assay.

Results
SwIAV induced a rapid cell death at a considerably higher rate than PRRSV (95% vs 30%) and intracellular influenza protein was detectable as early as 6hpi, whereas the intracellular detection of PRRSV was delayed to 18hpi. We then included the factor age and our results indicate that swIAV-infected MDMs from young pigs showed a significantly higher cell death rate than MDMs from sows. In contrast, there was no age-dependent difference in infection or cell death rate for PRRSV. The TCID50 assay indicated that the release of infectious viral particles of both viruses takes place in macrophages.

Conclusions
The comparison of swIAV and PRRSV on macrophages revealed striking differences, including an age-dependent effect with potential implications on viral neutralization and inflammation. The underlying mechanism will be part of future research, eventually leading to a better understanding of the host inflammatory response and its influence on lung pathology.

Financial Support
Saskatchewan Agriculture Development Fund; Natural Sciences and Engineering Research Council of Canada

Notes:

P151 - Investigations into factors that drive the emergence of novel influenza reassortants in pigs under field conditions

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Session: Virology

Objective
Influenza A virus (IAV) is one of the most important respiratory pathogens with high economic impact. Pigs can become infected with IAV of different origin including avian, pigs and humans. The introduction of human-origin IAV into pigs, and the coexistence with swine endemic strains of different lineages in pig populations create conditions for IAV to reassort. Therefore, is imperative to carry out investigations to explore reassortant events at the pig level under field conditions and investigate which factors may drive the emergence of new reassortant viruses. The aim of this study is to Identify and characterize IAV reassortants at the farm level, and evaluate the association of pig subpopulations and immunity with the emergence of IAV reassortants.

Methods
We will target an IAV positive nursery farm and its associated flows from weaning to market. A farm coinfected with more than one IAV subtype will be selected. 60 will be selected at each sampling event and nasal swabs collected at four different time points (serial cross-sectional study). Samples will be tested with a screening RT-PCR and characterized with specific subtype RT-PCR to confirm the co-circulation of more than one IAV subtype. Viral RNA for complete genome sequencing will be obtained from the viral plaques and submitted for sequencing. Genomes will be considered whole when complete sequences for eight segments are obtained. Additionally, blood sample will be collected from all pigs to evaluate impact of immunity on reassortment events. Three variables will be considered for statistical analysis: (a) pig subpopulation, (b) farm, and (c) immunity to predict frequency of reassortants detected.

Results
We expect to:
1.- Identify, quantify, and characterize the amount and type of reassortment events taking place in the pigs
2.- Provide understanding on the emergence, persistence, and subsidence of reassortant viruses under field conditions
3.- Identify factors that may drive influenza reassortment under field conditions
4.- Provide a glimpse on the impact that reassortment has as a source of new and evolving IAV and factors that drive the emergence of reassortants in pigs.

Conclusions
This study is crucial to have effective protocols to control. eliminate influenza in pigs, and prevent the risk of influenza transmission to people.

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

Notes:

P152 - In vitro studies of viral resistance to FIPV protease inhibitors

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Session: Virology

Objective
Feline infectious peritonitis virus (FIPV) is a virulent feline coronavirus that causes a fatal systemic infection, feline infectious peritonitis, in cats. We have previously developed small molecule inhibitors against 3C-like protease (3CLpro) of FIPV, including GC376. These inhibitors are active against multiple coronaviruses including SARS-CoV-2 and MERS-CoV. Viral resistance and drug efflux are important consideration in antiviral treatment for it may compromise drug efficacy. The objective of this study is to identify mutations in FIPV that confer resistance to GC376 and its derivative GC1003.

Methods
FIPV 79-1146 strain were serially passaged in CRFK cells in the presence of sequentially increasing concentrations of GC376 or GC1003, or mock medium (control). The changes in inhibitory effects of each compound were monitored by measuring 50% effective concentrations (EC50s) during serial viral passages, and the 3CLpro gene of passaged virus was sequenced. Once mutations in the 3CLpro were identified, recombinant 3CLpro with the identified mutations were generated, and the 50% inhibitory concentrations (IC50s) of each compound were determined in the enzyme assay and compared to that of the wild-type 3CLpro.

Results
After eight or twenty passages of FIP in the presence of GC376 or GC1003, the EC50s increased by about 13- or 5-folds, respectively, compared to those against FIPV passaged in mock medium. Sequencing of 3CLpros revealed mutations (G23V and G298S) in the virus passaged in the presence of GC1003 but none was identified with GC376. However, in the enzyme assay, only moderate increase in the IC50 of GC1003 was observed with the 3CLpro carrying the mutations. Interestingly, addition of inhibitors of P-glycoprotein (p-gp), a drug efflux pump, to viral passages in the presence of GC376 or GC1003 restored viral susceptibility to GC376 or GC1003.

Conclusions
Our results suggest that 3CLpro mutations confer partial reduction in drug susceptibility, and changes in p-gp efflux activity may contribute to the observed reduction in drug susceptibility to FIPV.

Financial Support
U.S. National Institute of Allergy and Infectious Diseases

Notes:

P154 - The presence and impact of bovine leukemia virus on a small research dairy

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Session: Virology

Objective
Bovine Leukemia Virus (BLV) is a retrovirus affecting bovine species that causes lymphocytosis in ~25% of infected cattle, and in terminal stages can cause lymphosarcoma. Estimates in the 1990s suggest BLV costs the United States dairy industry over $44 million annually, mostly attributed to replacement costs, reduced milk production, and removal of tumorous animals at the abattoir. BLV is present in more than 50% of dairy herds, of which, 70% of animals test antibody positive. Despite its impact, remarkably little is known about disease dynamics and transmission.

Methods
From Fall 2017 to present, data was collected from mastitic and periparturient cows and calves from the National Animal Disease Center research dairy herd. When possible, colostrum, CBC, and serum samples were collected from cows on day of calving. Calves are hand-reared and serum and CBC blood samples were collected pre and post-colostrum consumption. In cows with experimental or naturally occurring mastitis, milk samples were collected from infected quarters prior to treatment. Antibody testing of serum was performed in house by the Animal and Plant Health Inspection Service and virus presence was determined using a nested PCR of milk and colostrum samples.

Results
BLV positivity in cows was correlated with lower refractive index scores of colostrum quality and associated with increased number of dam lymphocytes on day of calving. Calves fed colostrum from BLV positive dams were positive for BLV antibody post colostrum consumption, however virus was only detectable in 22% of colostrum from BLV positive dams. Interestingly, mastitic milk from BLV positive cows was virus positive 55.6% of the time, indicating that milk more often than colostrum could be passing virus to future generations.

Conclusions
Persistent shedding of BLV impacts the production and animal health of the dairy industry. This work characterizes immune profiles of BLV positive cows through mastitis and calving events and suggests that producers should consider the BLV status of the animals they source colostrum and milk from before feeding to calves.

Notes:

P155 - Identification and characterization of nidovirus-host molecular interactions using global proteomic profiling

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Session: Virology

Objective
Porcine reproductive and respiratory syndrome virus (PRRSV) and porcine epidemic diarrhea virus (PEDV) are responsible for severe economic losses worldwide. We propose to investigate the molecular mechanisms of nidovirus-host interactions, because we believe that our insufficient understanding of these interactions hinders the development of new strategies for effective control of viral infections.

Methods
Virus-host interactions are highly dynamic, leading to important changes in the intracellular levels of cell proteins. Moreover, virus-induced modulations of the intracellular environment create more favorable conditions for viral infection and spread. Consequently, comparative proteomics of the nidovirus-infected cells in a time-resolved manner will provide global mapping of virus-host interactions. Growing evidence indicates that extracellular microvesicles (EMV) play an important role in viral pathogenesis and modulation of host immune responses to infection. The cellular proteins, specifically encapsidated into virions, could play important roles for viral pathogenicity. Specifically, we analyzed proteomic patterns of host cells during viral infection and characterized protein composition of the virions and EMV produced by virus-infected cells.

Results
We found that PRRSV and PEDV infections affected the abundance of numerous host proteins associated with EMV. Our data showed that viral infections resulted in significant alterations in the host cell proteome. We also found that both viruses induced specific changes, unique to their molecular pathogenesis. E.g., the abundance of proteins involved in immune responses was changed in PEDV infected cells. In PEDV infected cells, host proteins involved in cell cycle regulation and the cytoskeletal system were affected in abundance. Moreover, PEDV and PRSV significantly modulated biological pathways such as entry into the host cells, type I IFN signaling, defense response to viral infection, etc.

Conclusions
Further investigations are needed to evaluate the role of individual cellular proteins in nidoviral replication, assembly, and pathogenesis.

Financial Support
Natural Sciences and Engineering Research Council of Canada; Fonds de recherche du Québec

Notes:

P157 - Risk communication challenges in avian influenza

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Session: Virology

Objective
The goal was to evaluate knowledge and understanding of people about H5N1 and prepare the appropriate guidelines

Methods
Participants were select from the personnel Veterinary Scientific Research Institute. Information was collected using a self-administered questionnaire.
A total of 55 interviews were conducted. The main questions were obtained of knowledge about the self-protection and risk degree of virus

Results
More than 90 % of respondents answer were: don't know. In generally 95% of participants received H5N1 information via television and the Internet. Most the participants are generally not aware that government plans are in place; the people not understand the serious of threats which comes from virus.

Conclusions
Common Issues and Solutions includes:
Information Tools-Develop a risk communications tool-box; guidelines, procedures, and information to facilitate effective communication of pertinent information to the public and media; increasing of awareness and preparedness. Increase an interactive process of information and opinion exchange with individuals, groups, or institutions about real or perceived risks. Improving risk understanding, affecting risk perception, and/or equipping people or groups to act appropriately in response to an identified risk.

Notes:

P158 - Avian influenza virus phylodynamics – patterns of transmission between species and geography

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Session: Virology

Objective
A highly pathogenic avian influenza (HPAI) virus lineage with H5N1 subtype emerged in China in 1996, which has caused severe disease in poultry and wild bird species. In recent years, this lineage of HPAI H5 viruses have diversified by swapping different neuraminidase genes of various subtypes known as NX. In 2014/2015, outbreaks of HPAI H5N8/X devastated poultry in North America, Europe and Asia. Subsequently, in the 2016/2017 and 2020/2021 autumn/winter seasons H5N8/X again caused outbreaks in poultry in Asia, Europe and North America. The objectives of this research are to develop mathematical models of viral evolution to produce spread and species risk maps with genuine predictive value that can be used to inform vaccination and other control strategies.

Methods
Using phylodynamic and phylogeographic methods, the H5N8/X spreading pattern across continents has been reconstructed. This procedure uses viral sequence data at the field / population scale, and creates spatial-species time-scales phylogenetic trees from which sequence evolution parameters are inferred from nucleotide substitution rates, along with rates of cross species transmission, and spatial diffusion rates and routes using a combination of discrete trait, continuous trait and structured coalescent phylodynamic methodologies. Sequence data from public databases will be combined with the new data from China, Euope and U.S. and used to create the time scaled phylogenetic trees.

Results
Reconstructing the spatial spread with continuous trait phylogeography together with the transmission between bird species types, we demonstrate long-range transmission mediated by wild migrating anseriformes. We find that the highly pathogenic H5N8/X viruses undergo frequent reassortment with other co-circulating low pathogenic viruses in the wild bird population, and that H5N8 may persist in wild populations between breeding and wintering seasons.

Conclusions
This work provides preliminary insight into the global phylodynamics and transmission patterns of avian influenza viruses.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture; Biotechnology and Biological Sciences Research Council; China Agriculture Research System

Notes:

P159 - Characterization of canine respiratory epithelial cells system to study virus infection involved in kennel cough

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Session: Virology

Objective
Canine infectious respiratory disease complex (CIRDC) also known as 'kennel cough' is an acute, highly contagious respiratory infection of dogs. Viral, host and environmental factors determine the development of disease and its severity. Viral pathogens associated with CIRDC include canine adenovirus type 2 (CAV-2), canine distemper virus (CDV), canine herpesvirus-1 (CHV-1) and canine influenza virus (CIV). Viral infection initially damages the epithelium of the upper respiratory tract and increase the inflammation in the upper respiratory tract. Availability of a robust and ethical *in vitro* model representing the natural airway is critical to study pathogenesis in host responses associated with canine respiratory infections. Our hypothesis was, that in-vitro model of canine respiratory epithelial cells grown at air liquid interface (ALI-CRECs) can be establish and will resemble the natural airway morphologically and immunologically upon virus infection.

Methods
We collected epithelial cells from tracheas of 5 respiratory healthy dogs that were euthanized for unrelated reasons. Cells were isolated, cultured, characterized morphologically and immunologically before infecting them with CDV, CHV-1, CIV & CAV-2. Characterization of immune response included comparing expression of TLRs, interferons, cytokines and chemokines in dog's tracheas, freshly isolated CRECs and ALI-CRECs by Reverse transcriptase polymerase chain reaction and Reverse transcription-quantitative polymerase chain reaction (RT-qPCR). mRNA expression of interferon-stimulated genes in infected ALI-CRECs and non-infected ALI-CRECs done by RT-qPCR.

Results
We found that ALI-CRECs resembled the natural airway morphologically and immunologically. ALI-CRECs supported infection with CDV, CHV-1, CIV & CAV-2 and responded with increased expression of interferon-stimulated genes.

Conclusions
ALI-CRECs maybe ideal systems to study the molecular pathogenesis and induction of innate immune responses upon infection with kennel cough viruses and they can be used to test efficacy of antivirals aimed to prevent viral replication and spread.

Financial Support
Morris Animal Foundation; Michigan State University

Notes:

P160 - Competence of North American mosquitoes to Japanese encephalitis virus genotype II

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Session: Virology

Objective
The recent introduction of Japanese encephalitis virus (JEV) into piggeries across Australia caused significant reproductive and neonatal loss. This outbreak emphasized the risk of JEV introduction into North America. As JEV is primarily transmitted by Culex (Cx.) mosquito species, this study examined the competence of Cx. tarsalis to JEV.

Methods
JEV genotype II (GII) was propagated in porcine kidney epithelial cells (SK-RST) and C6/36 cells derived from Aedes albopictus mosquitoes. Mosquitoes were fed artificial infectious blood meals and sorted. Individual mosquitoes were collected immediately following feeding, homogenized and virus titrated using standard plaque assay. The abdomen, secondary tissues, and salivary glands were dissected from mosquitoes at 3, 7, and 14 days post infection (dpi). The tissues were homogenized, and infectious virus was examined by passaging the homogenate on BHK-21 cells and monitoring for cytopathic effect.

Results
JEV infection was detected in Cx. tarsalis mosquitoes with dissemination occurring by 3 dpi infection. Furthermore, JEV was present in the salivary glands at 7 dpi. To examine effects of host on the competence of Cx. tarsalis, virus dissemination was compared between swine- and mosquito-cell derived virus. Dissemination to secondary tissues was present at 3dpi in 40% and 20% of the mosquitoes infected with swine- and mosquito-derived virus, respectively. By 14 dpi, virus was detected in 20% of the salivary glands for mosquitoes infected with the swine-derived virus compared to less than 10% infected with the mosquito-derived virus. The mosquitoes ingested similar amounts of the different virus stocks; and therefore, the differences identified early in the infection were not due to the concentration of ingested virus.

Conclusions
This study suggests that Cx. tarsalis mosquitoes are competent vectors of JEV GII and different virus hosts could affect JEV transmission if introduced to North America. This data further demonstrates the risk of JEV spread in North America due to the presence of susceptible hosts and vectors.

Financial Support
U.S. Department of Agriculture

Notes:

P161 - Enhancing the production of type I interferons to create rationally-defined Marek's disease vaccines

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Session: Virology

Objective
Marek's disease (MD) is an oncogenic disease of poultry caused by Marek's disease virus (MDV), and commonly controlled by vaccination with a live attenuated virus strain. Vaccine breaks have been common in the past, making the need for novel vaccine development. Viruses encode gene products which inhibit secretion of the type I interferons (IFN-Is). We will ablate MDV genes which frustrate the production of IFN-Is during infection to create vaccine strains with improved protection. Our collaboration with the Boeke laboratory (NYU-Langone) will give us access to rapidly-assembled MDV viral genomes. This proposal will result in new and more protective vaccine strains for MDV.

Methods
In the initial stages of this proposal we will create stably-expression HD11 cells using lentivirus transfection. This cell line will be used to test MDV genes for their effects upon the production of IFN-Is in isolation (rather than integrated into a viral genome). Our first targets are MDV US3, UL46 (both non-essential), UL9, UL26 and UL48 (all essential for viral replication).

Results
The Dunn laboratory has hired a new postdoc and are creating a transformed avian monocytic cell line in which the initial genes in our study (see the previous paragraph for a list of genes) are stably expressed in HD11. The Boeke lab is currently attempting to assemble fragments of the MDV genome from a phage library supplied by ADOL.

Conclusions
We plan to have candidate viruses ready to evaluate during the next performance period and anticipate that this project will result in several new and highly effective vaccine candidates for MDV.

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

Notes:

P162 - Genetic variations of bovine viral diarrhea virus (BVDV) circulating in the Republic of Korea

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Session: Virology

Objective
Bovine viral diarrhea virus (BVDV) is the most important viral pathogen leading to substantial economic losses worldwide due to gastroenteritis, respiratory diseases, and reproductive problems in the cattle. BVDV is divided into three distinct species, BVDV1, BVDV2, and BVDV3. This study aimed to compare the sequences of three BVDV subtypes reported in the Republic of Korea (ROK) by year.

Methods
A total of 3,669 bovine sera were obtained from the Veterinary Service Laboratory in Gyeongbuk province, ROK and RNA was extracted from these samples. BVDV was screened by real-time RT-PCR and positive samples were sequenced to determine the subtypes of BVDV.

Results
By real-time RT-PCR, 846 sera (23.1%, 846/3,669) were positive and 87 were successfully sequenced. Among them, BVDV1b was the most detected (40/87), followed by BVDV2a (31/87), and BVDV1a (16/87). BVDV1a had the largest genetic variations in 2017 and thereafter gradually decreased. In the case of BVDV1b, significant variations were mainly found in 2018, and some of these variations were constant through 2021. The sequences detected in 2022 showed nucleotide substitutions only in specific locations, unlike others reported before 2022. The BVDV2a sequences identified in 2016 were similar to USA strains and to Korean isolates from 2017 to 2018. However, the BVDV2a found in 2021 had only three nucleotide substitutions. In all three subtypes, genetic variations appeared remarkably until 2018. Notably, BVDVs identified in the ROK were significantly different from other countries.

Conclusions
This study showed the presence of three BVDV subtypes in cattle in the ROK. Our findings suggest that there are genetic differences depending on the country, year, or both. The sequences of three subtypes detected in 2021–2022 revealed that the genetic variation was markedly decreased compared to those before 2021. This may be explained by the decline in international trade, such as animal and human movements due to COVID-19. Collectively, these results highlight the development of diagnostic kit or vaccine suitable for each country to eradicate BVDV.

Financial Support
Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, and Forestry (IPET) Grant No. 122017-02-1-HD020

Notes:

P163 - Severe hemorrhagic disease associated with bovine viral diarrhea virus2a in Korean native cattle

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Session: Virology

Objective
Bovine viral diarrhea virus (BVDV) is the most important pathogen causing diarrhea, respiratory disorders, reproductive failures, and immunosuppression in cattle worldwide [1]. To date, BVDV has been classified into three species, BVDV1, BVDV2, and HoBi-like virus based on the 5'-UTR region. In particular, BVDV2 has been associated with severe acute clinical signs and hemorrhagic disorders. This report describes spontaneous severe hemorrhagic disease outbreaks caused by BVDV2 infection in two steers in the Republic of Korea.

Methods
On July 7th and 22nd in 2022, two steers which are 1-year-old in the same herd successively exhibited severe hemorrhagic diarrhea. Outbreak 1 showed severe dehydration and died after three days, while outbreak 2 presented depression and astasia. Only outbreak 2 was autopsied, and blood and tissue samples were collected. Total RNA was extracted from collected samples, applied to RT-PCR, and directly sequenced. The tissue samples were used for histological examination and immunohistochemistry (IHC).

Results
BVDV was detected in all tissue samples by RT-PCR, and assigned to BVDV2a by phylogenetic analysis. The necropsy showed ulceration, inflammation, and hemorrhages in the mucosa of the pylorus, extensive petechiae and blood clots as well as broad hemorrhages in the colon, with brown and bloody fluid contents in the ileum. Histologically, there was ulcerative abomasitis and residual inflammatory exudates in the abomasum. Erosive enteritis was observed in the colon. In the small intestine, infiltration of macrophages, destruction of epithelial cells in the mucosa, and necrotic-hemorrhagic enteritis were found. In addition, BVDV Ag was widely detected in all tissues examined by IHC analysis.

Conclusions
These findings show that BVDV2a is associated with severe hemorrhagic diarrhea and extensive enteritis. It is speculated that astasia may be caused by BVDV2a infection in the cerebellum. Although it is not clear whether BVDV2a alone caused serious illness in steer, this report indicates that BVDV2a may contribute to severe disease and mortality in cattle in the ROK.

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Notes:

P164 - Permissiveness of the ZMAC cell line for African swine fever live attenuated or wild-type viruses

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Session: Virology

Objective
Traditionally, African Swine Fever virus (ASFV) isolation from clinical or field samples as well as propagation of laboratory strains is often conducted using swine primary monocyte-derived (MDM), lung (PAM), or bone marrow macrophages. In the current study we tested the USDA CVB-approved porcine alveolar macrophage ZMAC cell line for the ability to support stable passage and replication of live attenuated viruses (LAV (genotype II), and wild type isolates (genotypes I, II, V and X), including the current genotype II pandemic strain, ASFV-G.

Methods
ASFV live attenuated viruses ASFV-G-ΔMGF and ASFV-G-Δ9GL/ΔUK as well as wild-type strains: Georgia (ASFV-G), Haiti, Lisbon 60, Brazil '78, Dominican Republic I, Tengani, Lee, Uganda, and Zaire were cultured in either ZMAC cells or PAMs for growth comparison. Viral titers were determined by hemadsorption (HAD50). DNA from ASFV-G-Δ9GL/ΔUK ZMAC passages 5 and 10 was extracted and sequenced on an Illumina Nextseq. Alignment of both sequences were analyzed in TABLET for discrepancies against the reference sequence ASFV Georgia 2007/1 (GenBank: FR682468.2).

Results
Following serial virus passages of each of the 11 ASFVs on the ZMAC cell line, results showed higher titers on ZMAC cells compared to PAMs or MDMs. In addition, HAD50 titers were higher following virus incubation at 37°C than at 30°C. Viral DNA extracted from ASFV-G-ΔMGF -infected ZMAC cell passages 5 or 10 had no detectable sequence differences following next generation sequencing analysis, suggesting that the ZMAC cell line supports stable passaging of live, attenuated viruses.

Conclusions
Our results confirm previous studies and demonstrate diagnostic application of the ZMAC cell line for ASFV isolation from naturally-infected field samples such as spleen and blood, and for the stable propagation and production of ASF LAV vaccine candidates.

Financial Support
U.S. Department of Homeland Security; National Pork Board

Notes:

P165 - Transmission of SARS-CoV-2 in dogs and pathological changes in the lung and brain

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Session: Virology

Objective
Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is a causative agent of COVID-19 pandemic. Human and various animal species are susceptible to SARS-CoV-2 and zoonotic transmissions have been occurring. Dogs, one of the most popular companion animals, are also susceptible to this virus. A few studies had detected SARS-CoV-2 in pet dogs which were almost asymptomatic.

Methods
Here, dogs were experimentally infected with SARS-CoV-2 (delta variant, GK clade, AY.69 lineage) and naïve dogs were placed in the same cage as sentinels (contact group). Nasopharyngeal, oropharyngeal, blood, and fecal samples were collected periodically until 35 days post infection (dpi). SARS-CoV-2 RNA was detected from 4 to 14 dpi in nasopharyngeal and oropharyngeal samples and from 11 to 18 dpi in serum and fecal samples.

Results
Seroconversions of IgM and IgG antibodies to the spike S1 protein of SARS-CoV-2 were identified in all dogs in the infection and contact group and neutralizing antibodies were detected after 14 dpi. Two dogs, one dog each from infection group and contact group, were sacrificed at 10, 12, and 14 dpi for further tissue analyses. Hematoxylin and Eosin stain of respiratory tissues revealed infiltration of inflammatory cells in the trachea and lung tissues of all dogs of both groups. Immunohistochemistry assay showed the viral particles and the activation of immune cells in the lungs. SARS-CoV-2 proteins were also detected in the brain tissues, suggesting transmission of the virus to the central nervous system. Astrocyte activation and perivascular cuffing in the brains would imply the inflammation in the neurological system.

Conclusions
Collectively, we confirmed that dogs could be infected with delta variant of SARS-CoV-2 causing pathological changes in the lower respiratory tracts and brains. Direct dog-to-dog transmission of SARS-CoV-2 had also been demonstrated. This study would help for better understanding of transmission and pathogenesis of SARS-CoV-2 in the infected dogs.

Financial Support
Animal and Plant Quarantine Agency, South Korea (grant number: Z-1543085-2022-23-02)

Notes:

P166 - Role of IFITM3 in PRRSV replication in vitro

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Session: Virology

Objective
The main objectives of this study are to investigate the roles of IFITM3 in PRRSV replication and to elucidate its underlying mechanisms by which IFITM3 restricts PRRSV.

Methods
MARC-145 cells were transfected with either HA tagged IFITM3 or vector control. At 72 hours post transfection, cells were infected with PRRSV 23983 at an MOI of 1 for 24 h. Western blot and $TCID_{50}$ assays were performed to examine the expression of IFITM3, Actin, PRRSV nucleocapsid protein, Akt, phosphorylated Akt, and LC-3 proteins and to determine the supernatant virus titer. Immunofluorescence staining and confocal microscopy were used to determine the co-localizations of IFITM3 with PRRSV. Silencing RNA (siRNA) induced knockdown of IFITM3 was performed to confirm the role of IFITM3 on PRRSV replication. Flow cytometry was used to determine whether Amphotericin B can reverse the effect of IFITM3 on PRRSV replication.

Results
Over-expression of HA tagged exogenous IFITM3 reduced PRRSV replication compared to vector control. Silencing of endogenous IFITM3 slightly enhanced PRRSV replication. A dose-dependent enhancement of IFITM3 mRNA expression by treatment of MARC-145 cells with interferon-alpha was observed, which was negatively correlated with virus replication in MARC-145 cells. Amphotericin B partially restored the replication of PRRSV in cells over-expressing IFITM3. Over-expression of IFITM3 in MARC-145 cells resulted in a reduced level phosphorylation of Akt compared to vector control. An increase in LC-3II expression was observed in IFITM3 overexpression compared to vector control.

Conclusions
IFITM3 exhibits an antiviral role against PRRSV in MARC-145 cells. Over-expression of IFITM3 partially restricts virus entry into host cells. The reduced phosphorylated Akt induced by IFITM3 over-expression may also contribute to reduced virus replication efficiency in MARC-145 cells. IFITM3 over-expression also slightly increased the expression of LC-3II, a marker of autophagy. Collectively, multiple mechanisms may contribute to the reduced PRRSV replication in MARC-145 cells by overexpression of IFITM3.

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

Notes:

P167 - A Multi-pronged approach to study the mechanisms of differential rotavirus-host glycan interactions

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Session: Virology

Objective
Rotaviruses of group A (RVA) are the primary cause of acute viral gastroenteritis in children and young animals globally; however, their replication and pathogenesis remain poorly understood. We have previously demonstrated contrasting modes of interactions with the host cell glycans for two prevalent porcine RVA strains: OSU G5P[7] (historically associated with severe disease in piglets) and G9P[13] (globally emerging variant in humans and swine). Specifically, OSU G5P[7] and G9P[13] strain replication was significantly decreased and significantly increased, respectively, following removal of terminal sialic acids (SA) by neuraminidase (NA) treatment, which coincided with the presence of distinct mutations found in the VP4 fusion region of these strains.
In this study, we sought to clarify the host cell response mechanisms behind the RVA dichotomic pattern of interactions with SAs and to generate a reverse genetics system (RGS) to identify RVA genetic determinants of these interactions.

Methods
We compared transcriptome responses of the porcine small intestinal enteroids (PIEs) to OSU G5P[7] and G9P[13] infection. We have also established a reverse genetic system (RGS) carrying 11 RVA OSU genes.

Results
Our data demonstrated that there was a drastically different RVA genotype/strain-specific host transcriptome response resulting in 3,539 and 227 differentially expressed genes following infection with G9P[13] and G5P[7], respectively. Further, we observed that while infection of PIEs with G5P[7] did not significantly modulate the expression of the sialyltransferase genes, inoculation of G9P[13] affected their expression drastically, often down-regulating it.
The newly established RGS system will be used to determine the minimal required number of the VP4 mutations to achieve SA- or NA-dependent RVA phenotype.

Conclusions
Thus, our study has expanded our understanding of the mechanisms of RVA cell attachment and entry and generated a robust RGS platform to study RVA pathogenesis which can be used to identify novel therapeutic targets focusing on the RVA-host glycan interactions.

Notes:

P169 - Study of molecular-biological characteristics of rabies virus spread in Azerbaijan (2018-2021)

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Session: Virology

Objective
The presented study shows the virus characterization during 4 years (2018-2021) in the country.

Methods
During 2018-2021, 241 pathological material samples of wild and domestic animals were examined for rabies in CVL and rabies virus was detected in 180 of them (FAT and PCR).

Results
In order to study the molecular-biological characteristics of the virus, 15 RNA extractions obtained from positive brain samples and belonging to different regions of the country were sequenced, and as a result, phylogenetic relationship was found between the rabies virus spread in Azerbaijan and in the neighboring countries (Turkey, Georgia, Iran, as well as in Kazakhstan).

Conclusions
The results show that the strains spreading in neighboring countries should be taken into account in the prevention of rabies.

Notes:

P170 - Experimental infection of domestic pigs with African swine fever virus isolated in 2019 from Mongolia

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Session: Virology

Objective
African swine fever virus (ASFV), which belongs to the *Asfarviridae* family and genus *Asfivirus,* is a highly contagious pathogen that can cause high mortality in domestic swine and wild boar (*Sus scrofa)*. Currently, outbreaks are mitigated through strict quarantine measures and culling of affected herds, resulting in massive economic losses to the global pork industry. In 2019, an ASFV outbreak was reported in Mongolia describing a rapidly progressing clinical disease and gross lesions consistent with the acute form of ASF; the virus was identified as a genotype II ASFV strain. In the current study we evaluated the clinical disease, virulence, and pathology of an ASFV Mongolia/2019 field isolate (ASFV-MNG19) by experimental infection of domestic pigs.

Methods
Six domestic pigs were challenged intramuscularly with 1 mL of ASFV-MNG19 at 360 HAD_{50}/mL. Daily body temperatures and clinical signs were recorded to assess disease progression following challenge. Clinical samples were collected on -1, 1, 3, 5, and 7 days post challenge (DPC), consisting of EDTA blood, oropharyngeal (OP) swabs, and oral fluids. The clinical samples were used to follow viremia and viral shedding in bodily fluids over the course of disease using both quantitative real-time PCR and viral titrations. All pigs were necropsied for pathological evaluation and tissue collection.

Results
Clinical signs and viremia were observed starting at 3 DPC following inoculation with ASFV-MNG19. Clinical disease rapidly progressed, resulting in the humane euthanasia of all pigs by 7 DPC. ASFV-MNG19 infected pigs had viremic titers of 10^8 $TCID_{50}$/mL and shed virus in oral secretions late in disease, as determined by infectious virus from OP swabs. Moderate to severe gross lesions consistent with acute ASF included splenomegaly, hemorrhagic lymph nodes, and pulmonary congestion and edema were consistently observed.

Conclusions
The present study confirms that the ASFV-MNG19 strain is a virulent genotype II virus that has similar clinical disease presentation and progression as other historic and regionally circulating genotype II ASFV isolates.

Financial Support
U.S. National Institute of General Medical Sciences; State of Kansas

Notes:

P171 - Rescue and characterization of porcine circovirus type 3 from a recombinant infectious clone

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Session: Virology

Objective
Porcine circovirus type 3 (PCV3), a recently discovered circovirus, is epidemiologically associated with reproductive failure, porcine dermatitis, and nephropathy syndrome (PDNS) and respiratory distress in pigs. In infected pigs, PCV3 results in generalized inflammatory lesions which include vasculitis and myocarditis. There is little experimentally generated information on the pathogenesis of the PCV3 as traditional laboratory culture and isolation of PCV3 to obtain a pure culture is a challenge. The primary goal of this study is to develop and characterize a PCV3 infectious clone to enable the exploration of the role of PCV3 in primary and coinfection studies.

Methods
The entire genome of PCV3 strain USMN 2016 cloned into a shuttle vector using an enzyme with a single cut in the viral genome.

Results
Transfection of PK-15 cells with the excised and circularized genome resulted in production of recombinant viral particles with a titer of 10^6 $TCID_{50}$/ml. The identity of the rescued virus was verified by sequencing and PCV3-specific monoclonal and polyclonal antibodies. The recombinant virus remained infective over 5 serial passages in PK-15 cells. Further, the PCV3 ORF2 protein was expressed and purified in both mammalian and bacterial expression systems to enable the development of serological assays.

Conclusions
Currently, the recombinant PCV3 virus is being tested in weanling pigs to determine whether characteristic lesions are produced in singular infection and exacerbation of porcine respiratory diseases syndrome virus (PRRSV) infection occurs in dual challenge. Future studies will focus on testing the efficacy a novel PCV3 vaccine construct in preventing PCV3 using the optimized single and dual challenge models.

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

Notes:

P172 - Susceptibility of primary white-tailed deer cells to Japanese encephalitis virus

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Session: Virology

Objective
Japanese encephalitis virus (JEV), a mosquito-borne zoonotic pathogen, is the leading cause of human vaccine preventable encephalitis in Southeast Asia. It is maintained in an enzootic transmission cycle between mosquitoes, mammals, and wading birds. JEV has a wide host range with some wildlife species implicated. North American white-tailed deer (WTD; *Odocoileus virginianus*) can be infected by other significant zoonotic pathogens such as the closely related flavivirus West Nile virus as well as the organisms that cause tuberculosis, brucellosis, and COVID-19. The susceptibility of WTD to JEV is unknown.

Methods
In two independent experiments we examined the *in vitro* susceptibility of primary white-tailed deer brain (WTDBr) and lung cells (WTDLg) to an attenuated strain of JEV, SA14-14-2. Using known JEV-susceptible baby hamster kidney cells (BHK-21) as positive controls, both WTDBr and WTDLg cells were infected with SA14-14-2 at a 0.1 multiplicity of infection (MOI) in duplicate and observed for 120 hours. Cytopathic effects (CPE) were noted, monolayers were photographed, and supernatants were collected at timepoints 0, 24, 48, 72, 96, and 120 hours post infection (hpi). To quantify the number of infectious virus particles in cell supernatants, standard plaque assays were performed.

Results
In the supernatants of both infected WTDLg and WTDBr cells, infectious virus was detected by 24 hpi, with peak titers occurring between 48 and 72 hpi. CPE was detected in WTDLg cells at 72 hpi, onward, and titers were comparable to BHK-21. In WTDBr cells, no CPE was observed and peak titers were approximately 2 $\log_{10}$ pfu/ml lower than in BHK-21 and WTDLg cells.

Conclusions
Together, these results show that both WTDBr and WTDLg cells are susceptible to JEV SA14-14-2. Future studies assessing the susceptibility of WTD to JEV, their possible role in the maintenance of JEV in nature, and potential extent of host range for JEV are warranted.

Financial Support
U.S. Department of Agriculture

Notes:

P173 - Experimental infection of domestic pigs with an ASFV field strain isolated in 2021 from the Dominican Republic

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Session: Virology

Objective
African swine fever (ASF), a disease of domestic and wild swine that has spread throughout Africa, Central Europe, East and Southeast Asia. The clinical presentation of the disease heavily depends on the virulence of the ASFV strain. Recently, ASFV was detected in the Dominican Republic (DR) and Haiti, constituting the first diagnosis of ASFV in more than 40 years in the Western hemisphere. In this report, the clinical presentation of the disease in domestic pigs inoculated with an ASFV field strain isolated from samples collected in the DR (ASFV-DR21) was evaluated.

Methods
Two groups of pigs were inoculated either intramuscularly (IM) or oronasally (ON) with ASFV-DR21 (10^4 HAD_{50}). A group of naïve pigs (contact group) was co-housed with the ASFV-DR21 IM-inoculated animals to evaluate ASFV transmission.

Results
Animals inoculated IM showed an acute disease being euthanized by 7 dpi. Animals inoculated on developed a heterogeneous disease. One animal developed an acute form being euthanized on day 7 pi, another animal showed a protracted disease with euthanasia by day 16 pi, and the remaining two pigs survived through the 28-day observational period. Contact animals presented with heterogenous disease: three showed severe but protracted ASF and were euthanized at 14, 15, and 21 dpi, two pigs showed milder disease and survived the observational period." Suggest "Results suggest that ASFV-DR21, unless inoculated parenterally, produces a heterogeneous disease, with some animals showing acute ASF and others a mild transient disease with development of a strong antibody response

Conclusions
Results suggest that ASFV-DR21, unless inoculated parenterally, produces a heterogeneous disease, with some animals showing acute forms while others a mild transient disease along by the induction of a strong antibody response. This is the first report on the virulence phenotype of an ASFV field strain isolated in the DR in 2021 and provides information that may be used in developing epidemiological management measures to control ASF on the island of Hispaniola.

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

Notes:

P174 - Lights, camera, biofilm! A look at *Leptospira* biofilm formation at different temperatures

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Session: Bacteriology

Objective
Leptospirosis is a global zoonic disease of great economic and veterinary concern to agricultural livestock, companion animals, and humans alike. Persistence of the disease is driven by reservoir hosts wherein bacteria colonize the kidney and subsequently are shed in the urine. This leads to direct (animal to animal) and indirect (environmental contamination) disease transmission in incidental hosts who typically present with acute and sometimes severe life-threatening disease. *Leptospira* can persist for long periods of time colonized in host tissue as well as in the environment, some propose by forming biofilms. Transitioning between host and environment means leptospires must adapt to many conditional changes including different temperatures.

Methods
Three strains of *Leptospira* were evaluated for biofilm growth in HAN media at 29 ℃ and 37 ℃: *L. borgpetersenii* serovar Arborea strain LR131 and serovar Tarassovi strain MN900 and a *L. interrogans* serogroup Icterohaemorrhagiae strain. Biofilm development was analyzed by scanning and confocal microscopy and quantified with crystal violet (CV) staining. Biofilms were further evaluated with a susceptibility assay testing environmentally relevant parameters such as NaCl, UV light, tetracycline, and pH.

Results
CV staining and microscopy approaches showed that temperature and strain had an effect on biofilm growth. Strains LR131 and the *L. interrogans* strain out preformed strain MN900 in all conditions while within strain, all grew better at 37 ℃ than 29 ℃. The *L. interrogans* was the least susceptible to environmental challenges, while MN900 was the most susceptible by metabolic activity.

Conclusions
This work verifies biofilm formation in both the *L. interrogans* as well as *L. borgpetersenii* species. Notably, while all three tested strains developed biofilms in both temperatures, all grew better at 37 ℃ than the classic culture temperature of 29 ℃. Collectively these findings suggest biofilm formation may be an important factor in *Leptospira* host and environmental interactions, and should be considered for its implications in disease pathogenesis.

Notes:

V002 - Pentose cycle activity in intestinal mucosal leukocytes of hybrid striped bass

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Session: Immunology

Objective
This study determined the metabolism of glucose via the pentose cycle (PC) in intestinal mucosal leukocytes of juvenile hybrid-striped bass (HSB).

Methods
A Ficoll (density = 1.077 g/ml at 25°C) gradient was employed to isolate leukocytes from the mucosae of the entire intestine of HSB [with a body weight (BW) of either 14.2 ± 0.7 or 41.0 ± 2.7 g; means ± SEM; n = 8], and three equally divided segments (proximal, mid, and hind) of the intestine of HSB with a BW of 41.9 ± 1.8 g (means ± SEM, n = 3). Cells (0.5×10^6) were incubated at 26°C for 2 h in 1 ml of oxygenated (95% O_2/5% CO_2) Krebs-Henseleit bicarbonate buffer (pH 7.4) containing 5 mM D-glucose and either D-[1-^{14}C]glucose or D-[6-^{14}C]glucose (20 dpm/nmol carbon). At the end of the incubation, $^{14}CO_2$ produced from each ^{14}C-labeled substrate was collected.

Results
In HSB with the mean BW of 14.2 g, the rates of production of CO_2 from 5 mM D-[1-^{14}C]glucose and D-[6-^{14}C]glucose were 47.5 ± 1.8 and 11.9 ± 0.4 nmol/2 h/10^6 cells (means ± SEM, n = 8), respectively. In HSB with the mean BW of 41.0 g, the rates of production of CO_2 from 5 mM D-[1-^{14}C]glucose and D-[6-^{14}C]glucose were 42.4 ± 3.3 and 10.7 ± 1.1 nmol/2 h/10^6 cells (means ± SEM, n = 8), respectively. The fluxes of glucose to the PC (nmol/2 h/10^6 cells; means ± SEM, n = 8) in the leukocytes of HSB with the mean BW of 14.2 g and 41.0 g were between 35.6 ± 1.8 (lower limit) and 47.5 ± 1.8 (upper limit) and between 31.7 ± 3.0 (lower limit) and 42.4 ± 3.3 (upper limit), respectively. There was no difference ($P > 0.05$) in the rates of glucose metabolism between HSB with the two different BW groups. Either the rates of production of CO_2 from 5 mM D-[1-^{14}C]glucose and D-[6-^{14}C]glucose or the rates of glucose metabolism via the PC did not differ ($P > 0.05$) among the three different segments of the intestine of HSB with a mean BW of 41.9 g (n = 3).

Conclusions
Glucose is actively metabolized via the PC in the intestinal mucosal leukocytes of HSB to support their immune function.

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

Notes:

V003 - Role of glutamate and other crystalline amino acids in diets for the growth of juvenile hybrid striped bass

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Session: Aquaculture

Objective
This study evaluated the feasibility of using crystalline AAs to replace fishmeal protein in aquafeeds and the effect of dietary L-glutamate (Glu) on fish growth.

Methods
Juvenile hybrid-striped bass (HSB) with a mean initial body weight of 7.6 g were used for this study. In Exp. 1, HSB were fed either a 60%-fishmeal (control) diet or a crystalline AAs-based, purified diet with the same content of nutrients. The content (g/kg; as-fed basis) of AAs (all L-isoforms except for Gly and taurine) in both 60%-fishmeal and purified diets (containing 92.6% dry matter) was: Arg, 20.2; Asn,12.5; Asp, 18.3; Cys, 3.7; Gln, 20.1; Gly, 23.5; His, 7.8; Ile, 13.3; Leu, 24.8; Lys, 24.6; Met, 10.9; Phe, 12.8; Pro, 20.6; Ser, 14; Thr, 13.8; Trp, 3.9; Tyr, 10.4; Val, 16.7; taurine, 2.5; and Ala, 20. The content of dry matter in both diets was 92.6%. In Exp. 2, HSB were fed a crystalline AAs-based, purified diet containing 0, 2, 4, 6, or 8% Glu, with Ala being used as the isonitrogenous control. In both experiments, fish were maintained on a recirculating aquaculture system and fed their diets for 4 weeks; there were 5 tanks per treatment group (14 fish per tank with 55 L of water, 26°C).

Results
In Exp. 1, at the end of the 4-week trial, fish in the 3% Glu group had a 25% more weight gain ($P < 0.05$) and 8.7% higher body weight (13.8 g vs 12.7 g; $P < 0.05$) than fish fed the 60%-fishmeal diet, as analyzed by the unpaired t-test; no fish died in the 60%-fishmeal group, and 1 fish died in the purified diet group. In Exp. 2, increasing the dietary Glu content from 0 to 8% increased ($R^2 = 0.972$; $P < 0.05$) the weight gain and body weight of fish in a dose-dependent manner, as analyzed by regression analysis; 2 fish died in the 4%-Glu group, and 1 fish died in each of the other groups.

Conclusions
Fishmeal protein can be effectively replaced by crystalline amino acids in the diet of HSB and dietary Glu is essential for their maximum growth.

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

Notes:

V004 - Simulations suggest that migratory animals can exert positive or negative effects on parasite loads in residents

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Session: Parasitology

Objective
Migratory animal populations can impact resident host populations by shedding parasites within resident home ranges (transport effects), and by exerting trophic effects that either promote or reduce local parasite exposure risk for residents. Both of these effects can in turn be impacted by the number of migratory animals (intensity) and the amount of time spent within resident home ranges (duration), yet relatively little is known about how migration intensity and duration change migrant-host macroparasite dynamics. Our study has two objectives: 1) to quantify the potential impact of migratory animals on resident parasite loads; and 2) to evaluate the impact of intensity and duration on migratory-resident parasite dynamics.

Methods
We developed a dynamic model consisting of three coupled differential equations to explore the dynamics of parasites in resident hosts, the density of free-living larvae, and the vegetation biomass that hosts the larvae. We treated migration intensity and duration (and parasite loads in migratory animals) as a forcing function on this system. We used gastrointestinal nematodes as a model system, and parameterized the model primarily with data from the Serengeti wildebeest migratory system.

Results
Across a range of the explored parameter space, migratory events were predicted to either enhance or diminish pre-migration resident-host parasite loads, with migratory host parasite loads playing a dominant role. Increases in migratory intensity and duration were predicted to amplify these positive or negative effects.

Conclusions
Variation in migration intensity and duration can be integrated into our understanding of disease dynamics in migratory systems via a framework that links resident and migrant animal hosts through trophic and transport effects. The response of resident parasite loads to migration events will be determined by the strength of trophic interactions between host species and the ability of migrants to transport shared pathogens that are in turn impacted by trophic interactions.

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

Notes:

V005 - Effect of pH and lipopolysaccharide concentration in vitro on tight junction regulators and inflammatory markers

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Session: Immunology

Objective
To determine the effect of combinations of various pH and LPS concentrations on epithelial tight junction regulators (ETJR) and selected pro-inflammatory markers

Methods
The experimental design consisted of a 2 × 3 factorial arrangement of treatments (pH: 6 and 7.4; LPS concentration: 0, 0.05, 10 ng/mL). Human colon carcinoma Caco-2 line was cultured in 6-well plates and exposed to the treatments for 3 h. A pellet was collected to determine protein abundance (Toll-Like Receptor 4, TLR4; Myosin Light Chain Kinase, MYLK; and β-actin as housekeeping) using Western Blot and gene expression using rt-qPCR. The target genes included Interleukin 8 (IL-8), MYLK, Nuclear Factor κ-B (NF-κB), and Peroxisome Proliferator Activated Receptor Gamma (PPARγ). Cycle threshold (Ct) of target genes corrected by Ct of housekeeping genes (RPLP and PPIA) were used (ΔCt) for statistical analysis. Data were analyzed using a mixed model with significance declared at $P \leq 0.05$, and tendency at $P \leq 0.10$.

Results
Protein abundance of TLR4 tended to reduce at pH 6 compared to pH 7.4 ($P = 0.09$). Cells cultured at pH 6 showed a greater expression of MYLK gene ($P < 0.01$) than pH 7.4. Protein abundance of MYLK was reduced by pH 6 compared to pH 7.4 ($P = 0.01$) and tended to reduce when LPS concentration was increased ($P = 0.07$). The expression of IL-8 gene was increased in cells cultured at pH 7.4 compared to pH 6 ($P < 0.01$). In addition, there was a pH × LPS interaction for NF-κB gene expression ($P = 0.04$), which was greater in cells cultured at pH 6 combined with 0 or 10 ng/mL of LPS than the same LPS doses at pH 7.4. The gene expression of PPARγ was not significantly affected by the treatments ($P > 0.11$) under these experimental conditions.

Conclusions
Overall, gene expression of the inflammatory markers and MYLK, as well as protein abundance of ETJR were affected by the pH levels, LPS concentration, and pH × LPS interaction.

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

Notes:

V006 - Cellulose nanomaterials: A novel adjuvant and delivery system for aquaculture vaccine applications

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Session: Vaccinology

Objective
Disease outbreaks are a major impediment to aquaculture production. Vaccines are integral for disease management in aquaculture but they can be expensive, vary in effectiveness, and come with adjuvant-induced adverse effects causing fish welfare issues and negative economic impacts. The goal of this interdisciplinary project is to develop a new generation of vaccines for sustainable aquaculture. Our project uses novel nanomaterials produced from renewable wood fiber as depots/adjuvants in vaccine formulations to modulate the immune response of Atlantic salmon in a biocompatible, environmentally friendly, and cost-effective manner.

Methods
We are elucidating the role of cellulose nanomaterials (CNM) as a vaccine depot and mobile immunostimulant, the extent of CNM migration *in vivo*, and the efficacy of CNM bound antigen as an immunostimulant for protection against two Atlantic salmon pathogens. To date our interdisciplinary research team has prepared and conducted *in vitro* characterizations of CNM hydrogels and CNM/antigen (vaccine) formulations by using fluorescent CNM variants (CNM-FL) and *in vivo* durability and migration using confocal FL microscopy. Our next steps are to conduct *in vivo* studies to quantify the antibody kinetics in vaccinated fish serum using enzyme-linked immunosorbent assays, and finally to evaluate the efficacy of the CNM vaccine(s) in protecting against *Vibrio anguillarum* in Atlantic salmon.

Results
Initial results demonstrated TEMPO CNF hydrogels could be a potential vaccine adjuvant for fish but delivery was a primary obstacle. Alternative shear- thinning injectable hydrogels are being investigated. Performance of CNM formulations in stimulating antibody response and efficacy in preventing disease mortalities compared to a commercial vaccine and a negative vehicle control will be determined.

Conclusions
We anticipate the CNM vaccine formulations will perform as well or better than commercially available vaccines while being more cost-effective and sustainable for long-term aquaculture.

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

Notes:

V007 - Impact of prenatal and postnatal inflammatory conditions on the molecular pathways of the pig hypothalamus

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Session: Immunology

Objective
Virally-induced maternal immune activation during gestation can cause neurodevelopmental changes that modulate brain plasticity to infection later in life. Inflammatory response can alter the role of the hypothalamus on the release of hormones and associated behaviors. The effects of prenatal and postnatal inflammatory response on the hypothalamic transcriptome of female and male pigs were studied.

Methods
RNA-sequencing was used to profile the hypothalamus transcriptome of 116 pigs exposed to porcine reproductive and respiratory virus (PRRSV) during gestation (versus matching controls) and to a viral mimetic (Poly(I:C)) (versus saline injection) at two months of age.

Results
Over 2000 genes were differentially expressed (FDR-adjusted P-value <0.05 and |log2(fold change between pig groups)| > 1.2) in response to PRRSV, Poly(I:C), and sex. Exposure to a single inflammatory challenge in males prompted the highest number of differentially expressed genes, suggesting a protective effect. The TNF signaling pathway was enriched among genes over-expressed in Poly(I:C)- relative to saline-treated pigs, irrespective of PRRSV and sex group. Interaction effects were characterized by the enrichment of TNF signaling pathway among genes under-expressed in Poly(I:C)-treated females from PRRSV-challenged relative to Control gilts and among genes over-expressed in saline-treated males from PRRSV-challenged relative to Control gilts.

Conclusions
Management strategies can consider the present findings about the impact of prenatal inflammatory exposure and sex on the postnatal hypothalamus response to infection.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture; Agriculture and Food Research Initiative grant number 2018-67015-27413

Notes:

V008 - MDV pathogenesis: the role of MDV genome integration on disruption of host chromosome architecture

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Session: Omics

Objective
Marek's disease (MD) is a highly contagious lymphoproliferative disease of chickens, causing significant economic loss in poultry industries. MDV genomic integration into host chromosomes is essential for tumor formation. An unexplored consequence of MDV integration events is effects on the host chromosome architecture. We will examine the role of somatic cell integration in MDV tumorigenesis via disruption of 3D host chromosome architecture.

Methods
Genomic studies using capture Hi-C (cHi-C), Hi-C, RNA-seq, and ChIP-seq will be used to examine the effects of MDV integration on genomic interactions and host transcriptome profiles. In aim 1, tumor cell architecture will be compared among several MDV+ chicken B-cell, chicken T-cell, and turkey B-cell lines. In aim 2, tumor cell architecture will be examined in tumor samples obtained from MDV infected chickens. CD4+ T cells from uninfected chickens will serve as controls. For each sample, cHi-C will be used for enrichment of rare heterotypic MDV-host genomic interactions, while conventional Hi-C will be used to examine abundant homotypic host genomic contacts.

Results
cHi-C, genome-wide Hi-C and RNA-seq studies were conducted on an assortment of 6 MDV+ chicken B-cell, chicken T-cell, and turkey B-cell lines. In addition, tumor samples were generated from 10 experimentally infected chickens which were processed through the same analysis pipeline as for the MDV+ cell lines. The animal studies included CD4+ T cells from five uninfected chickens to serve as controls. Datasets have been obtained and are currently under bioinformatic analysis and data integration.

Conclusions
Conclusions are pending completion of our bioinformatic analysis of the datasets. However, if successful, the results will contribute to our understanding of how integrated viral genomes may contribute to the establishment of a unique and specific genomic architecture to support continuous cell growth and will aid in our understanding of the underlying mechanisms.

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

Notes:

V009 - Genomic screens to identify causative polymorphisms accounting for Marek's disease genetic resistance in chicken

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Session: Omics

Objective
Marek's disease (MD), a lymphoproliferative disease of chickens caused by the pathogenic Marek's disease virus (MDV), is a serious disease problem of chickens. Despite widespread use of vaccines, more virulent MDV strains have repeatedly arisen. Consequently, alternative control methods, such as improving MD genetic resistance, are needed. In prior work, we demonstrated that genes showing differential gene expression in response to MDV infection account for 83% of the genetic variance. This submission is designed to identify the causative variants.

Methods
Integrating Hi-C, ChIP seq for MDV Meq and chromatin marks that identify promoters and/or enhancers, and RNA seq to identify transcripts, we identify candidate regulatory elements that contain the causative polymorphisms. The samples surveyed are splenic-derived lymphocytes from uninfected and MDV-infected chickens. Then the results will be validated using progeny testing.

Results
Despite clear differential gene expression and clustering of samples, Hi-C results do not indicate clear differences in topologically associating domains (TADs) between sample groups. Two contingencies are being pursed: (1) query samples from cultured cells (e.g., control and MDV-infected fibroblasts) and (2) using recently identified enhancer-gene combinations, computational test for enriched-motifs and polymorphisms in the corresponding transcription factors.

Conclusions
There are two likely possibilities for the lack of Hi-C differences. First, our approach is not sensitive enough to pick up the subtle chromatin differences when only a small fraction of the cells are infected. Or second, differential transcriptional start sites (TSSs) and not enhancers are the regulator elements that we should focus on. [Results from this work was used in a 2021 Science (372:984) publication - 3D genomics across the tree of life reveals condensin II as a determinant of architecture type.]

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

Notes:

V010 - Genomic analysis of two aquaculture pathogens reveals a conserved T6SS and overlapping effector repertoires

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Session: Omics

Objective
The Type VI Secretion System (T6SS) augments the virulence and fitness of pathogenic *Vibrio* species by allowing them to deliver effectors that intoxicate prokaryotic and eukaryotic targets. Here, we investigate the role of T6SS in the aquaculture pathogens *Vibrio coralliilyticus* RE22 and *Vibrio parahaemolyticus* PSU5579, both of which cause mass mortality events in shellfish aquaculture. We identify several putative effector/immunity pairs encoded by RE22 and PSU5579, and compare the effector arsenals of these two species. We also assess the antibacterial activities of T6SS in RE22 and PSU5579 using *E. coli* as prey.

Methods
Rapid Annotation using Subsystem Technology (RAST) was used for genome annotation of RE22 and PSU5579. BLASTp was used to search the nonredundant protein database at NCBI to identify putative effector and immunity proteins. Nucleotide and amino acid sequence alignments were made using BLASTn and BLASTp on the NCBI website. Contact-killing of *E. coli* SM10 by RE22 and PSU5579 was tested using a filter membrane competition assay.

Results
Both RE22 and PSU5579 possess two complete T6SS gene clusters, with one located on each chromosome. It is hypothesized that these distinct T6SSs exhibit different target specificities, with one serving an antibacterial function while the other mediates virulence towards eukaryotes. A comparison of the T6SS clusters encoded by RE22 and PSU5579 reveals that the antibacterial T6SSs are remarkably similar, while the anti-eukaryote T6SSs are distantly related and harbor different effector candidates. Both strains possess homologs of several previously reported and experimentally validated antibacterial effector/immunity pairs found to be widely distributed throughout the *Vibrio* genus. RE22 and PSU5579 both demonstrate the ability to antagonize *E. coli* SM10 in a contact-dependent manner, yet differ with regard to killing efficiency.

Conclusions
Antibacterial T6SS gene clusters of *V. parahaemolyticus* PSU5579 and *V. coralliilyticus* RE22 are highly conserved and include several effector/immunity gene pairs.

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

Notes:

V011 - The impact of heat stress on the broiler chicken ileal enteric nervous system

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Session: General health and physiology

Objective
Research is needed to identify the mechanisms underlying emergence of avian pathogenic Escherichia coli (APEC) in the broiler intestinal tract. Heat stress may elicit an enteric neuroendocrine stress response to drive pathogenicity of resident E. coli strains. The enteric nervous system (ENS) releases neurochemicals into the gut lumen that have recognized roles in affecting bacterial pathogenicity. The present study sought to evaluate whether heat stress alters the broiler chicken ENS.

Methods
Broiler chicks were randomly allocated to control or heat stress (HS) groups. At 6 weeks of age, the control group was kept at standard conditions, while the HS group was subjected to a 12-h daily cyclic HS (35°C) for either 1 or 6 consecutive days. Birds were sacrificed at 1 or 6 day(s) following HS. The control group was sacrificed on the same days as the HS group. Ileal wholemounts were prepared and the distribution of HuC/D (a pan-neuronal marker) and neuronal nitric oxide synthase (nNOS; nitrergic neuron marker) was determined (N=6 birds/group) using tissue clarification and double-labelling confocal immunofluorescence. Ileal glial fibrillary acidic protein (GFAP; enteric glial marker) and ionized calcium binding adaptor molecule 1 (Iba-1; enteric muscular macrophage marker) was analyzed using confocal microscopy. All datasets were analyzed with two-tailed unpaired Student's t test with Welch's correction.

Results
Dendritic and axonal processes of nNOS-containing neurons were significantly altered due to HS. The number of HuC/D+ neuronal cells in the ileal myenteric ganglia was not impacted by HS. Density of neuromuscular GFAP+ processes was increased ($p<0.05$) in chickens that underwent 1 day of HS; these changes did not persist in the ileum of chickens that received 6 days of HS. Iba-1 immunoreactivity in the chicken ileum was not impact ($p>0.05$) by HS.

Conclusions
Heat stress impacted the chicken ileal ENS, with changes observed in enteric neurons and glial cells. Further investigation is warranted into determining how these changes may impact APEC infection in the broiler chicken gut.

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

Notes:

V012 - PARTNERSHIP: Single-cycle replicon-based African swine fever virus subunit vaccine

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Session: Vaccinology

Objective
Evaluate protective efficacy of a live-vectored replicons encoding multiple ASFV antigens

Methods
African Swine Fever Virus (ASFV) is a major swine pathogen, but there is no vaccine or treatment available. Protective immunity depends on antibody and probably cytotoxic T lymphocytes (CTLs). Development of a subunit vaccine requires identification of protective antigens. Immunization of pigs with replication-incompetent adenovirus encoding a few antigens induced CTL responses, but protective efficacy was low. To improve efficacy, adenovirus replicons encoding multiple multicistronic expression cassettes of vaccine candidate antigens containing putative CD8 T cell epitopes were generated and protein expression was validated using ASFV convalescent serum. The putative CD8 T cell epitopes were validated by flow cytometric analyses of peptide-stimulated PBMCs isolated from immunized pigs to enumerate IFN-γ+ and Granzyme B+ cells. The recombinant replicons were scaled up, quality control tested, and a cocktail of the viruses were used to conduct a dose escalation study [10^9; 10^10; and 10^11 ifu/replicon] in pigs to evaluate safety, immunogenicity, and protective efficacy following challenge.

Results
The adenovirus-expressed antigens were recognized by ASFV convalescent serum and the putative CD8 T cell epitopes were recognized by T cells from pigs immunized with ASFV antigens. The immunogen was well tolerated and induced ASFV antigen-specific immune responses. The pigs immunized with the 10^10 ifu/replicon performed better as judged by longevity of survival post-challenge and overall pathologic evaluation.

Conclusions
The adenovirus replicons encoding multicistronic ASFV vaccine candidate antigens containing putative CD8 T cell epitopes elicited immune responses in pigs. A prototype vaccine formulated using replicons expressing the selected CD8 T cell targets has potential to induce ASFV-specific CTL responses in pigs and confer protection. This platform is being optimized to allow empirical identification of a protective antigen combination needed to develop an efficacious subunit vaccine.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture; **National Bio and Agro-Defense Facility (NBAF) T**ransition Funds; MEDIAN Diagnostics Inc., S. Korea

Notes:

V013 - Improved vaccine platforms for safe and effective control of bovine viral diarrhea virus

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Session: Vaccinology

Objective
Develop novel prototype BVDV subunit vaccine capable of inducing broadly protective antibody and $CD8^+$ T cell responses.

Methods
The Bovine Viral Diarrhea Virus (BVDV) plays a significant role in causing Bovine Respiratory Disease Complex. Current BVDV vaccines, which contain a mixture of representative BVDV-1 and -2 viruses, are inefficient at conferring broad protection. To develop a broadly protective prototype vaccine, we combined strain-specific as well as shared neutralizing epitopes by designing novel mosaic antigens using data from all sequenced BVDV-1 & -2 genomes. The approach entailed design of E2 and NS2-5 mosaic antigens that incorporate protective epitopes conserved among BVDV-1a, b, and BVDV-2 genotypes, as well as strain-specific protective epitopes. In addition, we identified and incorporated twenty-eight novel IFN-γ-inducing CD8 T cell epitopes that are highly conserved among all the genotypes. A Bovine Parainfluenza-3 Virus [BPI3V] vector was developed for antigen delivery. Protein expression and authenticity was tested using anti-BVDV polyclonal sera and neutralizing mAbs. Processing and presentation of the T cell epitopes in the mosaic antigens was evaluated using lymphocytes from BVDV immune cows.

Results
Five novel mosaic antigens [E2-NS2-3^a, E2-NS2-3^b, E2-NS2-3^2, NS4-5^1, and NS4-5^2] were generated. BVDV neutralizing mAbs and convalescent sera confirmed protein expression and antigen authenticity. In addition, lymphocytes from cows immunized with irradiated wildtype BVDV-1 and -2 viruses confirmed that the epitopes contained in the mosaic antigens were processed and presented for recognition by CD4 and CD8 T cells. The BVDV neutralizing mAbs and convalescent sera showed that recombinant BPI3V viruses encoding the mosaic antigens expressed authentic antigens.

Conclusions
The findings support feasibility for the development of a broadly protective subunit vaccine capable of addressing virus heterogeneity more effectively than current vaccines. Safety, immunogenicity, and protective efficacy of the novel BPI3V-vectored BVDV prototype vaccine will be evaluated in calves.

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

Notes:

V014 - Maintenance of bovine herpesvirus 1 (BoHV-1) latency by viral and cellular factors

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Session: Virology

Objective
The focus of this study is to characterize cellular signaling pathways differentially expressed during latency.

Methods
Differentially expressed genes (DEGs) were identified in neurons within trigeminal ganglia (TG), an impotant site for latency, using RNA-sequencing studies. To confirm key mediators of cellular signaling pathways that were differentially regulated in TG neurons of latently infected calves, immunohistochemistry studies were performed.

Results
Following acute infection of calves, bovine herpesvirus 1 (BoHV-1) establishes life-long latency in neurons. Maintenance of latency is crucial for preserving a pool of latently infected neurons that are capable of reactivating from latency and transmitting the virus. Exciting new unpublished studies revealed the phosphatidylinositol-3-Kinase (PI3K)/Akt/mTOR signaling axis, axonal guidance, neuronal survival, and cAMP response element-binding (CREB) protein signaling pathways are more active during latency. Support for these findings came from studies indicating the Akt3 protein kinase is expressed in more TG neurons during latency compared to uninfected TG. Additional studies demonstrated Akt1 and Akt2, but not Akt3, impaired glucocorticoid receptor (GR)-mediated transcriptional activation. In contrast, Akt3, but not Akt1 or Akt2, stimulated neurite formation, which is a prerequisite for neurogenesis. Interestingly, 157 differentially expressed genes (DEGs) were identified that directly mediate neurogenesis: the cutoff for these genes was arbitrarily set at 4-fold.

Conclusions
Surprisingly, cellular signaling pathways that mediate neurogenesis, neuronal survival, and prevent neurodegeneration are more active in TG neurons of calves latently infected with BoHV-1 when compare to TG from uninfected calves. Since the latency related (LR) gene is the only viral gene expressed during latency, we predict LR gene products, directly or indirectly, activate these cellular signaling pathways during latency. A protein encoded by the LR gene (ORF2) impairs apoptosis suggesting this protein is important for maintaining latency.

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

Notes:

V015 - *Salmonella* Dublin transmission and control in a heifer-raising operation: a mathematical modeling assessment

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Session: Vaccinology

Objective
Evaluate the transmission of *Salmonella* Dublin (SD) in a heifer raising operation (HRO) and the cost-effectiveness of vaccination and cleaning as control strategies compared to "doing nothing" (no control).

Methods
A Susceptible-Infected-Recovered model was developed to describe SD spread in a HRO in the Northeastern United States; stochasticity was introduced via Monte Carlo simulations. Vaccine efficacy (VE) was modeled through reductions in SD shedding and probability of death in calves. Cleaning with an alley scraper was modeled at different scraping frequencies per day (once, thrice, six, twelve, and twenty-four times) with 95% of the feces removed from the barn after each scrape. The validated model was used to evaluate epidemiologic (probability of an outbreak post introduction of a carrier, number of SD-related deaths, SD carriers leaving the farm, and abortions) and economic outcomes (cost-effectiveness, measured as the cost per prevented death, and the farm operating income calculated as the difference between the income from sales and the operating costs). These outcomes were compared between a control strategy and "doing nothing" over the 2-year simulation period.

Results
Model outcomes were estimated for a herd size of 250 heifers. Evaluated outcomes were most sensitive to the duration of periods of infectiousness and immunity and the level of fecal shedding and cleaning. Under the "doing nothing" scenario, the probability of a SD outbreak was 88% (264/300 iterations). This probability was reduced to 63% with VE on shedding and to 53% if feces are removed twenty-four times per day. Compared to "doing nothing", vaccination reduced the number of carriers leaving the operation from a mean of 14 (interquartile range (IQR)=8—23) to 7 (IQR: 0—14) and led to a median increase in operating income of USD 12,618 over 2-years.

Conclusions
Both vaccination and improvements in cleaning can be profitable measures to improve the operating outcome of HROs affected by *S.* Dublin and reduce its dissemination to other operations through movement of carriers.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture; Cornell Institute for Digital Agriculture (CIDA)

Notes:

V016 - Phage endolysins to control clostridia in poultry: the lytic activity of PlyCP41 enzyme using an *ex vivo* method

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Session: Bacteriology

Objective
The endolysin PlyCP41 is known to exhibit lytic activity against *Clostridium perfringens* (CP) cells *in vitro* and, therefore, could potentially be used *in vivo* to achieve similar results within the gastrointestinal tract (GIT). The objective of this study was to measure the inhibitory capacity of naked PlyCP41 enzyme in an assay containing gut fluids from the upper (crop, proventriculus, and ventriculus), middle (duodenum, jejunum, and ileum), and lower (ceca) GIT of 21d broiler chickens.

Methods
A positive control (PC) was developed using a pure strain of *C. perfringens* (CP509) incubated at 37°C for 24h in FTG broth. The pellet was resuspended in 2 mL of PBS, then split into two aliquots: a PC containing CP cells only; and PC+enzyme containing the CP cells + 5% PlyCP41 enzyme. The PlyCP41 enzyme had a stock concentration of 15 mg/ml and was added to 1.0 ml of the PC assay at a 5% inclusion rate (15 mg/mL * 0.05 = 0.75 mg of enzyme). Chicken gut fluids were harvested and pooled by region (upper, middle, and ceca) from 3 birds at 21d of age. Contents were homogenized, then PBS buffer was added to half the contents from each region at a rate of 5% and vortexed to combine. The contents+PBS were incubated anaerobically at 40°C for 20 min, then plated to quantify the existing CFU/g of *Clostridium perfringens* without enzyme treatment. The remaining half of the gut contents had naked PlyCP41 enzyme added at a rate of 5%, then were vortexed to combine. The contents+enzyme were cultured using the same method to calculate CFU/g after treatment with the enzyme.

Results
PlyCP41 naked enzyme reduced CP509 by >1.0 log (7.08 to 5.89 log10 CFU/g) in a pure CP assay. 0.75 mg of PlyCP41 per gram of gut fluids reduced CP by >1.0 log in the upper GIT (4.81 to 3.78 log10 CFU/g). A less significant effect (0.2 log reduction) was observed in the small intestine, and there was no reduction of *C. perfringens* in the ceca

Conclusions
These results indicate that the naked enzyme may not be effective in cecal contents but does elicit lytic activity in the low pH and lower viscosity environment of the upper GIT.

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

Notes:

V017 - Development and testing of *Mycobacterium avium* subsp. *paratuberculosis* DIVA vaccines in ruminants

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Session: Vaccinology

Objective
Johne's Disease (JD), caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP), is a significant problem in animal health. We generated live-attenuated strains that can differentiate vaccinated from infected animals (DIVA) and engender protective T-cell responses. The objective of this study is to test apoptotic properties of MAP mutants and also to develop unmarked mutants of DMAP52 and DMAP56.

Methods
Strains were transformed with pYUB870 (Kan^R) that carries the *sacB* gene and γδ-resolvase (removes the hygromycin gene outflanked by inverted repeats). Selected colonies had pYUB870 removed via counterselection on 2% sucrose, confirming strains by the Kan^S and Hyg^S phenotypes. For apoptotic assays, MAP strains (1.2×10^6 CFU) were grown to mid-exponential phase in Middlebrook media to infect RAW 264.7 macrophages at a MOI of 10. Apoptosis was quantified by DAPI stained nuclei morphology at 6 hours post-infection. We also determined bacterial morphology by transmission electron microscopy (TEM).

Results
The unmarked mutants were verified by PCR amplification. DMAP52 and DMAP56 have a deletion of most of the ORF and the additional hygromycin gene resulted in a larger band than the wild type. Their unmarked mutants have a smaller band than both of these since the hygromycin region was removed. DAPI staining showed a ca. 8.6- (DMAP52), 7.7- (DMAP52-unm), 11.9- (DMAP56) and 9.8- (DMAP56-unm) fold increase in apoptotic nuclei compared to wild type UNL K-10 ($P < 0.001$). For TEM, both wild types (UNL and NADC K-10) showed the same shape and morphology. In contrast, the attenuated deletion mutants DMAP52 and DMAP56, and their unmarked counterparts DMAP52-unm and DMAP56-unm, were significantly elongated (ca. 1.8-fold).

Conclusions
Apoptosis is considered a key marker of bacterial virulence. The mutants clearly showed a significant increase in apoptosis compared to both wild type strains. Unmarked deletion mutant properties are those associated with good vaccine candidates. We plan to test antigens for DIVA capabilities and assess the immunogenicity and pathogenicity of unmarked mutants in calves.

Financial Support
U.S. Department of Agriculture, National Institute for Food and Agriculture; Hatch Capacity Grant Funding

Notes:

V018 - Field surveillance of influenza virus infection of equine populations in Mongolia during summer and fall 2021

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Session: Epidemiology

Objective
Field campaigns were conducted in 2021 to determine how prevalence of influenza strains in equine populations was affected by (i) proximity to migratory waterfowl, (ii) horse age, (iii) horse sex, (iv) primary use of horse, (v) herd size, (vi) season. These preliminary data are part of a larger cohort study to be conducted during the project lifetime.

Methods
A total of 25 herder households were selected, including 15 herder households where horses mix with wild birds, and 10 herder households with little or no contact between horses and wild birds. Questionnaires were administered and collected to provide metadata on the geography, livestock numbers, and diseases. Blood samples were taken from 10 horses from each herder household during each campaign. The samples were tested in Mongolia for influenza antibody detection by the ELISA test. Geographical maps of the location of the herds were created. Data analysis and laboratory results were shared with, and jointly developed with, research partners at University of Georgia and University of Glasgow.

Results
Prevalence among horses was relatively high (overall mean prevalence = 40%, with 51% in summer and 39% in fall). Prevalence tended to increase with horse age, though data suggest a possible interaction with age and season whereby older animals have the highest prevalence in summer and the lowest in fall. Prevalence was similar across horse sex and primary horse use, and showed some evidence of being higher in small herd sizes. Herds with high contact rate with birds had higher prevalence in summer.

Conclusions
The data demonstrate the feasibility of using the study system to explore factors influencing prevalence. The summer had appreciably higher prevalence, indicating either higher exposure rate or more efficient within-herd transmission. The fact that the high exposure group in summer had the highest prevalence, which then switched in the fall could indicate some herd immunity developing over time. The higher prevalence observed in small herds could be related to population density, which will be explored further.

Financial Support
U.S. Department of Agriculture, **National Institute of Food and Agriculture (**NIFA)/ Biotechnology and Biological Sciences **Research Council**

Notes:

V020 - Improving dairy cow health monitoring and management using automated sensors

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Session: General health and physiology

Objective
Our objectives are to: (1) Characterize parameters recorded by sensors during health and disease in dairy cows; (2) Demonstrate data integration and machine-learning methodology for synthesizing multiple parameters to create Health Status Indexes that identify cows with health disorders (HD); (3) Demonstrate that automated health monitoring can promptly and accurately identify cows with HD.

Methods
The health status of Holstein cows was monitored daily while cows had wearable sensors to monitor physical activity, resting time, body temperature, rumination, and eating time. Non-wearable sensors monitored milk volume, milk fat to protein ratio, milk conductivity, body weight, and environmental conditions. All data were used for development and testing of machine learning algorithms [XGBoost (XGB), Multi-Layer Perceptron (MLP), Recurrent Neural Networks(RNN)] to predict cow health status. An automated real time data aggregator software infrastructure was built to integrate data from all sensors and other herd management data for estimation and delivery of Health Status Indexes.

Results
The pattern of sensor parameters around HD varied depending on the parameter and the type of HD. Cows with all early lactation disorders of interest had alterations to the patterns of sensor monitored parameters for the 5 d before and after clinical diagnosis of disease (all $P<0.05$). The sensitivity and specificity of Health Status Indexes on a testing dataset were 88% and 88%, 43% and 96%, and 70% and 67% for XGB, MLP, and RNN, respectively. The data aggregator received and integrated data automatically from all sensor systems and herd management software.

Conclusions
Substantial variation in sensor parameters in cows with HD could be used to automate health monitoring. Individual HD had signature patterns for sensor parameters. Combination of sensor and non-sensor data in machine learning algorithms may result in reasonable prediction of cow health status. A software system that automatically integrates heterogeneous data from diverse sources at a dairy farm could be used for generation of Health Status Indexes.

Financial Support
U.S. Department of Agriculture-National Institute for Food and Agriculture-Agriculture and Food Research Initiative - Foundational and Applied Science; Project 2017-67015-26772

Notes:

V024 - A host-pathogen approach to GWAS for enhanced resistance to bacterial mastitis in U.S. dairy cattle

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Session: Omics

Objective
The primary goal of this study is to identify genetic variation that is statistically predictive and/or causal for differential susceptibility to *Escherichia coli* clinical mastitis (CM) in U.S. Holstein dairy cows. We hypothesize that a genome-wide association analysis (GWAA) using pathogen-specific mastitis phenotypes together with host (bovine) and bacterial (*E. coli*) genome-wide variants will facilitate Holstein genetic improvement via existing genomic selection models.

Methods
Using > 9,000 commercial Holstein dairy cows, we attempted to sample from the extremes (multiple *E. coli* CM, n ≥ 300; zero lifetime *E. coli* CM, n ≥ 500); with the diagnostic isolates characterized via genome sequencing. Bovine DNA was genotyped using the Illumina BovineHD assay, thus producing host and pathogen genotypes to investigate the joint effects on risk for *E. coli* CM. GWAA with genomic relationship matrix (GRM) heritability estimates were produced, and genomic best linear unbiased prediction (GBLUP) was used to investigate the potential for reducing Holstein susceptibility to *E. coli* CM by performing genomic predictions with cross validation.

Results
GRM heritability estimates for differential susceptibility to *E. coli* CM were moderate ($h^2 \geq 0.16 \pm 0.07$). GWAA revealed quantitative trait loci (QTL) on BTA17, BTA25, BTA16, and BTA29. Based on the proportion of phenotypic variance explained (PVE), most QTL ($P \leq$ 5e-05) were estimated to have large effects (PVE ≥ 2.0%) on the risk of *E. coli CM* in U.S. Holstein cows. Likewise, some *E. coli* virulence loci were also associated with recurring *E. coli* CM. Genomic predictions with cross validation produced mean accuracies (≥ 0.60) that were similar to bovine health and production traits previously investigated.

Conclusions
Bovine QTL and *E. coli* virulence loci detected herein positively augment existing knowledge related to host-pathogen physiology in U.S. Holsteins. Genomic predictions with cross validation support the reduction of Holstein susceptibility to *E. coli* CM by employing large reference populations for single-pathogen (*E. coli*) genomic predictions.

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

Notes:

V025 - The role of exosomes in Marek's disease virus pathogenicity and immunity

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Session: Immunology

Objective
Marek's disease (MD) is a T-cell lymphoma of chickens caused by Marek's disease virus (MDV). Losses due to MD are controlled via the use of live, apathogenic vaccines, however the mechanisms mediating life-long protection from tumor formation are not fully understood. Chickens vaccinated *in ovo* or at hatch are protected from tumor formation, but not superinfection with oncogenic MDV field strains. The purpose of our research is to identify the contributions of serum exosomes to lymphomagenesis, tumor progression, immune suppression and conversely, systemic anti-tumor immunity. Our hypotheses are that (1) serum exosomes expressed during MDV latency contribute to tumorigenesis and systemic immune suppression and (2) serum exosomes produced during vaccine virus replication elicit lifelong systemic anti-viral and anti-tumor responses.

Methods
To address these hypotheses, we have purified exosomes from the serum of tumor-bearing, vaccinated/challenged, and vaccinated/not challenged chickens using size-exclusion chromatography. These were characterized by TEM, nanotracking analysis (NTA) and protein expression, as well as whole transcriptome sequencing. Purified exosomes were labeled via CFSE for examining uptake by macrophages and dendritic cells to assess their effects on immune signaling and antigen presentation. Complete proteomes of chicken monocytic cell line HD11 and HD11 cells patterned to become macrophages and dendritic cells were determined prior to and 24 hrs post-treatment with purified exosomes.

Results
Our data show that upon differentiation to macrophages and dendritic cells, distinctive proteomic changes were consistently detected that affected the metabolism of the patterned cells. Moreover, we found that exosomes from vaccinated chickens (VEX) and tumor-bearing chickens (TEX) affected the proteomes of these cells in distinct ways.

Conclusions
Our data suggest that in addition to providing antigens for presentation to the immune system, exosomes may be important to the regulation of immunometabolism during a sustained immune response.

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

Notes:

V026 - Host-pathogen interaction in bovine cryptosporidiosis

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Session: Immunology

Objective
Cryptosporidiosis, a disease caused by *Cryptosporidium parvum* (*C. parvum*) in cattle, is one of the most economically significant production limiting diseases in calves worldwide. The differential immune dynamics responsible for resistance to infection in adult cattle, but susceptibility in calves, have not been fully explored. Intestinal epithelial cells (IEC), the target cell for *C. parvum*, initiate innate mucosal immune responses after colonization. Dendritic cells (DC) then propagate the response, activating and shaping the adaptive immune response. Age-related differences in IEC-DC interactions in calves and their correlation with immunity to *C. parvum* are unknown. The objective of this study was to substantiate the culture system to examine *C. parvum* -induced IEC-DC crosstalk for future use to evaluate age-related differences.

Methods
Healthy adult cattle were used to establish a culture system consisting of enteroid-derived 2D monolayers generated on transwell inserts and monocyte-derived DCs (MoDCs) cultured in the well below. The apical intestinal monolayer surface was stimulated with Phorbol-Myristate-Acetate and Ionomycin (PMA/I) or *C. parvum*, and cellular responses were assessed. Flow cytometry was used to evaluate MoDC activation, and basolateral cytokine secretion was quantified using a multiplex cytokine panel.

Results
Following infection of the apical compartment, changes in MoDC phenotype and basolateral cytokine secretion were detected. The degree of cytokine secretion following C. parvum exposure was greater with the culture system than when IEC or MoDC were cultured alone, suggesting that thy quantified cytokine secretion originated from cellular crosstalk—communication between the monolayer and MoDC.

Conclusions
The altered cytokine secretion and MoDC phenotype following stimulation of the culture system demonstrates the model's functionality for use in this project's future aims.

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

Notes:

V027 - Exploring the associations between perceived self-efficacy, subjective norms, and the intention to use antibiotics

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Session: Antimicrobial Use

Objective
Human values and attitudes on antibiotic resistance are important when seeking to understand antibiotic use among U.S. dairy farmers. Using Schwartz's theory of basic human values and Ajzen's planned behavior theory, we hypothesized that different human values, and attitudes towards perceived ease or difficulty of administering antibiotics influence the use of antibiotics, and the relationship is mediated by what others think of their use.

Methods
For this study, 269 non-organic dairy farmers were selected from a 2021 survey consisting of 45 questions completed by 315 farmers from FL, MI, OH, VT, and WI. SPSS was used for descriptive, exploratory, and confirmatory analysis. Structural Equation Modeling was used for model fit, mediation, and differences between human values. The construct of perceived self-efficacy included items addressing effort needed to measure and improve how antibiotics are used, availability and use of protocols, and situations meriting the administration of antibiotics in the herd. Intended use was measured by two vignettes of a metritis case warranting antibiotics and whether intramammary antibiotics are used at dry-off. Subjective norms included referencing product labels and the degree of value placed on veterinarian and peer recommendations. The Portrait Values Questionnaire was used to examine differing human values.

Results
Model fit was appropriate ($p \leq .001$; CFI = .96) indicating those with greater self-efficacy for administering antibiotics are more likely to consider advice regarding antibiotic use when necessary. Likewise, the human values model fit was appropriate ($p = .04$; CFI = .97) indicating those who value stimulation, self-direction, and hedonism perceived themselves as capable of appropriate antibiotic use, would consider advice, and use antibiotics when necessary.

Conclusions
Results indicate human values can influence the administration of antibiotics as those who value obedience, tradition, benevolence, egalitarianism, security, and stability are less likely to reference product labels and consult veterinarians and peers prior to using antibiotics.

Notes:

V028 - The combined effect on the hypothalamic transcriptome of fasting and maternal immune activation on pigs

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Session: Omics

Objective
Fasting can elicit molecular changes in the hypothalamus that can influence appetite behavior and hormonal levels. Inflammatory conditions during development can also affect the hypothalamic molecular mechanisms and modulate the response to fasting. The objective of this study was to evaluate the effects of fasting on the hypothalamic transcriptome of male and female pigs exposed to maternal infection during gestation.

Methods
Gilts were inoculated with the porcine reproductive and respiratory virus (PRRSV) during the final third of gestation, and among 80 offspring, half underwent 24-hour fasting at 60 days of age while the rest served as control. Transcriptome analysis using RNA-seq enabled the testing of the effects of maternal immune activation (MIA), fasting, and sex and associated interactions.

Results
Altogether, over 1900 genes were differentially expressed (FDR-adjusted P-value <0.05 and |log2(fold change between pig groups)| > 1.2) in response to fasting, MIA, or sex. Females exposed to MIA had more differentially expressed genes in response to fasting relative to unexposed offspring. This result suggests that MIA may sensitize the hypothalamus transcriptome response to fasting females. Genes annotated to the type I diabetes mellitus pathway were prevalently under-expressed in fasting females exposed to MIA while over-expressed in fasting males. The glycoprotein hormone alpha polypeptide gene (CGA) was under-expressed in fasting relative to saline-treated males, irrespective of MIA exposure. The thyrotropin-releasing hormone (TRH) gene was under-expressed in fasting relative to control males exposed to MIA.

Conclusions
Our results contribute to understanding the interplay between MIA, sex, and fasting to advance management and health practices in swine herds.

Financial Support
U.S. Food and Drug Administration; U.S. Department of Agriculture-National Institute for Food and Agriculture-Agriculture and Food Research Initiative grant number 2018-67015-27413

Notes:

V029 - Effect of inactivated vaccines against coliform mastitis on milk when applied during the lactation period in cows

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Session: Vaccinology

Objective
Vaccines against mastitis are generally applied during lactation; despite being safe, one of the main concerns is their possible adverse effect on milk production. The aim of this study was to compare adverse effect on the milk production of multi and monovalent inactivated vaccine indicated for coliform mastitis.

Methods
The study was conducted in a farm located in Mexico. Healthy cows at the first month of lactation were recruited for the study and randomly allocated into 2 groups. Group A (n=19) was vaccinated with Startvac®, a multivalent vaccine against mastitis; group B (n=18) was vaccinated with a commercially available monovalent vaccine indicated against coliform mastitis. Even if both vaccines share same J5 antigen, this trial aimed to access if the different formulations can have different safety profiles. Rectal temperatures were recorded before and up to 1 day post vaccination (dpv). The individual milk production was recorded daily for 4 dpv.

Results
None of the animals involved in the study showed fever and both groups showed similar rectal temperatures. In terms of milk production, group A produced on average during the entire period 1.61 Kg/day/cow more compared to group B (33.62 vs. 32.01 Kg/day/cow) (t-student, p=0.0234); this corresponded to a cumulative difference of 6.25 Kg/cow between groups (134.49 vs 128.04 Kg).

Conclusions
The results of this study confirm that any of the tested vaccines produce fever or increase of rectal temperatures when applied during lactation. Additionally, Startvac® had less impact in terms of milk drop, in the days following vaccination. This can be due to the difference in the formulations. Further studies are required to develop this hypothesis.

Notes:

V030 - Characterization of genetic mechanisms of antimicrobial resistance identified in *Edwardsiella ictaluri*

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Session: Aquaculture

Objective
Edwardsiella ictaluri is an important pathogen of farmed raised catfish. Recently, we showed that resistance to tetracycline and florfenicol in the *E. ictaluri* MS 17-156 strain isolated from channel catfish was facilitated by acquisition of a 135 kb-sized plasmid (named pEIMS-171561).

Methods
In this study, we describe the genetic structure of pEIMS-171561. Plasmid copy number and stability within *E. ictaluri* strain MS-17-156 was determined. We also investigated the *in vitro* and *in vivo* transferability of pEIMS-171561 using catfish as a model for *in vivo* transfer.

Results
pEIMS-171561 belongs to the IncA/C group and contains florfenicol efflux major facilitator superfamily (MFS) (*floR*), sulfonamides (*sul2*), and tetracycline efflux MFS (*tetD*) genes. The plasmid contains two conjugative transfer-associated regions and encodes six transposases and insertion sequences. *In vitro* conjugation experiments demonstrated that the IncA/C plasmid can transfer from *E. ictaluri* to *Escherichia coli*. The plasmid is stable in *E. ictaluri without selection pressure for 33 days.* We showed that pEIMS-171561 did not transfer from *E. ictaluri* MS 17-156 to endogenous microbiota in catfish. Moreover, we could not detect *in vivo* conjugal transfer of pEIMS-171561 from *E. ictaluri* to *E. coli*. Results from real-time PCR revealed upregulation of *floR* gene in catfish intestine when receiving florfenicol medicated feed compared to fish receiving unmedicated feed.

Conclusions
This study demonstrated that pEIMS-171561 did not disseminate from *E. ictaluri* to gut microbiota under selective pressure. This result suggests a limited role of the fish microbiota as a reservoir for this plasmid and for spread of resistance.

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

Notes:

V031 - Effect of dietary trans-cinnamaldehyde on susceptibility of catfish to *Edwardsiella ictaluri*

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Session: Aquaculture

Objective
Edwardsiella ictaluri is the causative agent of enteric septicemia of catfish (ESC) and one of the most significant pathogens of US catfish aquaculture. The current therapeutic strategies to prevent ESC have their limitations, and catfish operations continue to suffer significant losses due to ESC. The problem is exacerbated by the increasing emergence of *E. ictaluri* multidrug-resistant (MDR) strains. The objective of this study is to find alternative intervention strategies to conserve antimicrobial use.

Methods
Minimum Inhibitory Concentrations (MIC) of trans-cinnamaldehyde (TC) was assessed against *E. ictaluri* 93-146 using broth microdilution. An efficacy trial was conducted to evaluate effectiveness of TC to treat *E. ictaluri* infection in channel catfish.

Results
Results demonstrated that TC inhibited the growth of *E. ictaluri* at concentrations of 20 µg/ml. Morphologies of *E. ictaluri* in response TC were observed using scanning electron microscopy (SEM) and transmission electron microscopy (TEM). After *E. ictaluri* challenge, significantly higher survival was found in catfish that received dietary-TC at the levels of 15 and 20 mg/kg compared to control group (49.12% and 65.52% survival vs 11.11% survival). Bacterial concentrations in spleen and anterior kidney were significantly lower in fish fed with TC (20 mg/kg diet) compared to control at 5-day post-infection.

Conclusions
Results indicate that supplementation of catfish feed with TC reduces *E. ictaluri* infection. A safe and efficacious alternative to antimicrobials will reduce the emergence and spread of antimicrobial resistant strains.

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

Notes:

V032 - Inactivation of veterinary-relevant viruses in decomposing tissues under simulated environmental conditions

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Session: Biosecurity and Infection Control

Objective
The introduction of foreign animal diseases affecting livestock into the U.S. will likely lead to significant animal mortality. Understanding the inactivation dynamics of relevant veterinary viruses in decomposing animal tissues is essential for successful pathogen mitigation efforts. We evaluated the inactivation of five veterinary viruses in decomposing tissues subjected to simulated environmental conditions.

Methods
The tested viruses included Senecavirus A (SVA), Feline Calicivirus (FCV), Bovine Herpesvirus type 1 (BoHV-1), Bovine Virus Diarrhea Virus (BVDV), and Porcine Epidemic Diarrhea Virus (PEDV). Each virus was homogenized with three tissue types: bone marrow (from young and adult animals) and spleen. In addition, tubes containing cell culture supernatant of infected cells were added for comparison purposes. The samples were incubated at two temperatures, 42°F (5.5°C) and 85°F (29.4°C), simulating winter and summer average temperatures in central Oklahoma. Samples were collected on days 0, 5, 10, 14, 20, 25, 31, 45, and 60. Two independent studies were conducted, and samples were evaluated by virus titration and virus isolation.

Results
Viruses remained viable for a longer period at 42°F compared to 85°F. At day 60, viruses with measurable titers at 42°F were SVA, FCV, and BoHV-1, with titers reaching over 10^8, 10^5, and 10^4 median tissue culture infectious dose ($TCID_{50}$/mL). In the tissue matrix, BVDV was viable up to day 45 (titer $<10^{1.8}$ $TCID_{50}$/mL), whereas PEDV was viable for 10 days. At 85°F, SVA was the only viable virus in the tissue matrix on day 60. The survival period was 15 days for BVDV and BoHV-1 and 5 days for FCV and PEDV. These results also demonstrated the variable inactivation length based on the matrix type. Notably, in samples kept at 85°F, SVA was viable for 14 days in spleen samples while over 60 days on bone marrow from mature animals.

Conclusions
These findings support databased risk assessment and the rational development of countermeasures during an eventual security breach in sites managing animal mortality.

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

Notes:

V033 - A comparison of 2 point-of-care glucometers in healthy ewes (*Ovis aries*)

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Session: Diagnostic Testing

Objective
Point of care (POC) devices are becoming commonly used for large animal research. Blood glucose is essential in the investigation multiple areas of animal health, from sepsis to nutrition. POC glucometers are readily available for use by veterinary practitioners; however, the performance of certain veterinary marketed POC glucometers have not been evaluated for sheep. Because of this, researchers are left with uncertainty when it comes to the performance or bias if using these devices in ovine patients. Our objective was to compare the performance of a POC blood glucose (BG) measuring device validated for small animals (Alphatrak 2; AT2) to a POC glucometer already evaluated for healthy ewes (Precision Xtra; PX).

Methods
Four healthy ewes had blood samples collected at various time points across 3 different days within a 3-week period. Once collected, blood samples were simultaneously evaluated by both devices. The AT2 analysis compared both the canine and feline settings. 88 blood samples were collected – 44 comparing the AT2 canine setting with the PX device and 44 comparing the AT2 feline setting with the PX. The results were evaluated via regression and Bland-Altman analysis.

Results
Pearson R values for feline and canine settings were 0.7269 and 0.4710 respectively. With both canine and feline settings, the AT2 overestimated BG concentrations compared to the PX. The AT2 canine readings showed increased bias (canine bias: 21.24 ± 8.087) compared to the AT2 feline readings (feline bias: 14.54 ± 5.878).

Conclusions
Veterinarians should be aware of the bias if using the AT2, in either the canine or feline settings, for BG evaluation in sheep compared to the PX device.

Notes:

V034 - Methicillin-resistant *Staphylococci* (MRS) distribution in shared public health bathrooms at workplaces

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Session: Antimicrobial Use

Objective
Staphylococci are commensals of skin, respiratory, and digestive tracts. They can cause skin infections, respiratory diseases, and food poisoning of the digestive tract. Some *Staphylococci* have acquired methicillin resistance and become a public health threat, as they are untreatable. MRS *Staphylococci* strains spread through contact, nasal discharges, and feces to the ecosystem. Reports on MRS are rising from diverse host ranges, various ecologies, and many surfaces but MRS studies at workplaces on different surfaces are scarce. This study evaluated MRS at workplaces on publicly shared bathroom sinks and toilets.

Methods
Ten sink and 10 toilet swabs from male and female bathrooms (N=40) were collected from a college compus and spread plated on a CHROMagar MRSA media. MRS colonies show mauve, red, dark pink, and light violet but colorless, blue, green, yellow, etc. colonies may not be MRS as per the manufacturer.

Results
42 isolates showing 14 different colony color appearances were isolated. The 14-colony color appearances included red (21%), blue (14%), gray (7%), green (7%), purple (7%), white (7%), and yellow (7%). The rest 7 isolates had 7 different colony colors i.e. each 2%. All green colored isolates were from the sink (n=3) and toilet (n=1) of males whereas all purple colored isolates were from the toilets (n=2) and sink (n=1) of females. Majority of the red colored isolates (n=9) were from the toilet (56% = 5/9) and sink (n= 33%) of males but one isolate (11%) was from the sink of females. The isolates were more abundant on the sink (60% =25/42) than on the toilet (40%). Of 42, 14, 11, 9, 5, and 3 isolates were from the sink of males, the sink of females, the toilet of males, the toilet of females, and the toilet of both genders, respectively.

Conclusions
Isolates with diverse colony appearances were detected on the sink followed by on the toilet indicating people “silently” shed them to pose a risk. We recommend sequencing of the isolates for their species/genotype determination and PCR for their *mec*A and SCC*mec* typing. Improving general hygienic practices at workplaces are needed.

Financial Support
Long Island University and New York Institute of Technology

Notes:

V035 - Transmission of H9N2 low pathogenicity avian influenza virus (LPAIV) in a challenge-transmission model

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Session: Virology

Objective
Migratory birds are major reservoirs for LPAIV, which can be transmitted to poultry and mammals. The H9N2 LPAIV has become prevalent in poultry over the last two decades. However, there is scarcity of detailed information on how this virus can be transmitted. The current study aimed to establish a direct contact model using seeder chickens infected with H9N2 LPAIV as a source of the virus for transmission to recipient chickens.

Methods
Seeder chickens were inoculated with two different inoculation routes either directly (combination of occular, nasal and tracheal) or via the aerosol route. To infect via aerosol route chickens were held in the aerosol chamber for a period of 20 min for maximum inhalation of aerosol particles. Twenty four hours post-infection, the recipient (naive) chickens were grouped with the respective inoculated seeder chickens in each model. Both the inoculated seeder and recipient groups were housed together for a period of 14 days.

Results
Shedding was observed to be higher in aerosol-inoculated seeder chickens, with a greater percentage of chickens infected at each time point. In terms of transmission, the recipient chickens exposed to the aerosol-inoculated seeder chickens had higher oral and cloacal virus shedding compared to the recipient chickens of the directly inoculated group. Furthermore, the aerosol route of infection resulted in enhanced antibody responses in both seeder and recipient chickens compared to the directly inoculated group. Overall, the results confirmed that the aerosol route is a preferred inoculation route for infecting seeder chickens in a direct contact transmission model.

Conclusions
In conclusion, the results signify that the aerosol and direct inoculation routes can be used as effective methods for experimentally infecting chickens with H9N2 LPAIV. Transmission occurs more readily from chickens that were infected via the aerosol route as compared to the direct inoculation route. Future studies should focus on determining the relative contribution of different routes of LPAIV transmission and various factors that can affect the transmission.

Financial Support
Egg Farmers of Canada; Natural Sciences and Engineering Research Council of Canada; University of Guelph; Arrell Food Institute; Ontario Ministry of Agriculture, Food and Rural Affairs; Chicken Farmers of Saskatchewan; Canadian Poultry Research Council

Notes:

V036 - Using Toll-like receptor (TLR) ligands to prevent H9N2 avian influenza virus (AIV) transmission in chickens

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Session: Immunology

Objective
Transmission of H9N2 AIV in poultry has been a major concern globally. H9N2 AIV outbreaks in chickens necessitate development of effective control strategies. Alternatives such as immunostimulatory molecules can be considered to enhance host immune responses against H9N2 AIV to prevent its transmission. The present study investigated the efficacy of TLR ligands CpG 2007 and poly(I:C) to reduce H9N2 AIV transmission from TLR treated seeder (trial 1) or inoculated chickens (trial 2) to naïve chickens in a direct contact transmission model.

Methods
We hypothesized that treatment with TLR ligands reduce oral and cloacal shedding and thereby affect transmission. Two independent trials were conducted to test the efficay of ligands to limit H9N2 AIV transmission in a direct contact transmission model.

Results
Results from trial 1 revealed that transmission of H9N2 AIV was reduced in all TLR treated groups. TLR ligand dosage affected virus shedding, with CpG 2007 low dose being effective at reducing oral shedding and the poly(I:C) high dose group showing the highest reduction in oral and cloacal shedding. In terms of transmission, recipient chickens exposed to CpG 2007 low dose treated seeder chickens showed maximum reduction in oral and cloacal shedding at different time points with the lowest number of animals being AIV positive. Results from trial 2 suggested that TLR treated recipient chickens were protected when exposed to the H9N2 AIV inoculated seeder chickens. Maximum reduction in oral and cloacal shedding was observed in the TLR treated chickens (recipient) of the poly(I:C) high dose group followed by chickens in the low dose CpG 2007 group. In the above two groups, an upregulated expression of antiviral and pro-inflammatory genes, including type I interferons (IFNs), protein kinase R (PKR), interferon induced transmembrane protein 3 (IFITM3), viperin, (interleukin) IL-1B, IL-8, and 1L-18 in the spleen, cecal tonsils and lungs were observed.

Conclusions
These results suggest that TLR ligands can be employed as effective anti-viral compounds for reducing AIV transmission in chickens.

Financial Support
Canadian Poultry Research Council; Egg Farmers of Canada; Natural Sciences and Engineering Research Council of Canada; University of Guelph; Arrell Food Institute; Chicken Farmers of Saskatchewan; Ontario Ministry of Agriculture, Food and Rural Affairs

Notes:

V037 - Comparison between ELISA and RT-QuIC to detect chronic wasting disease in retropharyngeal lymph nodes of deer

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Session: Diagnostic Testing

Objective
Current chronic wasting Disease (CWD) testing protocol requires submission of retropharyngeal lymph nodes (RPLN) samples reacting by ELISA testing to be confirmed by immunohistochemistry (IHC), a methodology that involves a degree of subjectivity. Furthermore, some studies have demonstrated the lower sensitivity by IHC, especially in samples with lower concentration of CWD prions, this can be a disadvantage with samples yielding borderline ELISA results. Real-time quaking-induced conversion (RT-QuIC) holds promise for testing tissues from dead animals, or clinical fluids or excretions from live animals. The aim of this study was to compare ELISA and RT-QuIC for CWD diagnosis.

Methods
A total of 227 RPLN were tested by ELISA (TeSeE® Detection Kit, BioRad) and by (RT-QuIC) with a truncated recombinant Syrian golden hamster PrP (HarPrP 90-231) and a BMG Labtech FLUOstar Omega reader. Agreement between ELISA and RT-QuIC was determined by Cohen's Kappa test. Pearson's correlation coefficients were determined between ELISA OD's and time to threshold or amyloid rate formation. Intra and inter-assay coefficient of variation was determined in six samples.

Results
Very good agreement between both methods was determined by Cohen's Kappa test, with a coefficient of 0.879, complete agreement was observed with 214 of the samples (94.27% of the observations). A Pearson's correlation coefficient (r) of -0.8178 with a r squared of 0.6688 was observed between ELISA OD's and time to threshold obtained by RT-QuIC. The correlation between ELISA OD's and amyloid rate formation was lower with a Person's coefficient of 0.5820 with a r squared of 0.3388. The RT-QuIC intra-assay coefficient of variation ranged from 9.73% to 43.39%, and the inter-assay coefficient of variation ranged from 26.36% to 39.50%. Comparison between RT-QuIC and IHC was conducted with 31 samples with agreement in 30 samples.

Conclusions
The results suggest that RT-QuIC may be useful as a confirmatory testing for CWD diagnosis in RPLN samples of CWD affected deer.

Financial Support
U.S. Department of Agriculture, National Animal Health Laboratory

Notes:

V038 - ASF virus neutralizing antibodies is highly associated with protection against virulent challenge in domestic pigs

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Session: Virology

Objective
To detect ASF virus neutralizing antibodies and its association with protection against virulent challenge in domestic pigs.

Methods
This study describes an ASF virus neutralization method to assess levels of virus neutralizing antibodies in domestic swine inoculated with live attenuated ASFV.
A total of 84 sera from pigs that were inoculated with two different ASFV attenuated mutants: ASFVG-deltaI177L and ASFVG-delta9GL/ deltaUK. The ASFVG-deltaI177L strain were inoculated at 10^2 by intramuscular route; 10^6 inoculated oral and nasal; and one group inoculated intramuscular with 10^6 from virus that growth in cell adapted. All animals from ASFVG-deltaI177L group were challenged at 28 days post inoculation. The ASFVG-delta9GL/ DUK strain were inoculated at 10^2, 10^4 or 10^6 dose by intramuscular route followed by challenged at 7, 14, 21 or 28-days post inoculation.
Detection of ASFV specific antibody was performed with In-house ELISA.
Virus neutralization was accessed in a Vero-adapted ASFV growth.

Results
ASFV specific antibody titer values increased in groups of animals challenged at 28 dpv compared to earlier challenge at 7, 14 and 21 dpv ($p<0.0001$, <0.0001, 0.0003). Only 2.38% of pigs were protected in the absence of antibody.
In the group of animals surviving the challenge, 95.7% of them demonstrated neutralization index (NI) activity and only 3 out 70 (4.3%) failed to induce neutralizing antibodies against ASFV. Therefore, there was a strong association between survival after the challenge and NI activity.
The Pearson correlation demonstrated that there was a positive correlation between survival and antibody neutralizing activity ($r=.68$; $P=0.0421$).

Conclusions
ASF virus neutralization activity was present in almost 100% of animals vaccinated with live attenuated vaccine candidates that survived to the ASV challenge. The humoral immune response plays a role on ASFV protection. But a complete correlates of protection may relies in other components of immune system, such as, Th1 and regulatory immune response.

Notes:

V039 - Evaluation of a surgical cannulation method for measurement of abomasal pH with a smartphone pH meter in ewes

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Session: General health and physiology

Objective
To evaluate the use of human percutaneous gastrotomy (PEG) tube for surgical cannulation of the abomasum in adult sheep, and evaluation of a point-of-care smartphone-based pH meter for abomasal fluid analysis in adult sheep.

Methods
PEG tubes were implanted under general anesthesia in four adult ewes. The abomasum was pexied to the body wall and the PEG tube was inserted via a stab incision and fixed in position with a purse-string suture.
Abomasal fluid was collected via the PEG tube. Determination of fluid pH was performed immediately after sample collection. Both smartphone-based pH meter and conventional benchtop pH meter were used for each sample. Results were compared by commercial statistical software.

Results
Four of four PEG tubes remained in place throughout the entirety of the study. Remaining PEG tubes were patent throughout the entire 31-day study period and 252 collections. All ewes remained healthy at the conclusion of the study.
Regression analysis of the smart-phone pH meter compared to the standard pH meter indicated a relationship of $Y = 1.011X - 0.1137$. R^2 was 0.9849. Bland-Altman analysis indicated a bias of -0.05917 ± 0.1951 and 95% limits of agreement were -0.4416 to 0.3233.

Conclusions
Ewes tolerated implanted PEG tubes well, and both standard and smartphone pH devices demonstrated high agreement.

Notes:

V040 - Effects of infection and anti-inflammatory supplementation on the behavior of gilts

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Session: Biosecurity and Infection Control

Objective
The inflammatory response of pigs to an immune challenge can be modulated by diet supplementation with omega-3 polyunsaturated fatty acids. This therapy is valuable because inflammation can increase the likelihood of health imbalances that become chronic. Changes in the levels of inflammatory indicators have been associated with changes in behavior. A study of the simultaneous effect of infection and omega-3 polyunsaturated fatty acid supplementation on the behavior of gestating gilts was undertaken.

Methods
The effects of porcine reproductive and respiratory syndrome virus infection and fish oil supplementation during gestation were measured on multiple behaviors. The behaviors of twelve Camborough gilts were video recorded for five hours during seven days along the final third of gestation. All gilts received a corn and soybean meal-based diet that matched the nutritional requirements. Measurements were collected on control gilts that were compared to gilts infected on day 76 of gestation and to gilts supplemented with docosahexaenoic and eicosapentaeonic fatty acids. The time displaying a behavior was described using a generalized linear mixed effects model including the effects of infection and supplementation and the day of measurement.

Results
The day of measurement had a significant effect (P-value < 0.05) on most behaviors, and the day-by-treatment effect was non-significant. Most behavioral changes between pre-infection and post-infection measurements became non-significant close to farrowing. The effect of day encompasses the effects of gestational progress, infection, and recovery processes. The time laying was influenced by the treatment received (P-value < 0.05) and non-supplemented infected gilts were observed laying more frequently than non-infected gilts.

Conclusions
The findings from this study indicate that infection and omega-3 fatty acids can modulate the behaviors of gilts during gestation.

Financial Support
U.S. Department of Agriculture, **National Institute of Food and Agriculture,** Agriculture and Food Research Initiative grant number 2018-67015-27413

Notes:

V041 - Transcriptome analysis of BT cells infected with typical and high virulence bovine viral diarrhea virus strains

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Session: Omics

Objective
The study evaluated the differentially expressed genes (DEGs) in primary bovine turbinate (BT) cells infected with typical or high virulence strains of bovine viral diarrhea virus (BVDV).

Methods
The typical virulence (TV) BVDV strains used were the non-cytopathogenic (ncp) BVDV2 RS886 and the cytopathogenic (cp) BVDV1 Singer. In addition, the study included the high virulence (HV) strain BVDV2 1373. BT cells inoculated with the described BVDV strains (multiplicity of infection of 1), and control cells were harvested 12- and 24-h post-infection (hpi). RNA was purified and submitted to poly (A) RNA sequencing. Gene expression analyses were performed, and DEGs were subjected to gene ontology and (GO) and KEGG pathways.

Results
The majority of DEGs at12 hpi were associated with the HV BVDV. DEGs were primarily associated with positive regulation of the respiratory chain complex (involved in mitochondrial ATP synthesis and proton transport), including genes MT-ND1, 2, 3, 4, MT-ATP6, and 8). In addition, HV BVDV downregulated several genes involved in the regulation of transcription by RNA polymerase II (ZMYM5, BCOR, RIF1, EGR1, and ZNF174), apoptosis pathway (PPP1R2, SUMO1, ATR), and immunity (MAVS, NSMAF, and JAK2). At 24 hpi, genes involved in regulating the respiratory chain continued upregulated for the HV strain. At 24 hpi the TV cp BVDV Singer upregulated genes mainly assigned to biological processes associated with inflammation pathway (CXCL3) and apoptosis (IRF7).

Conclusions
Characterizing DEGs in primary cells of the upper respiratory tract infected with different BVDV strains is critical for deciphering disease mechanisms and host responses. TV and HV BVDV strains modulate gene expression in unique ways. For example, HV BVDV distinctly induced the inhibition of genes responsible for the anti-inflammatory and apoptosis pathways. Interestingly, the HV BVDV seems more efficient in downregulating RNA polymerase II, a known mechanism to counteract host antiviral responses.

Financial Support
Oklahoma State University; Oklahoma Agriculture Experiment Station, U.S. Department of Agriculture Hatch

Notes:

V042 - TaqMan PCR assays with an internal control for detection of *M. bovis* and *M. avium paratuberculosis* in FFPE tissues

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Session: Diagnostic Testing

Objective
To develop improved TaqMan PCR (duplex/ triplex) assays by incorporating endogenous internal controls (IC) for rapid and reliable detection of *Mycobacterium bovis* (M. *bovis*) and *Mycobacterium avium* subsp. *paratuberculosis* (Map) in formalin-fixed paraffin-embedded (FFPE) tissues.

Methods
FFPE tissues from 37 tuberculosis,11 paratuberculosis and 16 negative controls were tested. Crude FFPE tissue extracts (CFTE) were used for both duplex and triplex TaqMan PCR with an IC (host origin) to monitor DNA integrity and PCR inhibition. Two IC targets: a highly conserved region of mammalian gene encoding interphotoreceptor retinoid-binding protein (IRPB) and a highly conserved region of eukaryotic 28S rDNA were evaluated. *M. bovis* PCR targeted multicopy IS1081 (present in all members of *M. tuberculosis* complex), single copy Locus 3 (*M. bovis* specific) and IC. Map PCR targeted multicopy IS900, single copy F57 (Map specific) and IC.

Results
Thirty-six of 37 tuberculosis cases were IS1081-positive. Of these only 18 were L3-positive in duplex assays. Fifteen of the 18 cases tested by *M. bovis* triplex PCR were both IS1081 and L3-positive while the other three were only positive for IS1081. For paratuberculosis, 10/11 cases were positive for both IS900 and F57 in duplex and triplex assays. Both ICs had shown stable amplification apart from one paratuberculosis case where IRBP failed to amplify. C_T values for IC IRBP were similar in singleplex, duplex, and triplex reactions and consistent between samples (28 ±3 with one exception). C_T values for 28S ranged from 15-30 except for two samples.

Conclusions
The optimized duplex/triplex TaqMan PCR assays performed directly on CFTE can detect *M. bovis* and *M. avium* subsp. paratuberculosis. IC data suggests that IRBP and 28S are stable IC's and viable options to rule out false negative results associated with the detection of *Mycobacterium* spp. This proof-of-concept study has shown that an endogenous IC incorporated in the TaqMan assay can significantly improve Mycobacteria diagnostics in FFPE tissues. More work is needed on a larger sample size to validate this assay.

Notes:

V043 - Frequencies and interactions of animals on Vermont dairy farms

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Session: Biosecurity and Infection Control

Objective
There is a growing need for a collaborative effort among researchers, farms, and the dairy industry to be better prepared to handle and survive a bio-disaster should such occur in Vermont. To achieve this, we designed a survey for Vermont dairy farmers. We aimed to use results from this survey to build simulated computerized models of how a disease could spread under various scenarios.

Methods
266 dairy farms within Vermont responded to the survey, which was divided into four sections: (a) Farm description (farm size and type) (b) Animal movements (c) Farm contacts (d) Farm biosecurity. Using the data obtained, we analysed the possible associations of the farm descriptors with animal movements, farm contacts, and farm biosecurity information. The data analysis was done using SAS® Studio version3.8 (Enterprise Edition) and R® Studio version 4.2.1 (2022-06-23 ucrt), and Microsoft® Excel® (Version 2209 Build 16.0.15629.20200) 64-bit.

Results
Of the 266 survey participants who completed the survey and had dairy cows on their farms, 39% (33%, 45%) of them had a herd size of 100-199 dairy animals, with most having 50-99 mature dairy cows. 28% (22%,35%) of surveyed farms were organic, none of which had above 500 dairy animals. 93% (89%, 96%) of survey respondents reported deer (86%, 93%) and moose (7%, 15%) hunting activities within 500 feet of their livestock/pastures in the fall, winter, and spring sighting 132(96, 144) deer and at least 2 (1, 3) moose in 2009. 98% (95%, 99%) of Vermont dairy farms provided information on their replacement heifers' direct contact history with other farms. 14% (10%, 19%) of them commingled these heifers with animals from other farms, of which 85% (68%, 94%) were not quarantined before introduction into their dairy herd.

Conclusions
Vermont produces about two-thirds of New England’s milk. Therefore, proper knowledge of farm management decisions driving animal interactions in the disease transmission dynamics of Vermont dairy farms would be invaluable to enhancing emergency preparedness.

Financial Support
Vermont Agricultural Experiment Station

Notes:

V044 - Assessing the gut microbiome as a tool for the mitigation of respiratory disease in nursery pigs

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Session: Omics

Objective
Porcine reproductive and respiratory syndrome virus (PRRSV) causes the most costly disease to swine production in the United States. Disease caused by this virus often involves secondary bacterial pathogens, which exacerbates respiratory disease severity and increases antimicrobial administration in young growing pigs. Although commercial vaccines are used to reduce the effects of PRRSV on swine health, the currently available vaccines are considered inadequate for disease control. Alternative strategies for control of PRRSV is needed to maintain swine health and welfare while lessening the economic effects of this disease on pork producers.

Methods
The goal of this study is to investigate the gut microbiome as an alternative prevention tool for PRRSV control due to its impact on the immune system and clinical outcome after infection. Fecal microbiota transplant (FMT) material was collected from donor sows with several health and production characteristics, processed to concentrate microbes, and administered to weaned pigs for microbiome modulation prior to co-infection with PRRSV and porcine circovirus type 2d (PCV2d). Litter and sex-matched controls were administered saline. Following a 7-day administration of FMT or saline orally, the population of pigs (n = 100) were co-infected and followed for 6 weeks post-infection.

Results
Objectives of this study include investigating the effects of microbiome modulation on outcome of swine with respiratory disease, including PRRSV and PCV2d replication, morbidity and mortality, average daily weight gain, lung and lymphoid pathology, and humoral immune response. Further, a second objective includes the identification of beneficial gut microbes within the FMT material, including correlations with presence and abundance, which are associated with improved health outcomes in growing pigs under a porcine respiratory disease complex model.

Conclusions
Our goal is to determine how beneficial gut microbes may be used as a preventative medicine tool to reduce the effects of respiratory disease and decrease the need for antimicrobials in swine.

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

Notes:

V045 - A framework for multi-player decision-making on the allocation of limited resources on simulated FMD outbreaks

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Session: Epidmiology

Objective
Necessary response for a livestock transboundary infectious disease outbreak will likely outpace available resources. Consequently, policymakers need strategies to inform decisions about allocating limited resources. This study aimed to develop a framework that strategically examines decision-positions from a series of simultaneous multi-player decision sets, using desired criteria and rules of allocation.

Methods
We examined the impact of vaccine dose allocation decisions on simulated foot-and-mouth disease (FMD) outbreaks. We modeled six stochastic FMD epidemiological scenarios using the InterSpread Plus tool. Stakeholders were denoted by two decision-makers (DMs). DM1 represented the index state, and DM2 represented a group of three neighboring states. We selected two outcome criteria, outbreak size for DM1 and outbreak duration for DM2. The vaccine dose allocation strategies were determined by a third party that evaluated requests according to established rules. Rule 1 prioritized vaccine dose allocation to the index state, rule 2 prioritized allocation to the neighboring states, and rule 3 provided equal prioritization among DMs. 300 simulations of each scenario were run and the ranking of the 25th, 50th and 90th quantile of the scenario outcomes were compared. Those rankings were treated as the payoffs of DMs and were analyzed as static games with perfect information.

Results
Nine feasible decision-positions were evaluated per allocation rule according to equilibrium principles for game theory. The decision-position of sharing doses was both a Pareto optimal and a Nash equilibrium solution in every case. Under rule 3, more outcomes resulted in Pareto optimal payoffs than under rule 1 or 2. For 50th quantile results, there were more action combinations that resulted in Nash equilibrium solutions.

Conclusions
The proposed modeling framework incorporates epidemiological data and accounts for the payoffs resulting from multiple stakeholders' choices. This approach can aid in decision-making for scarce resource allocation in contexts where individual payoffs depend upon others' choices.

Financial Support
Kansas State University; U.S. Department of Agriculture, Animal and Plant Health Inspection Services; U.S. Department of Agriculture, Agriculture and Research Services, Center for Epidemiology and Animal Health

Notes:

V046 - Epigenetic changes associated with increased phagocyte functions demonstrate trained immunity in catfish leukocytes

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Session: Aquaculture

Objective
Trained Immunity (TI) is the immunomodulation of innate immune cells that provides non-target protection following stimulation. It is determined by epigenetic reprogramming. TI is characterized by metabolic changes that modify immune cell functions. Our study was performed to determine if TI occurs in leukocytes from catfish exposed to beta glucan (Bg), a known inducer of TI. We evaluated this using functional assays, differential transcriptome analysis and differential chromosome modification analysis.

Methods
Catfish were injected with saline or beta glucan (bg). After 1 month, studies were performed to determine the effects on the in vivo, leukocyte, and genomic immune functions. In the in vivo experiment, tank survival trials were performed by injection of *Edwardsiella ictaluri* and *E. piscicida*, and survival determined. In the leukocyte experiments, flow cytometry analyzed phagocytosis or binding of *E. ictaluri* and *E. piscicida* by cells labeled with monoclonal antibodies L/CD207, mpeg-1, 51a, nccrp-1, 9E1, or C24a for dendritic cells, macrophages, neutrophils, non-specific cytotoxic cells, B-cells or T-cells, respectively. To evaluate genomic functions, Chromatin immunoprecipitation (ChIP) and deep sequencing analyses of H3K4 and H3K27 were performed. KEGG pathway analyses were performed on differentially expressed genes.

Results
Fish that received bg followed by an IP injection of *E. piscicida* had higher survival. Fish that received an IP injection of bg followed by an IP injection of *E. ictaluri* had higher survival. Flow cytometry demonstrated neutrophils phagocytosed more *E. piscicida* than neutrophils from saline exposed fish. Neutrophils and macrophages from bg exposed catfish phagocytosed more *E. ictaluri* than control neutrophils and macrophages. Genomic analyses demonstrated modifications at H3K4 and H3K27.

Conclusions
Our study demonstrated the hallmarks of trained immunity. Beta glucan induced epigenetic reprogramming in leukocytes. This resulted in enhanced macrophage and neutrophil cell signaling and phagocytosis which provided protection against bacterial infections.

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

Notes:

V047 - Investigating the role of dairy farm antimicrobial use on the bovine fecal resistome

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Session: Antimicrobial Use

Objective
The rising impacts of antimicrobial resistant infections emphasize a need to better understand the emergence and dissemination of antimicrobial resistance across environments. While antimicrobial use (AMU) is considered a main driver of emergence in livestock and human healthcare, its impacts on the fecal resistomes of dairy cattle are less resolved, largely due to the difficulty of AMU quantification on dairy farms. Here we sought to leverage both the quantification of farm AMU and shotgun metagenomic sequencing to compare the antimicrobial resistance gene (ARG) ecology of cattle feces from commercial dairy farms in Wisconsin, USA with high and low AMU. We hypothesize there to be no significant difference in fecal ARG diversity between cattle from high and low AMU farms.

Methods
Fecal samples were collected from individual cattle on 8 conventional WI dairy farms, 4 having high AMU and 4 with low AMU, monthly for 4 months. Samples were collected from 4 animal groups: calves, cull cows, sick cows, and healthy lactating cows, and were pooled by animal group per sampling. DNA was extracted from the resulting composite samples and submitted for shotgun-metagenomic sequencing. ARGs were annotated from quality reads and their abundances were compared by farm AMU.

Results
We report a 2.9-fold difference in mean AMU between low and high AMU farms in our study. Our analyses of ARG carriage to-date have found calf feces to carry distinct ARG compositions from adult cull cattle, with calves carrying more ARG-classified reads and a higher proportion of tetracycline resistance genes than cull cows. Our current investigations are focused on comparing ARG carriage between high and low AMU farms and evaluating the roles of animal group, time, and individual antimicrobial use in ARG-carriage to better elucidate the dynamics of antimicrobial resistance emergence on dairy farms.

Conclusions
Thus far we conclude that calves in this study carry higher abundances of ARG than cull cows. We will look to see if this pattern continues, and if it is impacted by farm AMU, once we complete sequencing for all samples.

Notes:

V048 - Genomic analysis and transfer dynamics of ICE carrying multidrug resistance in *H. somni* from feedlot cattle

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Session: Omics

Objective
The study examined whole genome sequences of *Histophilus somni* isolates and mobile genetic elements carrying antimicrobial resistance genes. An isolate of *H. somni* harboring an Integrative Conjugative Element carrying multiple antimicrobial resistance genes was used for a series of in vitro experiments to determine ICE transfer frequency and host range.

Methods
H. somni isolates from Alberta feedlot cattle were used to construct whole-genome assemblies from Illumina short-reads. Mobile genetic elements and AMR genes were identified using *in silico* methods. A large mobile genetic element was identified in multi-drug resistant *H. somni* strains. One strain carrying an ICE was selected for use in in vitro conjugation assays to examine transfer frequency and host range. Susceptibility of recipients was done using microbroth dilution. Competition experiments and long-term passage experiments were used to examine fitness costs of ICE acquisition.

Results
We identified *ICEHs02* which is 72,914 base pairs, and 79 genes including tetracycline, aminoglycosides, florfenicol, sulfonamide, and multicopper oxidase resistance genes. The ICE was transferable to *H. somni* and *P. multocida*. Multi-drug resistance was transferred. Transfer rates increased upon tetracycline and ciprofloxacin induction. There was a nominal fitness cost.

Conclusions
The conjugal transfer of a multi-drug resistance ICE between bacterial species associated with bovine respiratory disease elucidates one mechanism of dissemination of antimicrobial resistance among pathogens. Observed increased transfer frequency when exposed to antimicrobials provides information about drivers of the dissemination of resistance.

Financial Support
Alberta Agriculture and Forestry

Notes:

V049 - Translating the genomic and environmental landscape of BRDC for genetic improvement of U.S. dairy cattle

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Session: Omics

Objective
The objective of this study is to catalyze a long-term reduction in bovine host risk and production losses due to Bovine Respiratory Disease Complex (BRDC) across multiple U.S. dairy intensive regions by selecting against variants that significantly increase susceptibility (i.e., removal of highly susceptible breeding stock), and by integrating genomically estimated breeding values into selection indexes.

Methods
Collectively, 1000 Midwestern preweaned commercial Holstein calves (n = 500 BRDC cases, n = 500 controls) will be enrolled in the present study using the McGuirk Holstein Calf Health Scoring System (clinical scores). Bacteriology and virology will be performed for all calves using nasal swabs, as in the BRD-CAP project. Midwestern genome-wide association analysis with genomic relationship matrix heritability estimates will be produced using high-density genotypes and BRDC phenotypes (i.e., binary case-control, clinical scores; with and without pathogen data). Genomic predictions with cross validation will be deployed for the Midwest. Thereafter, Midwestern Holstein calf data will be merged with Western Holstein calf data from the BRD-CAP (n = 2800); to determine if training and prediction should occur for individual populations or regions (due to GxE interactions; differing primary BRDC pathogens), or if prediction accuracy generally increases with inclusion of sequentially larger U.S. training sets.

Results
Collectively, 347 preweaned commercial Holstein calves from two Midwestern facilities (WI, OH) have been enrolled in the present study (n = 180 cases, n = 167 controls); with bovine DNA isolated, diagnostics performed, and enrollment ongoing. A genomic prediction pilot study with cross validation using a custom 10K Illumina array demonstrated that mean prediction accuracy was relatively high (0.76) on two independent commercial populations, despite regional differences.

Conclusions
BRDC pathogen profiles differ across U.S. dairy intensive regions; yet our initial findings provide support for reducing susceptibility to BRDC in commercial U.S. Holsteins via genomic prediction.

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

Notes:

V052 - Evaluation of the abundance of ESBL-producing bacteria in shared public bathroom sinks and water bodies

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Session: One Health / Public Health

Objective
Morbidity and mortality of antimicrobial resistant bacteria (ARB) cause the highest economic and public health burden due to poor response to treatments. ARB are spreading globally in humans, animals, and the environment. Human digestive tract harbors microbiota including diverse ARB that are excreted with feces to toilet sink. Ill-treated bathroom sewages pollute the environment such as water bodies. Thus, this study evaluated the abundance of ESBL-producing bacteria from shared public bathroom sink samples and four lakes in Bishoftu, Oromia region, Ethiopia.

Methods
63 samples were collected from 10 locations in Bishoftu, Ethiopia. They included 37 sink swabs from five health facilities (a hospital and 4 health centers), 10 sink swabs from college of Veterinary Medicine, and 16 water samples from four lakes. We diluted each sample serially 10-fold using saline solution, spread plated 100 µl of 10-4 dilution on ESBL CHROMAgar, and differentiated bacterial colonies to genera by their unique color. The representative isolates of each genus were tested by anti-CTX-M antibody coated lateral flow assay device to determine the responsible enzyme for ESBL.

Results
Of the 63 samples, 49.2%, 30.2%, and 22.2% of *Pseudomonas*, *E. coli*, and *Klebsiella* were grown on CHROMagar ESBL media, respectively. However, 73.7% (14/19), 50% (7/14), and 3.2% (1/31) of *E. coli, Klebsiella*, and *Pseudomonas* were positive for CTX-M enzymes, respectively. Based on sample source, *Pseudomonas* was the most prevalent ESBL-producer bacteria in water bodies (100% of water samples). Whereas *E. coli* was the most prevalent ESBL-producer bacteria in toilets (n =19). Male toilets seem to carry ESBL-*E. coli* and ESBL-*Klebsiella* but female toilets had higher proportion of ESBL-*Pseudomonas*.

Conclusions
Three ESBL-producer bacterial genera are abundant in public toilet sinks; thus, droplets from sink splashes put the public at risk. Water bodies are reservoirs of ESBL-*Pseudomonas* putting swimmers and fishermen at risk. ~75% of ESBL-*E.coli* and 50% ESBL-*Klebsiella* utilize CTX-M enzymes for ESBL but 96.8% of ESBL-*Pseudomonas* did not use CTX-M.

Financial Support
NGB (France), LIU and CVMA AAU

Notes:

V054 - Methods for error control when a new trial is motivated by the results of an existing network meta analysis

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Session: Modeling, Methods, & Study Design

Objective
Both pairwise and network meta-analysis can be used to inform the design of a new trial. However, there is concern that this idea could be used inappropriately. When the decision to conduct a new trial is dependent on the results from an existing network, analyzing the new trial with the existing network leads to inflated type one error. To avoid such a problem, one could analyze the new trial on its own. While this traditional approach controls type one error, it is still desirable to develop methods that allow the new trial to be analyzed with the existing network. The motivation for this is two-fold: first, eventually trials might be added to a network regardless of a researcher's intentions in research synthesis, and second, leveraging information in NMA is more powerful than analyzing a trial on its own.

Methods
We propose two resampling based methods that allow a new trial that has been motivated by an existing network to be analyzed with said network. Both methods are assessed through simulation studies designed to evaluate error control and power.

Results
Simulation results show that the two proposed methods control the type one error rate at the desired level and are more powerful than analyzing the new trial on its own. Under the first scenario, where the criteria to conduct the new trial depends on a p-value for the comparison of interest from the existing network less than .1, the first method is more powerful than the second. Under the second scenario (p-value between .05 and .1) the two methods perform similarly.

Conclusions
Both proposed methods allow the new trial to be added to the existing network meta-analyses when the new trial is motivated by the existing network. When the criteria for conducting the new trial is known, the first method is preferred, otherwise, the second method can be used. Our proposed methods control the type one error rate and are more powerful than the naive approach of analyzing the new trial by itself.

Notes:

V055 - Public health implication of *Brucella abortus* strain-19 vaccine in milking cows: a shedding of *brucella* organisms.

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Session: Food Safety

Objective
Therefore, the study is aimed at detecting the brucella organisms in milk following vaccination with reduced-dose of *Brucella abortus* Strain-19 vaccine in milking cows. This will explore the public health threats associated with the vaccination of milking cows even with a reduced-dose.

Methods
The presence of *Brucella* DNA in milk samples was detected by *BCSP31* gene-targeted PCR and by amplification of IS711-genes by conventional PCR. Confirmation of the PCR positive samples was done by qPCR using Taqman^{R} assay.

Results
A total n=120 milk samples were collected from the vaccinated milking cows during days post-vaccination (DPV) at 30PV, 60PV, 90PV and 120PV (n=10 each). Some were found positive for both *Brucella* genus and species-specific genes in subcutaneously (s/c) vaccinated cows. Significantly higher positives were detected by the qPCR. BCSP31 sequence was deposited at GenBank NCBI and assigned accession no. MK881173-6. The presence of brucella organisms in milk of vaccinated cows revealed the risk of zoonotic transmission of brucellosis to humans, if raw milk was consumed and the public health threats associated with that.

Conclusions
These results strongly suggested that use of both conventional PCR and qPCR techniques could lead to more reliable diagnosis of brucellosis from vaccinated bovine milk samples. There is a public health of contracting brucellosis in milking cows

Notes:

V056 - Engineering a recombination resistant PEDV by targeting the TRS for live attenuated vaccine development

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Session: Vaccinology

Objective
Porcine epidemic diarrhea virus (PEDV) is a deadly coronavirus of neonatal pigs, but no safe and effective vaccines are available. Due to the ability to induce protective immunity in sows and passively protect piglets against PED via colostrum and milk, live attenuated vaccines (LAVs) are urgently needed to control PED worldwide. However, the potential recombination between vaccine and field strains raises a safety concern for LAVs. During coronavirus replication, a discontinuous transcription mechanism is employed, and transcriptional regulatory sequences (TRSs) are critical elements regulating the process. We hypothesized that a recoded TRS that is incompatible with wildtype (WT) TRSs can block recombination between the engineered virus carrying the recoded TRS and field strains carrying WT TRSs because a recombinant carrying incompatible TRSs is nonviable. This may serve as a strategy to design safe LAVs. Our objective is to engineer a recombination resistant PEDV by remodeling the TRS.

Methods
We used an infectious clone-derived reporter PEDV dORF3-EGFP as a backbone to engineer one remodeled TRS (RMT) mutant that carries recoded TRSs for all ORFs, except the one for the EGFP gene.

Results
The RMT mutant and dORF3-EGFP virus showed comparable replication profiles in vitro. No/extremely low EGFP level in the RMT-infected cells indicated the incompatibility between the recoded and WT TRSs. In neonatal gnotobiotic pigs, the RMT mutant had similar attenuated phenotype and replication efficiency to dORF3-EGFP. Challenge study showed that both RMT and dORF3-EGFP retained good immunogenicity. The modified TRSs were genetically stable after passaging in vitro and in vivo. In pigs co-infected with RMT (or dORF3-EGFP as a positive control) and a PEDV S-INDEL strain Iowa106, potential recombinants were found from the pigs co-infected with dORF3-EGFP and Iowa106, but not the group co-infected with RMT and Iowa106.

Conclusions
The RMT mutant is resistant to recombination due to incompatibility between the recoded and WT TRSs and can serve as a platform for LAV development.

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

Notes: