PHI ZETA

The Honor Society of Veterinary Medicine
Epsilon Chapter

November 13, 2024

Research Emphasis Day

AUBURN UNIVERSITY
COLLEGE OF VETERINARY MEDICINE

PHI ZETA
EPSILON CHAPTER
COLLEGE OF VETERINARY MEDICINE
AUBURN UNIVERSITY

welcomes you to our

PHI ZETA RESEARCH DAY FORUM
November 13, 2024

We want to thank all the presenters, their co-investigators and mentors for their participation in this annual event.

We also want to thank the following departments for their support:

Office of the Dean
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Department of Anatomy, Physiology, and Pharmacology
Department of Clinical Sciences
Department of Pathobiology
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PROGRAM

PHI ZETA RESEARCH DAY FORUM

NOVEMBER 13, 2024 – VETERINARY EDUCATION CENTER

8:00: Opening Statement

Dr. Frank F. Bartol,
Alumni Professor and Associate Dean for Research & Graduate Studies

8:15-11:30 MORNING Presentations

Veterinary and Graduate Students -101 VEC (Moderator: Dr. Bruce F. Smith)

8:15	Grace Hurley	Evaluation of the ability of *Babesia rossi* to induce neutrophil extracellular trap(NET) formation
8:30	Blake Park	Prognostic Value of Lactate to Albumin Ratios in Horses with Enterocolitis
8:45	Allison Rucker	Effects of Prepubertal and Concurrent Exposures to Endocrine Disrupting Chemicals in the Male Gonad
9:00	Melikasadat Mollabashi	*In Vitro* Antimicrobial Activity of Canine Platelet Lysate with variable Leukocyte concentration, Plasma Content, and Heat Sensitive Proteins
9:15	Peter Neasham	Studying co-infection of IAV at the Human-Swine Interface in fully differentiated Primary Bronchial Epithelial Cells
9:30	J Fletcher North	Immunogenicity of a Universal Influenza Vaccine in Swine with Pre-Existing Immunity
9:45	Mana Okudaira	Inflammatory cytokine priming alters the biocargo of equine bone marrow derived mesenchymal stem cell extracellular vesicles (BM-MSC-EVs)

10:00-10:15 Break

10:15	Dimitrios Sakkas	Computationally Optimized Broadly Reactive Antigen (COBRA) Intranasal Vaccination offers protection against challenge with the pandemic 2009 H1N1 Influenza A Virus (IAV) in the porcine model
10:30	Jyoti Yadav	Antibody Gene Therapy Protects Against Lethal Rabies Encephalitis

Graduate Students -Overton Auditorium (Moderator: Dr. Jeff Huang)

8:15	Camila Cuadrado	Vaccination with Recombinant LaSota Virus Expressing IBV Spike Protein: Immune Response in Chickens with Maternal Antibodies
8:30	Nikolia Darzenta	The anti-inflammatory properties of Mesenchymal Stromal Cell derived Extracellular Vesicles on feline neuroinflammation
8:45	Lana Dedecker	Pulmonary disposition and pharmacokinetics of chloramphenicol in healthy fasted adult horses
9:00	Raimundo Espejo	Mucosal Immune Responses in the Harderian Gland Following Newcastle Disease LaSota Vaccination in Chickens with Maternally Derived Antibodies
9:15	Y Savanie Fernando	Extracellular Vesicle Delivery of Antiviral Therapy for Treating Rabies Encephalitis
9:30	Pankaj Gaonkar	Antimicrobial resistance in the different stages of commercial poultry production environment
9:45	Natasha Grabau	Therapeutic Potential of an Orexin 2 Receptor (OX2R) Agonist in Addressing Secondary Symptoms of Narcolepsy in Orexin Knock-Out (OX-KO) Mice

10:00-10:15 Break

10:15	Terri Higgins	Development of Next-Generation Oncolytic Virus and CAR T cell Immunotherapies for Treatment of Osteosarcoma in a Canine Model
10:30	Oscar Huertas	Nasal and bronchoalveolar fluid BRSV IgG-1 titers transferred from maternal colostrum or a colostrum replacement in dairy calves
10:45	Mary Douglass Kerby	Effects of iodine sterilization and storage on microbial count, histologic properties, and omega-3 fatty acid concentration for tilapia fish skin grafts
11:00	Alana Kramer	Immunotherapy on Demand: Tailoring a PD-1 Dependent 4-1BB Agonist for Combination Immunotherapy
11:15	Hannah Maxwell	Factors Associated with Survival in Goats Treated for Toxic Mastitis

11:30-12:00 Break

12:00–1:30 POSTER Presentations

1:45-3:30 AFTERNOON Presentations

Faculty/Postdoctoral/Staff – 101 VEC (Moderator: Dr. Vinicia Biancardi)

1:45	Amanda Gross	Dual site administration of AAV gene therapy for the treatment of feline GM1 gangliosidosis
2:00	Samantha Hagerty	Autoclave sterilization of polymer-based canine training aids for biodetection requires contextual odor learning due to altered signal to noise ratio
2:15	Jeba Jesudoss Chelladurai	Associations between the fecal microbiome and gastrointestinal nematode parasites in North American Bison
2:30	Stacey Sullivan	The Relationship Between Radiographic Spinal Score and FGF4L2 Genotype in Dachshunds
3:00	Chengming Wang	Feline Infectious Peritonitis Risk Factors and Diagnostic Insights from PCR Analysis of Large-Scale Nationwide Submissions
3:15	Xu Wang	Resident urinary microbiome in canine clinical samples: reference profiles, dysbiosis, and associations with disease states

3:45 KEYNOTE LECTURE-Overton Auditorium

5:00 INDUCTION AND AWARDS ANNOUNCEMENT

INDUCTION of new Phi Zeta Members

Research Awards Presentations

PHI ZETA KEYNOTE SPEAKER

"A Long and Winding Road: My Path Toward Becoming a Clinician-Scientist in Veterinary Medicine"

Steven Friedenberg, DVM, PhD, Dip. ACVECC
Associate Professor
Department of Veterinary Clinical Sciences
College of Veterinary Medicine
University of Minnesota

Dr. Steven Friedenberg is currently an Associate Professor at the University of Minnesota College of Veterinary Medicine. He earned his BS from Yale University, MBA from MIT, and DVM from Cornell University. Prior to joining the University of Minnesota, he completed a Small Animal Medicine & Surgery Internship and a Small Animal Emergency and Critical Care Residency at the Ohio State University College of Veterinary Medicine. He then earned his PhD in Comparative Medicine at North Carolina State University College of Veterinary Medicine.

Dr. Friedenberg's research focuses on understanding the genetic and immunologic mechanisms that cause autoimmune disorders such as Addison's disease and autoimmune hemolytic anemia in dogs. His lab is also interested in bioinformatics and applying "big data" methods to veterinary medicine to help improve patient care and outcomes across a wide variety of clinical disorders.

Dr. Friedenberg's research has been supported by competitive extramural grant funding from the NIH, Animal Health Foundations, and Veterinary Medicine Foundations. He has authored or co-authored more than 50 peer-reviewed journal articles and book chapters, and delivered numerous invited lectures. Dr. Friedenberg has served on various study sections and editorial boards. Over the years, he has mentored numerous postdoctoral researchers as well as graduate, undergraduate, and professional students.

Posters

Undergraduate Students

Christopher Consoli	Temporal and temperature-dependent volatile odorant emissions of *Popillia japonica*
Devin Cooper	Elucidation of CAV2 Tropism
Paige Drotos	Sexual dimorphism of fetal and placental weights of rat fetuses exposed to prenatal cannabinoids
Ellie Hundley	Assessing the Dosage Effect of AAV Gene Therapy in Intravenous Treatment of Feline GM1 Gangliosidosis
Henry Limbo	Age-Related Changes in Tau and Beta-Amyloid in 3xTg Mice: Implications for Alzheimer's Disease Pathology
Nathan Newman	Generation and Characterization of Canine Tumor-Associated Macrophages
Carly Parker	Sexual Dimorphism of the Glucose Transporter GLUT1 in THC Exposed Rat Placentas
Caroline Parrish	Investigating the tumor promoting characteristics of the STK11 and MSK1 genes in canine mammary cancer
Nathaniel Pike	Encapsulation of Bacteriophage within Chitosan Nanoparticles for Delivery of Pig-specific Contraceptives
Nicholas Renfree	Construction of Recombinant Hyr1 Vaccine Preparations for Induction of Hyr1-specific Antibodies
Gabrielle Schultz	Novel Anti-Luteinizing Hormone Antibodies and Their Biological Effects in Mice

Veterinary Students

Sydney De Lorenzo	Demonstrating effective odor capture of invasive species volatiles using polymer- based training aids for canine pest detection
Courtney Dunning	The Impact of the Non-Nutritive Sweetener Allulose on the Gut Microbiome and Metabolic Syndrome
Micah Goode	Retrospective Analysis of Hypertrophic Pododermatitis Treated Across Multiple Referral Institutes
Kristen Hoehler	Bilateral Pyelonephritis and Suspected Uremic Encephalopathy in a New-World Camelid

Jessica Meier	P-cresol inhibits hepatocellular proliferation *in vitro*
Zach Oestmann	Developmental toxicity of S-(1,2-dichlorovinyl)glutathione (DCVG) in zebrafish (*Danio rerio*)
Hannah Vaughn	Can SR9238 block hepatotoxicity in cases of chronic phenobarbital exposure: A One Health perspective
Meghan Yaffa	Antimicrobial activity of canine platelet lysate against *Staphylococcus in vitro*

Graduate Students

Kiranmayee Bhimavarapu	Environmental antimicrobial resistance threats to pollinators
Victoria Caravaggio	Interferon beta stimulation of 3D trophoblast spheroids
Deepika Goyal	Comparative Analysis of Expression of NECTIN1 in Canine Mammary Tumors
Maryam Hariri	Investigating the Impact of Lid Sealing Methods on Volatile Organic Compound Exposure Accuracy: Are the Results of Toxicity Studies Underestimated?
Shakiba Kazemian	An Enhanced cDNA Synthesis Method for Improved an Influenza Virus Sequencing: Overcoming Limitations of Traditional Techniques
Sumbul Khan	Exploring Oncolytic viral therapy to target Osteosarcoma
Pia LaPorte	Endoscopic Approach to the Canine Olfactory Area
Cecelia Lounsberry	Comparison of MOPP versus LOPP for first-line treatment of canine multicentric T-cell or hypercalcemic lymphoma
Emily Marshall	Assessing How Blood Serum Biomarkers Predict Biological Age in Chimpanzees
Shelby McAlister	Safety of Multi-site Ultrasound Neuromodulation to Improve Metabolic Function in Domestic Cats
Hayley Moore	The Interrelationship between anti-Müllerian hormone, antral follicle count, and hormone treatment in mares
Kira Noordwijk	Method for single nuclei isolation from equine sarcoid tissue
Jessica Prim	Effect of administration of intranasal minerals on immune and clinical outcomes of dairy calves experimentally challenged with bovine herpesvirus 1 (BHV-1)
Philip Strate	The Development of Virus-Like Particles Targeting Avian Origin Hemagglutinin 5 for Production of Hyperimmune Antiserum

Ji-Hang Yin	Exploring 1-Trichloromethyl-1,2,3,4-tetrahydro-beta-carboline (TaClo)-induced neurotoxicity following the early-life exposure in the zebrafish model (*Danio rerio*)
Farnaz Yousefi	*C. difficile* Infection Induces a Pro-Steatotic and Pro-Inflammatory Metabolic State in Liver
Ying Zhang	Independent origin of genomic imprinting in viviparous fish: parent-of-origin effects in placenta gene expression in matrotrophy Poeciliid
Yue Zhang	*Corynebacterium* enrichment is a hallmark of the vaginal microbiome in cows carrying persistently infected Bovine Viral Diarrhea Virus (BVDV) fetuses

Faculty/Postdoctoral Fellows/Staff

Murugadas Anbazhagan	Alcohol Administration Disrupts Intestinal Autophagy to Exacerbate Liver Injury
Ahmed Bakheet	Dysregulated Lysosome Biogenesis and Abnormal Lysosomal Activity Potentially Drive Human Alcohol-Associated Liver Disease
Kayla Corriveau	A Survey of the Clinical Usage of Non-Steroidal Intra-Articular Therapeutics in Dogs by Veterinary Practitioners
Darielys Diaz	Hematology and serum chemistry in the first inbred strain of Monodelphis domestica, a marsupial laboratory model for biomedical research
Johanna Ehrhardt	Characterization of the Transient Perinatal Rise in Luteinizing Hormone in Cats and Dogs
Rocio Gerez	Characterization of H9Nx avian influenza viruses in chicken embryo-derived primary cell cultures
Reid Hanson	Enhancing the Musculoskeletal System Course (VMED 9590) Assessments with AI and Respondus 4.0
Steven Kitchens	Genomic Analysis of a Bacteriophage-Resistant *Salmonella* Newport and its Attenuation in Calves
Thu Nguyen	A decade of insights: long-term clinical environmental microbiome changes in Bailey Small Animal Teaching Hospital from veterinary medicine and One Health perspectives
Mariana Pinheiro	Susceptibility of different avian cell lines to H4Nx avian influenza viruses
Xu Wang	Investigation of food marking and contamination behaviors informs feeding practice in research colonies of laboratory opossums

Rie Watanabe	Showcase! The Advanced Capabilities of Auburn University Flow Cytometry and High-Speed Cell Sorting Laboratory (RRID:SCR_025507)
Arthur Zimmerman	Alzheimer's Disease Histopathological Phenotype in Feline GM1 and GM2 Gangliosidosis

Veterinary Student Platform Presentations

O1 Evaluation of the ability of *Babesia rossi* to induce neutrophil extracellular trap(NET) formation

Grace Hurley[1], Liming Shen[1], Andrew Leisewitz[1], and Dana N.LeVine[1]
[1]Department of Clinical Sciences, Auburn University CVM, Auburn, AL

Introduction. *Babesia rossi* (*B. rossi*) causes the most severe disease of all babesia species infecting dogs. Babesiosis is a highly inflammatory disease that models malaria, causing hemolytic anemia and multiple organ failure. Neutrophil extracellular traps (NETs) are extracellular projections of histones, DNA, and granular proteins released by stimulated neutrophils. NETosis, the formation of NETs, drives inflammation in malaria. We hypothesized that *B. rossi* would stimulate NETosis; specifically, that serum from *B. rossi* infected dogs and cultured *B. rossi* parasite lysate would induce *ex vivo* NETosis in healthy dog neutrophils.

Methods. Using an *ex vivo* model, healthy donor dog neutrophils were isolated from whole blood and incubated with pre and post-infection serum from *B. rossi* infected dogs (n=6). Neutrophils were incubated with a NET inducer, phorbol-12-myristate-13-acetate (PMA), or media control (unstimulated). Neutrophils were then stained with cell impermeable dye (SYTOX orange), staining extracellular DNA, or NETs, and a permeable dye (SYTO green), staining intracellular DNA. Percent NET-forming neutrophils were determined using fluorescence confocal microscopy. The effect of serum treatment and volume on NETosis was determined by a two-way ANOVA followed by Tukey's multiple comparisons. To assess the direct effects of *B. rossi* on NETosis, *B. rossi* parasite lysate or red cell lysate control was also incubated with healthy donor dog neutrophils. Cells were then fixed and stained with antibodies targeting citrullinated histone 3 (citH3), a specific NETosis marker, and DAPI (DNA stain), and imaged with fluorescence microscopy. NETs were identified by dual DAPI and citH3 staining. Finally, *in vivo* NET generation was assessed by measuring circulating plasma cell-free DNA (cfDNA), a nonspecific NET marker, in *B. rossi* infected dogs. cfDNA was extracted from pre and post-infection plasma (QIAamp® MinElute® ccfDNA kit) and quantified with picogreen, which stains extracellular DNA. Plasma cfDNA was compared pre and post-infection by a Wilcoxon matched-pairs signed rank test.

Results. Pre or post-infection serum did not impact NETosis in unstimulated neutrophils. Unexpectedly, pre and post-infection serum significantly inhibited PMA-induced NETosis ($p<0.0001$). Dual antibody staining confirmed *B. rossi* parasite lysate induced NETosis in healthy canine neutrophils. Circulating plasma cfDNA was higher post *B. rossi* infection than pre-infection ($p=0.0312$), suggesting *in vivo* NETosis occurs in *B. rossi* infected dogs.

Conclusions. *B. rossi* parasite induced *ex vivo* NETosis combined with increased circulating plasma cfDNA in *B. rossi* infected dogs, suggests that NETosis plays a role in *B. rossi* pathogenesis. The unexpected inhibition of pre and post-infection serum on *ex vivo* NETosis has several possible explanations: serum albumin binding PMA, serum nucleases degrading NETs, or dogs being sampled before sufficient inflammation occurred to allow the production of NET-inducing cytokines (eg. IL-8). The role of NETosis in *B. rossi* infection warrants further investigation; NETs may be a future therapeutic target in *B. rossi* infection.

Acknowledgements. Boehringer Ingelheim

O2 Prognostic Value of Lactate to Albumin Ratios in Horses with Enterocolitis

Blake Park[1,2], Amelia S. Munsterman[1], and R. Reid Hanson[2]
[1]Department of Large Animal Clinical Sciences, College of Veterinary Medicine, Michigan State University, MI
[2]Department of Clinical Sciences, College of Veterinary Medicine, Auburn University, AL

Introduction. Equine enterocolitis is a gastrointestinal disease resulting in diarrhea and mucosal inflammation, often leading to endotoxemia and septic shock. In humans, blood lactate to albumin (L:A) ratios are predictive of the risk of morbidity and mortality in septicemia. The use of L:A ratios in equine medicine has not been reported. The aim of this study was to evaluate L:A ratios to determine if they were higher in horses with enterocolitis that did not survive to discharge.

Methods. This was a retrospective study, evaluating adult horses presented for enterocolitis to a university hospital over a five-year period (2019-2024). Horses were required to have a measured venous lactate and albumin level and to meet two of the criteria that identify systemic inflammatory response syndrome (fever, tachycardia, tachypnea, leukocytosis, or leukopenia) for inclusion.

Results. Results of this study showed that horses that died or were euthanized had a higher L:A ratio (P=0.001), higher blood lactate (P=0.005), lower serum albumin levels (P=0.048), but shorter hospital stays.

Conclusions. A higher L:A ratio on hospital admission may be useful as a prognostic indicator of mortality in horses with enterocolitis. Further research is indicated to identify a cut off value for increased risk of mortality, and the role of the L:A ratio in prediction of morbidity and complications.

Acknowledgments. Dr Amelia S. Munsterman DVM, MS, PhD, DACVS, DACVECC, Michigan State University CVM Summer Research Program, Funding- Boehringer-Ingelheim.

O3 Effects of Prepubertal and Concurrent Exposures to Endocrine Disrupting Chemicals in the Male Gonad

Allison Rucker, Bobby Willingham, Robert L. Judd, and Benson T. Akingbemi
Department of Anatomy, Physiology, and Pharmacology, College of Veterinary Medicine, Auburn University, AL 36849

Introduction. The consumption of soybeans and other legumes in the diet is common in the population. Soybeans contain isoflavones, which possess hormonal activity and are capable of interfering with androgen biosynthesis. Similarly, the population is exposed to other endocrine disrupting chemicals, including phthalates present in food, air and water. Therefore, experiments were performed to determine the effects of concurrent exposures to isoflavones and phthalates on androgen secretion in the male gonad.

Methods. Prepubertal male Long-Evans rats at 21 days of age were fed a control casein- or soybean-based diet (SBM) and were provided di (2-ethylhexyl) phthalate (DEHP) or diisononyl phthalate (DINP) in drinking water (1 µg/L). Animals were processed to obtain blood for serum after 14 days of chemical exposure. In addition, testicular tissue and Leydig cells were obtained and incubated in DMEM/F-12 culture media without (basal) or with 100 ng/mL ovine LH (LH-stimulated) for 3 h. Testosterone concentrations were determined in serum and spent media by radioimmunoassay.

Results. The results showed that serum T concentrations were increased in the DEHP ($P<0.05$), DINP ($P<0.001$) and SBM+DINP groups ($P<0.01$). However, basal testicular production was increased ($P<0.05$) only in the SBM+DEHP group while LH-stimulated testicular T production was increased by exposure to DINP ($P<0.01$), SBM+DEHP ($P<0.05$) and SBM+DINP ($P<0.01$). Basal Leydig cell T secretion was decreased ($P<0.05$) in SBM-fed animals but was increased in the DINP group ($P<0.01$). On the other hand, LH-stimulated Leydig cell T secretion was increased only in the SBM+DEHP group ($P<0.01$).

Conclusions. In conclusion, increased testicular androgen concentrations observed in the SBM+DEHP group was absent in the DEHP-only group, whereas this effect was apparent in both DINP and SBM+DINP groups. Together, the data demonstrate that concurrent chemical exposures have additive effects on androgen biosynthesis in the male gonad. These results have implications for risk assessment of populations exposed to environmental chemicals.

Acknowledgments. This study was supported in part by the Department of Anatomy, Physiology and Pharmacology and the Boehringer Ingelheim Veterinary Scholars Program at the AUCVM.

Graduate Student Platform Presentations

O4 Vaccination with Recombinant LaSota Virus Expressing IBV Spike Protein: Immune Response in Chickens with Maternal Antibodies

Camila Cuadrado, Cassandra Breedlove, Haroldo Toro
Department of Pathobiology, Auburn University College of Veterinary Medicine, Auburn, AL, 36830

Introduction. Despite extensive vaccination efforts using live attenuated vaccines, infectious bronchitis virus (IBV) remains highly prevalent worldwide. Use of live attenuated IBV vaccines have perpetuated the disease in the poultry industry from recombination events between vaccine and wild viruses (REF). Therefore, new IBV vaccine technologies are needed. Vaccination with a recombinant Newcastle disease (ND) LaSota (LS) strain expressing Arkansas (Ark) -type infectious bronchitis virus (IBV) spike protein (rLS/ArkS) was evaluated in chickens of commercial origin with ND virus (NDV) maternally derived antibodies (MDA).

Methods. Chickens with MDA were vaccinated ocularly either with rLS/ArkS or the empty LS virus at 2, 8, 15 or 30 days of age (DOA). In addition, specific pathogen free (SPF) chickens were vaccinated with each virus at 2 DOA. NDV RNA was determined in lacrimal fluids, indicating successful replication of the recombinant virus at periocular mucosal sites. IBV IgA in lacrimal fluids and serum IgG were determined by ELISA using recombinant IBV Ark S1-protein-coated plates. Antibody avidity was measured.

Results. Vaccination at 2 DOA with rLS/ArkS in chickens with MDA elicited a vigorous IBV IgA response in lacrimal fluids without significant differences between commercial and SPF chickens. Chickens with MDA vaccinated with rLS/ArkS at 8 DOA showed IgA levels in lacrimal fluids not differing significantly from levels achieved upon vaccination at 2 DOA. Vaccination at 30 DOA did not result in increased IBV IgA levels in tear fluids of commercial birds. Chickens with MDA vaccinated at 2 DOA with the empty LS vector developed significantly higher NDV IgA levels in tears compared to chickens vaccinated with rLS/ArkS. Chickens with MDA vaccinated with LS at 8 DOA show slightly higher NDV IgA in tears compared to chickens vaccinated with rLS/ArkS. LaSota vaccination in chickens with MDA at 15 or 30 DOA elicited an ND IgA response in tears similar to chickens vaccinated with rLS/ArkS. Vaccination with rLS/ArkS at 2 or 8 DOA in chickens with MDA resulted in absence of IBV IgG responses in sera. However, vaccination with rLS/ArkS at 15 or 30 DOA elicited IBV serum IgG response. Chickens with MDA vaccinated with rLS/ArkS at 15 DOA displayed increased NDV IgG antibody level in sera.

Conclusion. Vaccination with rLS/ArkS induces both IBV and NDV IgA at periocular mucosae and elicits serum IgG in chickens with NDV MDA.

O5 The anti-inflammatory properties of Mesenchymal Stromal Cell derived Extracellular Vesicles on feline neuroinflammation

Nikolia Darzenta[1,2], Emily Edelman[1,2], Douglas R. Martin[1,3], Maria C. Naskou[1,2]
[1]Scott-Ritchey Research Center, [2]Department of Pathobiology, [3]Department of Anatomy, Physiology and Pharmacology, College of Veterinary Medicine, Auburn University, Auburn, AL.

Introduction. Extracellular vesicles (EVs) are lipid nanoparticles secreted from all cell types that mediate intercellular communication and maintain cellular homeostasis. When EVs are being secreted from mesenchymal stromal cells (MSCs-EVs), they exert anti-inflammatory and immunomodulatory properties via inhibiting cytokine secretion, immune cell activation and via promoting T regulatory polarization, to name a few. However, their biological action is dependent on the cell culture conditions of the MSCs and which of them produces the most immunosuppressive EV isolates still remains undetermined. On top of that, EVs are able to diffuse through the blood-brain-barrier and exhibit low immunogenicity, so they are promising therapeutic candidates for neuroinflammation in neuropathology. Neuroinflammation is triggered by neuronal death and when persists exacerbates the progression of neurodegenerative diseases. Moreover, traditional anti-inflammatory drugs have side effects and offer minimal symptomatic relief. Therefore, there is a *critical need* to develop novel treatments that dampen neuroinflammation and reverse its detrimental role in neurodegenerative disease prognosis. Our goal is to evaluate the anti-inflammatory effects of MSC-EVs in the neuro- and systemic inflammation of Sandhoff Disease (SD), which is a fatal and aggressive inherited neurodegenerative disease. Our objectives was a) to identify the immunosuppressive properties of different isolates of MSCs-EVs on inflammatory mixed glia, b) to assess their anti-inflammatory properties in peripheral mononuclear blood cells (PBMCs) from SD cats c) and to determine their anti-inflammatory effects in the brains of SD cats.

Methods. Phenotypical characterization was performed in isolated mixed glia from normal cats via flow cytometry and immunofluorescence. Extracellular Vesicles isolated from umbilical cord derived MSCs were isolated under different culture conditions including a serum free environment (SF-EVs) or in the presence of FBS (FBS-EVs) or following priming of MSCs with IFN-γ and TNF-α (IF-EVs). Subsequently, different isolates of EVs were added to naïve and LPS-stimulated cells for 24 and 48 hours. Additionally, PBMCs were isolated from SD cats and were treated with IF-EVs for 48h. EVs cytotoxicity effects were assessed via 2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide (XTT) assay. Cell culture supernatants from mixed glia and PBMCs were collected and analyzed for pro- and anti-inflammatory cytokines, such as IL-6 and TNF-α, through ELISA while mRNA expression of inflammatory pathways was assessed via reverse transcriptase quantitative PCR. Furthermore, 10^{12} UCMSC-EVs were intravenously administered in SD and normal cats at 4 months old. Brain and peripheral tissues were collected three days post injection. Enzymatic activity assays, immunohistochemistry and gene studies via qPCR were performed for main inflammatory mediators.

Results. Isolated glia cells from normal cats were confirmed as a mixed glia population due to the expression of neuronal (anti-NeuN), astrocytic (GFAP), microglial (Iba-1) and pericyte (PDGFR-β) cell markers. We found that the addition of MSC-EVs onto inflammatory feline mixed glia suppressed the production and the mRNA expression of the inflammatory compared to those without the addition of EVs, especially after 48h. However, IF-EVs exhibited boosted anti-inflammatory effects, since they significantly reduced multiple inflammatory biomarkers. Similarly, IF-EVs reduced the expression of the same inflammatory factors in the SD PBMCs and no cytotoxicity effects were observed. Lastly, our preliminary *in vivo* data indicate that the IV administration of MSC-EVs suppresses neuroinflammation by decreasing the expression of Iba-1, GFAP and MHCII and CD4 inflammatory markers in parietal cortex, thalamus, and cerebellum.

Conclusions. Our findings reveal a potent clinically feasible anti-inflammatory therapeutic against neuroinflammation which is present in all neuropathologies. MSC-EVs also support the autologous use while their inflammatory profile boosts their therapeutic benefits via low-cost and engineer-free modifications.

Acknowledgments. Animal Health and Disease Research program.

O6 Pulmonary disposition and pharmacokinetics of chloramphenicol in healthy fasted adult horses

Dedecker, L.[1]; Ceriotti, S.[1]; Mora, M[1]; Knych, H., K.[2]; Zuber, E.[3]; Lascola, K., M.[1]
[1]Department of Clinical Sciences, College of Veterinary Medicine, Auburn University, Auburn, AL, USA.
[2]Kenneth L. Maddy Equine Analytical Chemistry Laboratory (Pharmacology), School of Veterinary Medicine, University of California, Davis, CA, USA.
[3]Department of Pathobiology, College of Veterinary Medicine, Auburn University, Auburn, AL, USA.

Introduction. Chloramphenicol is administered orally for treatment of bacterial pneumonia in horses. Plasma drug concentrations often fall below susceptibility breakpoints (2-8 µg/mL) for clinically relevant bacteria. Pulmonary pharmacokinetics are not described in horses.
Study Objective: To describe and compare pulmonary and plasma pharmacokinetics of chloramphenicol after oral and intragastric administration.

Methods. A randomized, cross-over study, using 5 healthy adult horses. Single-dose chloramphenicol (50mg/kg) was administered, as base tablets orally (TPO), and as compounded suspension orally (SPO) and intragastrically (SIG). Chloramphenicol concentrations were determined via liquid chromatography tandem mass spectrometry in plasma, and at 1, 4, and 8 hours in pulmonary epithelial lining fluid (PELF) for noncompartmental pharmacokinetic analysis. Pulmonary and plasma pharmacokinetic parameters were compared between routes and between formulations ($P<0.05$).
Results: For all formulations and routes of administration, chloramphenicol concentrations and drug exposure (AUC) were higher in PELF compared to plasma and concentrations remained above 8 µg/ml for ≥ 4 hours. No differences were observed between oral administrations, only SIG demonstrated higher maximum concentration and AUC compared to SPO (table 1).
Table 1-Mean ± standard deviation plasma and PELF pharmacokinetic variables.

	SIG	**SPO**	**TPO**
T_{max} (h)	2.4 ±1.34	2.90 ±1.43	3.60 ±1.52
C_{max} (µg/ml)	5.73[a,b] ±2.99	2.52[a,b] ±1.40	3.72 ±2.34
$AUC_{1\text{-}8hrplasma}$ (µg/ml*h)	8.19[b] ±2.68	11.95[b] ±3.20	10.54[b] ±2.84
$AUC_{1\text{-}8hrPELF}$ (µg/ml*h)	51.41[a,b] ±37.16	103.43[a,b] ±36.26	10.54[b] ±2.84
$C_{max\_obsPELF}$ (µg/ml)	12.92[a,b] ±10.23	39.31[a,b] ±27.04	32.16 ±34.34

[a]significant difference between SPO/SIG administration.

[b]significant difference between plasma/PELF, same administration route
Conclusion. Chloramphenicol achieves pulmonary concentrations above 2-8 µg/ml for at least 4-8 hours.

Acknowledgments. The study was funded by the Birmingham Racing Commission (G00015299).

O7 Mucosal Immune Responses in the Harderian Gland Following Newcastle Disease LaSota Vaccination in Chickens with Maternally Derived Antibodies

Raimundo Espejo[1], Cassandra Breedlove[1], and Haroldo Toro[1]
[1]Department of Pathobiology, College of Veterinary Medicine, Auburn University, AL

Introduction. Newcastle disease virus (NDV) is a significant pathogen in poultry, causing severe economic losses worldwide. Vaccination is the primary control measure, and while it is effective, maternally derived antibodies (MDA) can interfere with vaccine-induced immune responses, particularly in young chicks. The Harderian gland (HG) plays a critical role in the mucosal immunity of the ocular and upper respiratory systems, which are key entry points for NDV. This study aims to characterize the immune responses in the HG following ocular vaccination with the LaSota strain of NDV in specific pathogen-free (SPF) chickens and commercial-origin chickens with MDA. By comparing immune responses between these two groups, we seek to understand the impact of MDA on mucosal immunity in the HG and its potential interference with NDV vaccine efficacy.

Methods. Two trials were conducted to evaluate the immune responses in the HG post-vaccination. In a first trial, 13 days of age (DOA) layer-type SPF chickens were vaccinated ocularly with the LaSota strain of NDV. Serum NDV antibody levels were monitored post-vaccination, along with specific IgA responses in lacrimal fluids. Flow cytometry was used to assess the frequencies of B cells (Bu-1+), T-helper cells (CD4+), and cytotoxic T cells (CD8+) in the HG at 11- and 16-days post-vaccination.

In a second trial, chickens with MDA were vaccinated at either 2 or 15 DOA. Viral RNA was detected in lacrimal fluids and tracheal swabs. Both serum NDV antibody and specific IgA responses in lacrimal fluids were measured. The relative abundance of immune cell populations in the HG was assessed at days 10 and 16 post-vaccination.

Results. In trial 1, ocular vaccination with LaSota induced a detectable serum antibody response beginning at day 15 post-vaccination, while IgA was already present in lacrimal fluids by day 10. B cells, CD4+ T cells, and CD8+ T cells in the HG increased significantly at day 11, with maximum levels observed by day 16.

In trial 2, vaccination at 2 DOA did not result in seroconversion, while vaccination at 15 DOA induced a serum antibody response, likely due to the waning of MDA. However, strong mucosal IgA responses were observed at both 2 and 15 DOA, with IgA levels peaking on day 9 post-vaccination. B cell expansion in the HG was noted both at day 10 and day 16 post-vaccination in chickens vaccinated at 2 DOA, with greater responses in those vaccinated at 15 DOA. CD4+ T cells expanded more in the HG following vaccination at 15 DOA compared to 2 DOA, while CD8+ T cell expansion was similar regardless of vaccination age and MDA status.

Conclusions. NDV LaSota vaccination elicits robust humoral and cellular immune responses in the Harderian gland. Despite MDA interference with systemic serum antibody responses, mucosal immune responses in the HG, particularly IgA production, remain strong and unaffected. These findings highlight the importance of mucosal immunity in NDV protection and suggest that vaccination strategies targeting local immune responses in the HG can be effective even in the presence of MDA.

Acknowledgments. The authors wish to thank James Gillespie, Stephen Gulley, and Dr. Vicky van Santen for valuable suggestions. Research supported by a U.S. Department of Agriculture Non-Assistance Cooperative Agreement.

O8 Extracellular Vesicle Delivery of Antiviral Therapy for Treating Rabies Encephalitis

Y Savanie Fernando[1,2], Anna Cochran[1], Miguel Esteves[3], Henry Baker[2], Douglas Martin[2], and Maria Naskou[1,2]
[1]Department of Pathobiology, College of Veterinary Medicine, Auburn University, AL
[2]Scott Ritchey Research Center, Auburn University, AL
[3]Department of Neurology, UMass Chan Medical School, MA

Introduction. There are many neuropathic viruses that can cause viral encephalitis in human that are mostly lethal and incurable. Among these viruses, zoonotic rabies virus (RABV) is invariably fatal to humans following the onset of clinical manifestation and causes more than 60,000 deaths per year. Although there are preventable measures for rabies post-exposure prophylaxis, existing interventions do not work effectively once the virus reaches the central nervous system and neurologic symptoms become apparent. The main reason most of the treatments for rabies encephalitis are unsuccessful is that blood brain barrier limits the delivery of treatments into the brain. Therefore, there is a critical need of developing novel therapeutic strategies to treat rabies encephalitis. Extracellular vesicles (EVs) derived from cell plasma membranes offer a unique platform for developing novel therapeutics with their ability to shuttle molecules between cells and through crossing the blood brain barrier. Moreover, RNA interference (RNAi) methods such as synthetic short double stranded DNA (siRNA) can be used to selectively bind targeted viral messenger RNA and induce mRNA degradation. Our overall objective for this project is to engineer extracellular vesicles expressing rabies virus glycoprotein (RVG) derived peptide in order to increase brain tropism and encapsulate therapeutic antiviral siRNA for delivery into neuronal cells. We hypothesized that by engineering extracellular vesicles to express RVG we will increase their brain tropism and ultimately increase their efficiency to deliver anti-viral siRNAs to rapidly inhibit rabies virus replication in the central nervous system. This project aims to implement a novel therapeutic approach and advance the viral suppression therapy for rabies encephalitis.

Methods. We transfected HEK293 cells with a plasmid encoding Lamp2b-construct fused to the neuron specific RVG or FLAG peptide as the control. Following the transfection of cells to express RVG in the EVs they were loaded with a surrogate siRNA (GAPDH) for technique optimization. Subsequently, the efficacy of RVG modified EVs to deliver their cargo was tested in neuronal cells. Next, we screened 46 antiviral siRNA candidates targeting the rabies N gene for their high binding and gene inhibition *in vitro* using psiCHECK2 luciferase assay system. In addition, the gene expression was evaluated using RT-PCR.

Results. Our preliminary data showed successful modification of EVs with the RVG peptide and loading of a surrogate siRNA (GAPDH). Moreover, we found that EVs were able to deliver their load to neuronal cells *in vitro.* In addition, our preliminary data from Luciferase assay shows that 6 antiviral siRNAs candidates can induce more than 90% N gene interference in HEK293 cells.

Conclusions. We aim to evaluate the therapeutic effect of EVs to deliver antiviral siRNAs in rabies infected neuronal cells and mice via biodistribution and gene expression assays.

Acknowledgments. We extend our special thanks to Dr. Miguel Esteves from University of Massachusetts Chan medical school for collaboration, Scott-Ritchey research center and College of Veterinary Medicine, Auburn University for funding this project.

O9 Antimicrobial resistance in the different stages of commercial poultry production environment

Pankaj Gaonkar[1], Alinne Santana-Pereira[1], Alyssa Lambert[1], Reed Golden[1], Courtney Higgins[1], Yagya Adhikari[2], Matthew Bailey[2], Kenneth Macklin[3] and Laura Huber[1]
[1]Dept. of Pathobiology, College of Veterinary Medicine, Auburn University, AL
[2]Dept. of Poultry Science, Auburn University, AL
[3]Dept. of Poultry Science, Mississippi State University, MS

Introduction. Antimicrobial resistance (AMR) is a threat to the poultry industry, resulting in economic losses. AMR transmission can occur between poultry, humans, and the environment, yet environmental AMR remains understudied. Our study aimed to determine AMR in the environment in different stages of commercial poultry farms.

Methods. We included commercial poultry farms (n=29) in different stages of production practicing restricted antimicrobial use. Litter samples from inside and soil samples from outside the poultry house were collected. Carcass rinses were collected from the processing plant at post-pick and post-chill stages. The frequency of 3 mobile genetic elements (MGEs) and 14 antimicrobial resistance genes (ARGs) was assessed using qPCR. Shotgun metagenomics was performed on litter and soil to examine microbiome and resistome compositions.

Results. AMR to majority of antimicrobial classes was found in litter samples, and it was higher in broiler compared to breeder and pullet farms. AMR was lower in soil compared to litter and there was no difference among farm types. MGEs were most frequently found in litter and were consistent across the farm types. In the processing plant, AMR was comparable between post-pick and post-chill stages. Distinct microbial and resistome composition was observed between litter and soil (PERMANOVA=0.001). Pullet and breeder had similar profiles (PERMANOVA > 0.05), while broiler had distinct microbial (PERMANOVA < 0.002) and resistome (PERMANOVA < 0.01) compositions compared with other farm-types. Litter microbiome and resistome shifted along the production chain, with increased frequency of Staphylococcus and bacitracin resistance in broiler farms. Two broiler farms had soil composition similar to litter, indicating possible AMR contamination between inside and outside poultry houses.

Conclusions. Restricted AMU alone does not prevent AMR persistence in poultry production environments. Historic AMU may contribute to ARGs persistence. Cross-contamination between litter and soil poses a risk of AMR dissemination. The presence of ARGs in carcasses in the post-chill stage indicates a potential public health risk. Understanding AMR spread in the environment is essential to maintain poultry, human, and environmental health.

Acknowledgment. This study was supported by USDA-NIFA and Alabama Agricultural Experimental Station.

O10 Therapeutic Potential of an Orexin 2 Receptor (OX2R) Agonist in Addressing Secondary Symptoms of Narcolepsy in Orexin Knock-Out (OX-KO) Mice

Natasha Grabau[1], Vander LeKites[1], Danielle Forbus[1], Julia Peterson[1], Henry Limbo[1], Daniel Kroeger
[1]Department of Anatomy, Physiology and Pharmacology, College of Veterinary Medicine, Auburn University, AL

Introduction. Narcolepsy is a sleeping disorder resulting from the loss of orexin neurons in the lateral hypothalamus and the consequent loss of orexin signaling in the brain. Narcolepsy patients typically exhibit severe sleepiness during the day, fragmented sleep during the night, hypnagogic hallucinations, and cataplexy which are considered as primary symptoms. The secondary symptoms include memory impairments, anxiety, and depression. Current therapies utilize stimulants or antidepressants to enhance wakefulness to address the primary symptoms, but symptoms rarely fully resolve. Orexin knock-out (OX-KO) mice are a model system for Narcolepsy and previous research utilizing these mice demonstrated that an orexin agonist targeting the orexin 2 receptor (OX2R) can be used as an effective treatment for the primary symptoms of narcolepsy. This agonist improved narcolepsy in OX-KO by increasing wake bout durations and abolished cataplexy even in the presence of a strong cataplexy trigger. The current study tested whether this OX2R agonist can also alleviate the secondary symptoms of narcolepsy in OX-KO mice.

Methods. To assess the efficacy of our OX2R treatment we used three groups of 12 OX-KO mice (and three groups of 12 wild-type control mice). One group of each genotype received the OX2R agonist treatment, another vehicle treatment, and the third received Modafinil (a current narcolepsy treatment). One hour after administration, we assessed performance in the Novel Object Recognition (NOR) test (memory), Open Field Test (OFT; anxiety), and Forced Swim Test (FST; depression). The NOR test was used to assess working memory (5 minutes), short-term memory (30 minutes) and long-term memory (24 hours) by calculating the exploration index (EI). EI values >50 indicate that mice spent more time with the novel object implying they remember the previously explored 'familiar' object. The OFT was used to measure the percent time mice spend within the center zone, frequency of crossing over the center zone and total distance travelled in the arena. A less anxious mouse would spend more time exploring the arena and in the center zone compared to a more anxious mouse that would spend less time in the center zone and more time immobile. Higher time spent swimming in the FST indicate less depression-like symptoms as mice continue to struggle and escape water rather than give up and float.

Results. Although not significant, there was a trend for agonist treated OX-KO mice to spend less time in the center zone of the OFT arena compared to mice in other treatment groups suggesting an increase in anxiety-like behavior after agonist treatment. Assessment of 'working memory' in the NOR test at the 5 minutes time point after acquisition trial indicated a trend towards agonist treated OX-KO mice to have better working memory due to EI values >50. In the FST, both agonist ($p<0.0535$) and Modafinil ($p<0.019$) treated OX-KO mice spent more time swimming suggesting that both these drugs may reduce depression-like symptoms in the OX-KO mice.

Conclusions. Our results show that the OXR2 agonist may improve working memory and decrease depression-like symptoms but it may increase anxious behavior in OX-KO mice.

O11 Development of Next-Generation Oncolytic Virus and CAR T cell Immunotherapies for Treatment of Osteosarcoma in a Canine Model

Theresa A. Higgins[1,2] and Payal Agarwal[1,2]
[1]Scott Ritchey Research Center, College of Veterinary Medicine, Auburn University, AL
[2]Department of Pathobiology, College of Veterinary Medicine, Auburn University, AL

Introduction. Osteosarcoma (OS) is a highly aggressive and metastatic bone malignancy with a low survival rate and poor patient prognosis. Current treatments include chemotherapy, radiation, and tumor removal, though no significant changes in treatment and survival have been seen since the 1970s. In recent years, immunotherapies, such as immune checkpoint inhibitors, conditionally replicative Adenoviruses (CRAds), and chimeric antigen receptor (CAR) T cells, have shown promise as a new modality treating various cancers.

Methods. We have developed and characterized a next-generation conditionally replicative canine adenovirus CAV2-AU-M2 armed with anti-PD1 sdAb. The infectious and cytolytic effects of CAV2-AU-M2 were tested in four different canine OS cell lines in 2-dimensional (2D) and three-dimensional (3D) cell cultures. We also aim to develop CAR T cells targeting the cancer-specific B7H3 receptor.
Anti-B7H3 CAR T cells will be created by stimulating canine T cells and then transfection of anti-B7H3 receptor expressing lentivirus and retroviruses. The cytotoxic effects of anti-B7H3 CAR T cells will be tested in 2-dimensional (2D) and three-dimensional (3D) cell cultures. We expect cell lysis only in B7H3- positive cell lines. We will also assay the immunogenic effects of CAR T cells by analyzing IFNγ, TNFα, and IL-2 cytokine levels in OS cell lines post-CAR T therapy. We will compare osteosarcoma cell lysis and cytokine secretion between CAR T cells transfected with lentivirus against those transfected with retrovirus.

Results. CAV2-AU-M2 showed selective replication in OS cells and induced efficient tumor cell lysis. Moreover, CAV2-AU-M2 produced anti-PD-1 sdAb that demonstrated effective binding to PD-1 receptors.
We have successfully isolated and stimulated canine T cells in preparation for lentivirus and retroviral integration of the anti-B7H3 CAR construct.

Conclusions. The combination approach of CRAds armed with immune checkpoint inhibitors is intended to stimulate and enhance the anti-tumor immune response in the tumor microenvironment. Similarly, cancer-targeting CAR T cells will help stimulate immune response against osteosarcoma.

Acknowledgments. I would like to thank the Agarwal Lab group for their support, Sumbul Khan, Isabella Shimko-Lofano, Gracie Bunch, and Devin Cooper. I would also like to thank our collaborators Dr. Rimas Orentas, Dr. Maninder Sandey, Dr. Roberto Molinari, Dr. Yev Brundo, and Dr. Bruce Smith, as well as Dr. Rie Watanabe, Dr. James Gillespie, and Dr. Emily Graff for research support. Additionally, I would like to thank our funders, NIH NCI R15, Scott-Ritchey Research Center, and CVM orgs.

O12 Nasal and bronchoalveolar fluid BRSV IgG-1 titers transferred from maternal colostrum or a colostrum replacement in dairy calves

Huertas OF[1], Chamorro MF[1*], Passler T[1], Saucedo M[1], Taylor S[1], Schwartz D[1], Falkenberg S[2], Becker A[2], Bayne JE[1], Stockler J[1], Woolums AR[3].
[1]Department of Clinical Sciences and [2]Pathobiology College of Veterinary Medicine, Auburn University, [3]Department of Pathobiology and Population Medicine, College of Veterinary Medicine, Mississippi State University.

Objective. Bovine respiratory syncytial virus (BRSV) is an important cause of respiratory disease in young dairy calves worldwide. Clinical protection from maternal colostrum and vaccination with intranasal (IN) modified-live BRSV vaccines is inconsistent. The presence of passively transferred BRSV IgG-1in the respiratory tract of calves could affect the efficacy of IN vaccination. Currently, the initial titers and persistency of BRSV IgG-1 transferred from colostrum into the respiratory tract of neonatal calves are unknown. The objective of this study was to determine the initial level and persistency of BRSV IgG-1 titers transferred from maternal colostrum or a colostrum replacement into the upper and lower respiratory tract of dairy calves.

Methods. Nasal secretion (NS) and bronchoalveolar fluid (BALF) samples were collected from dairy calves that received either 6 L of maternal colostrum (MC; n=18) or a total mass of 300 g of IgG from a commercial colostrum replacement (CR; n=21) within 12 hours of life. Samples were collected at birth, 48 h of life, and 14, 28, and 90 days of age to determine BRSV IgG-1 titers by ELISA. Additionally, serum samples were collected from all calves at birth and 48 hours of life to determine total IgG levels by radial immunodiffusion. The apparent efficiency of absorption (AEA) of IgG was calculated for the CR group using the following formula: serum IgG concentration at 48 h of age (g/L) × plasma volume (L) ÷ total IgG intake (g).

Results. Titers of NS BRSV IgG-1 were detected at 48 h of life but had decayed completely by 14 days of age in all calves. The median NS BRSV IgG-1 titer at 48 h was greater in MC calves compared with CR calves (50 vs. 25). Preliminary BALF BRSV IgG1 titers persisted longer and were detected up to 28 days in MC calves and up to 90 days in CR calves; however, significant differences between MC and CR calves were not observed at any time point. The mean ± SD serum total IgG concentrations at 0 h of life were minimal and not significantly different between groups (P = 0.36). In contrast, the mean ± SD serum total IgG concentrations at 48 h of life were significantly (P = 0.003) greater in MC calves (30.8 ± 4.3 g/L) compared with CR calves (15.4 ± 2.6 g/L). The AEA of IgG for the CR group was 14.7%, much lower than the AEA reported in previous studies using the same colostrum replacer product in dairy calves.

Conclusions. The short persistency of BRSV IgG-1 titers transferred from colostrum into the upper respiratory tract of dairy calves may not play an important role in interfering with adequate immunization following IN vaccination. The similar respiratory BRSV IgG-1 concentrations and different total serum IgG levels observed in calves from this study reflect the limitations of total serum IgG to predict the transfer of adequate mucosal immunity against specific infectious agents.

O13 Effects of iodine sterilization and storage on microbial count, histologic properties, and omega-3 fatty acid concentration for tilapia fish skin grafts

Mary D. Kerby, DVM[1], Scarlett M. Sumner, DVM[1], Katelyn C. Hlusko, DVM, MS, DACVS-SA[1], Katherine A. Horzmann, DVM, PhD, MPH, DACVP[2], Carlos Eduardo Basos Lopes, DVM, MS[2], Donna Raiford[2]
[1]Department of Clinical Sciences, College of Veterinary Medicine, Auburn University, AL
[2]Department of Pathobiology, College of Veterinary Medicine, Auburn University, AL

Introduction. Wound management is common in both general and specialty veterinary practice. Many topical treatment options exist such as traditional dressings, extracellular matrices, or tissue-engineered biologics. Xenografts have been extensively used in human medicine to treat acute, chronic, non-healing, and burn wounds. Fish skin grafts (FSGs) are a type of xenograft that provides a unique scaffold rich in type I and type III collagen and omega-3 polyunsaturated fatty acids (PUFAs) with antibacterial and antioxidant properties. Commercially available FSGs are expensive, limiting their use in veterinary medicine. Fresh tilapia fish skin (TFS) has recently been investigated in human and veterinary medicine as a practical, economical topical wound dressing. Several sterilization techniques have been validated for fresh fish skin. No veterinary studies have reported PUFA concentration or storability of TFS. The objective of this study was to determine the effects of storage on iodine sterilized TFS by microbiological, histological, and spectroscopic assessment. It was hypothesized that iodine sterilization and storage would be effective and practical. Additionally, the bacterial load would increase, collagen integrity would decrease, and omega-3 fatty acid concentrations would decrease with storage.

Methods. This was an ex vivo, prospective, randomized, controlled study. Fresh tilapia fish were obtained from a local fish market. TFS were harvested and treated with 10% iodine or saline for 15 minutes. Each group was aseptically packaged and stored at 4°C for the duration of the study. The samples underwent microbiological, histopathological, and spectroscopic evaluation on days 0, 1, 3, and 7. Colony forming units were determined and disinfection efficacy was calculated. Structural integrity was graded based on collagen content and fiber organization. Fish oil was extracted via wet reduction and derivatized via transesterification. Chemical integrity was determined by PUFA concentration via gas chromatography mass spectrometry (GC-MS) of extracted fatty acid methyl esters.

Preliminary Results. Iodine was an effective sterilant resulting in a 100% disinfection efficacy compared to control TFS. Iodine sterilized TFS maintained sterility for the duration of the study. Omega-3 and omega-6 PUFAs were undetectable via GC-MS of extracted fish oil.

Conclusions. Iodine sterilized, cold stored, fresh TFS maintains sterility for 7 days. Extracted fish oil from iodine and saline treated TFS contains insignificant amounts of omega-3 fatty acids.

Acknowledgements. The authors thank Austin C. Conley and Melissa Boersma for assistance with microbiological evaluation and mass spectrometry, respectively.

O14 Immunotherapy on Demand: Tailoring a PD-1 Dependent 4-1BB Agonist for Combination Immunotherapy

Alana J. Kramer[1], Damien Ruiz[1], Jonathan Marable[1], Maninder Sandey[1]
[1]Department of Pathobiology, College of Veterinary Medicine, Auburn University, AL

Introduction. Immune checkpoint inhibitors (PD-1/PD-L1, CTLA-4) and immunostimulatory agonists (4-1BB, OX40) have emerged as promising cancer immunotherapies. These therapies were developed to enhance the cancer patient's immune response, modulate the tumor microenvironment (TME), and combat the immunosuppressive mechanisms prominent in patient's refractory to single agent therapies. 4-1BB is an inducible TNF receptor superfamily (TNFRSF) receptor that upregulates on the surface of NK and T cells following T-cell receptor activation. Primary functions of 4-1BB as a costimulatory receptor includes activation of CD8+ T cells, secretion of cytokines such as interferon gamma (IFN-γ) and interleukin 2 (IL-2), and upregulation of anti-apoptotic Bcl-2 members. As a result, 4-1BB elicits potent anti-tumor stimulation. Optimal signaling of 4-1BB requires trimerization of the receptor on the cell surface for activation. Despite its proven therapeutic efficacy, 4-1BB agonists elicit a dose-limiting hepatotoxicity by recruiting CD8+ T-cells to the liver in preclinical and clinical trials. Restriction of 4-1BB activation to the TME is hypothesized to limit hepatotoxicity while simultaneously triggering a robust antitumor response. The objective of this study is to develop and evaluate a set of bispecific immunostimulatory fusion protein constructs that consolidate PD-1 blockade and 4-1BB activation into a single therapeutic and activate 4-1BB signaling in the TME, only in the presence of PD-1 expressing T-cells. The results from this study will guide future studies testing caninized versions of 4-1BBL constructs in a cross-species approach.

Methods. Three murine specific bispecific fusion proteins were engineered, expressed and purified using Expi293-F cells. The configurations of the three constructs are as follows: Construct 1: 4-1BBL extracellular domain, isoleucine zipper trimerization domain (ILZ), altered Fc IgG region, and an anti- PD-1 nanobody (130 kDa). Construct 2: Analogous to construct 1 with replacement of the ILZ trimerization domain with a glycine linker (~122 kDa). Construct 3: Analogous to construct 2 with replacement of Fc domain with a second anti PD-1 attached to a glycine linker (~100 kDa). The binding ability of the bispecific fusion proteins to murine PD-1 and 4-1BB was evaluated using flow cytometry. Moreover, the ability of the bispecific fusion proteins to disrupt PD-1/PD-L1 interaction and activate 4-1BB signaling in a PD-1 dependent manner was evaluated using several functional assays.

Results and Conclusions. All three bi-specific proteins were successfully purified and showed high binding affinity and specificity to murine PD-1 and 4-1BB receptors. Additionally, the proteins successfully inhibited PD-1/PD-L1 interaction in a cell-based assay and activated 4-1BB signaling in a PD-1 dependent manner for the respective constructs. Future *in vivo* studies aim to test therapeutic efficacy and toxicity profiles in a mouse melanoma tumor xenograft model.

Acknowledgments. This project was supported by research funding from: Animal Health and Disease Research (AHDR) by AUCVM and a COHA Fellowship Grant: NCATS 1U01TR002953. We would also like to thank Rie Watanabe for flow cytometry support.

O15 Factors Associated with Survival in Goats Treated for Toxic Mastitis

Hannah Maxwell, Erin Harris, Lisa Johnson, Thomas Passler, Jessica Rush, and Manuel F. Chamorro
Department of Clinical Sciences, College of Veterinary Medicine, Auburn University, AL

Introduction. Toxic or gangrenous mastitis is a relatively uncommon but life-threatening disease of lactating does. Currently, scientific reports of prognostic indicators for survival of goats with toxic mastitis are unavailable. The purpose of this retrospective study is to evaluate records of goats presented to a veterinary teaching hospital for toxic mastitis in order to identify prognostic indicators for survival to discharge. We hypothesized that the physical exam findings of heart rate, respiratory rate, and rectal temperature upon presentation to the hospital would differ between survival and non-survival groups. Additionally, we hypothesized that medical management factors such as length of hospitalization, treatment with intramammary antibiotics, and treatment with dexamethasone would be positively associated with survival.

Methods. A retrospective study was conducted to evaluate medical records from 2014 to 2024 of goats treated for toxic mastitis at Auburn University Large Animal Teaching Hospital. Adult does with clinical mastitis and signs of systemic compromise (e.g., abnormal body temperature, dehydration, anorexia, etc.) were included in the study. A total of 26 medical records met the inclusion criteria, and the association between independent variables and outcome (discharge from the hospital vs. non-discharge) was assessed by Chi Square and Fisher exact tests.

Results. Of the 26 goats included, 76.9% (20/26) survived to discharge and 23.1% (6/26) died or were euthanized. Initial physical exam findings of heart rate, respiratory rate, and rectal temperature were not associated with the outcome ($p > 0.05$). In contrast, hospitalization length $\geq$ 10 days ($p = 0.006$), treatment with intramammary antibiotics ($p = 0.01$), and treatment with dexamethasone ($p = 0.04$) were independently associated with survival to discharge.

Conclusions. Treatment of goats with toxic mastitis at this institution carried a fair to good prognosis to hospital discharge. Inclusion of intramammary antibiotics and steroids in the treatment regimen may improve survival in some cases of toxic mastitis.

O16 *In Vitro* Antimicrobial Activity of Canine Platelet Lysate with variable Leukocyte concentration, Plasma Content, and Heat Sensitive Proteins

Melikasadat Mollabashi[1,2], Alonza Klopfer[3], Scarlett M. Sumner[3], Maria C. Naskou[1,2]
[1]Department of Pathobiology, College of Veterinary Medicine, Auburn University, AL
[2]Scott-Ritchey Research Center, College of Veterinary Medicine, Auburn University, AL
[3]Department of Clinical Science, College of Veterinary Medicine, Auburn University, AL

Introduction. Platelets play a crucial role during the inflammatory phase of wound healing due to the release of chemokines, proteins, cytokines, and growth factors. They also have antimicrobial peptides which contribute to their antimicrobial properties. Factors affecting the effectiveness of platelet products in wound healing include the manufacturing process, platelet concentration, leukocyte content, method for platelet activation, and pooling of individual donors.

Methods. In this study, blood was collected from six purpose-bred dogs. Platelet-rich plasma was produced using two centrifugation methods, one leukocyte-rich and one leukocyte-reduced. A portion of the samples was processed for plasma depletion and platelet lysate was subsequently generated through freeze-thaw cycles. A portion of platelet lysate samples underwent heat treatment. All treatment groups were tested against four common bacteria found in canine skin wounds: *Escherichia coli*, *Enterococcus faecalis*, *Staphylococcus pseudintermedius,* and *Staphylococcus aureus*. Effects on bacterial inhibition were evaluated via a bacteria spiking assay.

Results. Statistically significant differences were noted for the log reduction of *S. aureus* and *E. coli* with platelet lysate (PL)after 3 hours. No statistically significant differences were noted between the log reduction of bacteria with leukocyte-reduced and rich methods. After depleting plasma, the log reduction of S. pseud was statistically significantly less than before plasma depletion, whereas the opposite was seen for *E. faecalis* after 3 hours. Deactivating the complement proteins of plasma by heat led to a statistically significantly lower log reduction for *E. faecalis* after 24 hours.

Conclusions. According to the results, plasma and complement proteins seem to be critical for inhibiting the growth of some bacterial strains although leukocyte concentration is not essential for this goal. Our future experiments will evaluate the synergistic effects of PL with antibiotics against bacterial wounds in canines.

Acknowledgments. We would like to acknowledge the Scott Fund, the Swaim fund for Excellence in Wound healing and the Scott-Ritchey Research Center.

O17 Studying co-infection of IAV at the Human-Swine Interface in fully differentiated Primary Bronchial Epithelial Cells

Peter J. Neasham[1], J. Fletcher North[1], Dimitrios C. Sakkas[1], Vasilis C. Pliasas[1], Davis Chastain[1], Victoria Christopulos[1], Madelyn Krunkosky[2], S. Mark Tompkins[2] and Constantinos S. Kyriakis[1,2]
[1]Department of Pathobiology, College of Veterinary Medicine, Auburn University, AL
[2]Center for Vaccines and Immunology, University of Georgia, Athens, GA

Introduction. The human/swine interface is a critical transmission point influenza A virus (IAV) and for the evolution of novel IAVs with pandemic potential. Reverse zoonosis at this interface has enabled human IAV (huIAV) gene segments to reassort and be incorporated into the diverse pool of circulating swine IAVs (swIAVs) within the swine host. A significant example of this was the isolation of H3N2 variant (H3N2v) viruses from humans during the 2011-2012 season, which acquired the matrix (M) gene from the A/CA/09/H1N1 (pdmH1N1) strain. However, the specific roles of human and swine hosts in driving the evolution of H3N2v strains are still poorly understood. Our overall aim is to study the emergence of the H3N2v genotype at the human/swine interface, by employing an *in vitro* model using fully differentiated human and swine bronchial epithelial cells.

Methods. Human and swine bronchial epithelial cells were polarized using an air-liquid interface (ALI) system. Two parental strains, A/swine/MN/A01125993/2012/H3N2 (swH3N2) and A/CA/07/09/H1N1 (pdmH1N1), were chosen to model the evolution of H3N2v strains. The parental strains were co-infected at a high multiplicity of infection (MOI) of 10 in both the primary human and swine cells, to promote reassortment between the parental strains and to increase the probability of all possible genotypes being initially generated. Supernatants from the primary cells were harvested at 12 hours post-coinfection, prior to being passaged at an MOI of 0.1 to select for genotypes depending on the human or swine host cell line. At each passage (P1, P3, and P5), the genotype of clonal plaque isolates from the harvested supernatants were screened using High Resolution Melt (HRM) analysis. This allowed for the detection and quantification of reassortant strains.

Results. We observed differences in the frequency of H3N2v genotypes following selection in either human or swine bronchial epithelial cell lines. In addition, we observed most frequently that the pdmH1N1 polymerase acidic (PA) and M gene segments were incorporated into the swH3N2 backbone in primary swine cells. However, the parental swH3N2 genotype was most prevalent in both primary human and swine cells following the initial co-infection and during serial passaging experiments.

Conclusion. The findings from this study suggest that the human/swine interface plays a significant role in the evolution of novel IAV strains. The observed increase in swH3N2 strains incorporating the pdmH1N1 M gene highlights the potential threat posed by swine as a reservoir for generating and circulating viruses with zoonotic and pandemic potential. In the future, we aim to utilize this methodology to study past historical reassortment events that have shaped the evolutionary history of swIAVs across N. America. We believe this may provide valuable insights into the origins and evolutionary dynamics of swIAVs and contribute to better pandemic risk assessment and preparedness strategies by predicting the threat posed by co-circulating IAVs in swine herds.

Acknowledgments. This work was funded by the NIH/NIAID Centres of Excellence for Influenza Research and Surveillance (CEIRS - Emory-UGA CEIRS HHSN272201400004C) and the Alabama Agricultural Experiment Station (AAES).

O18 Immunogenicity of a Universal Influenza Vaccine in Swine with Pre-Existing Immunity

J. Fletcher North[1], Sangil Kim[2], Nicholas Bayless[2], Peter J. Neasham[1], Dimitrios Sakkas[1], Philip G. Strate[1], Jacob Glanville[2], Constantinos S. Kyriakis[1]
[1]Department of Pathobiology, College of Veterinary Medicine, Auburn University, AL
[2]Centivax Inc., San Francisco, CA, USA

Introduction. Influenza A viruses (IAV) remain one of the more important viral pathogens of humans, causing seasonal epidemics and occasional pandemics. Vaccination serves as the primary prophylactic measure against IAVs, however traditional IAV vaccines have several shortcomings. IAV vaccines rarely achieve efficacy rates above 60% with most years falling between 40-60%. Years in which failures during strain selection or vaccine production occur have been as low as 17%. Current production methods rely on testing of vaccine candidates using naïve animal models. While this is an important step, the overwhelming majority of the human population is already immunologically experienced against influenza, being exposed by two years of age on average. This first exposure has been shown to have long-lasting influence over an individual's ability to develop IAV-specific immune memory later in life, raising questions about the methods by which we assess vaccine candidates. The Centi-Flu vaccine platform has already been proven efficacious in naïve animals, but to better understand how it would perform in the general population it is necessary to test it in a model with pre-existing immunity.

Methods. Our group has previously demonstrated swine as a valid model for studying pre-existing immunity. As such, five groups of swine were enlisted in this study: three groups with pre-existing immunity against IAVs, and two IAV naïve groups. All pre-immune groups and one naïve group were administered the Centi-Flu vaccine adjuvanted with ISA-201 (W/O/W emulsion) in a prime-boost regimen, while the final naïve group was given a typical seasonal IAV vaccine. Animals were bled following each vaccination and up to three months post boost, to measure serum responses to vaccine antigens, as well as several strains which served as 'future' variants one might encounter. Serum responses were measured using hemagglutination inhibition assays (HAI), the standard assay for assessing IAV-specific protection.

Results. Animals with pre-existing immunity exhibited substantial increases in HAI antibody titers, as well as a large increase in the breadth of both H1- and H3-specific responses. Titers increased after only a single dose and were maintained up to two months following the last dose. In contrast vaccination of naïve animals with the Centi-Flu platform induced a similarly broad response against only H3 strains, but titers were transient and below the protective threshold (HAI >40).

Conclusions. Vaccine responses differed dramatically when comparing naïve vs. immunologically experienced animals, with pre-immune groups demonstrating higher antibody titers and wider breadth of immune responses. This is likely a better model of the responses one could expect from vaccination in humans. These results suggest the current reliance on naïve animal models may undersell a vaccine's immunogenicity during early studies. Moving forward it may be necessary to use both naïve and immunologically experienced models to fully assess seasonal vaccines, as well as characterize novel vaccine technologies.

Acknowledgments. This work was funded by Centivax Inc.

O19 Inflammatory cytokine priming alters the biocargo of equine bone marrow derived mesenchymal stem cell extracellular vesicles (BM-MSC-EVs)

Mana Okudaira[1,2], Lauren K Olenick[2], Alexandra IJ Usimaki[2], Hoda Elkhenany[2], Renata L Linardi[2], Angela M Gaesser[2], Luca Musante[2], Kyla F Ortved[2]
[1]Department of Clinical Sciences, College of Veterinary Medicine, Auburn University, AL
[2]New Bolton Center, University of Pennsylvania School of Veterinary Medicine, PA

Introduction. Extracellular vesicles (EVs) constitute a key component of the mesenchymal stem cell (MSC) secretome and have recently become a biotherapeutic target for the treatment of osteoarthritis. Standardized methods of EV isolation are not well established in equine tissues and characterization of EV-miRNomes are novel in this species. Although inflammatory priming of MSCs has shown potential in improving their clinical efficacy, the effect of inflammatory priming on the biocargo of EVs secreted by primed equine bone marrow-derived MSCs (BM-MSCs) is not well understood. The objective of this study was to investigate the effect of inflammatory priming agents, using interferon-gamma (IFN-γ) and/or tumor-necrosis-factor-alpha (TNF-α), on the immunomodulatory properties of MSC-EVs. We hypothesized that BM-MSCs primed with IFN-γ and TNF-α, or their combination, would yield an increased number of EVs with an enhanced immunomodulatory profile.

Methods. Equine BM-MSCs were cultured in three different conditions of primed cell media: 1) IFN-γ (50ng/mL), 2) TNF-α (10ng/mL) or 3) IFN-γ (50ng/ml) + TNF-α (10ng/mL). EV-depleted cell media without any added priming agent was used for the control group. The concentrations of immunomodulatory cytokines including IL-6, IL-8, IL-10 and IL-1β were measured in cell culture supernatants at 24h, 48h and 72h post-priming using an equine fluorescent bead based multiplex assay. At 72h, cells were harvested and characterized as MSCs by flow cytometry. Extracellular vesicles were isolated from cell culture supernatants using hydrostatic filtration dialysis followed by stepwise ultracentrifugation. Isolated EVs were characterized by nanoparticle tracking analysis (NTA), transmission electron microscopy (TEM), and Western blot. Following EV characterization, RNA was extracted from the EV preparations and submitted to Novogene Corporation Inc. (Sacramento, CA) for small RNA sequencing.

Results. EVs were successfully isolated from cell media supernatants in all groups and validated using standard methods of EV characterization. There was no difference in particle yield per cell across treatment groups. However, when particles were categorized by their size distribution, the number of particles in the exosome (0-100nm diameter) and microvesicle (101-1000nm diameter) size ranges were significantly greater in the TNF-α and IFN-γ+TNF-α groups. Additionally, MHC II expression was higher in IFN-γ treated cells. Levels of IL-1β and IL-10 were significantly higher in the IFN-γ group at T24 when compared to the control and TNF-α groups. Transcriptomic analysis yielded 18 differentially expressed miRNA in experimental conditions compared to the control group. The downstream functional enrichment analysis is pending.

Conclusions. Findings from this study show differences in relevant cytokines and the transcriptome that suggest inflammatory priming of BM-MSCs alters immunomodulatory profiles of EVs. Inflammatory cytokine priming can modify the miRNA profile of BM-MSC-EVs which may enhance their therapeutic potential.

Acknowledgements. Funding for this study was provided by the Raymond Firestone Trust Research Grant.

O20 Computationally Optimized Broadly Reactive Antigen (COBRA) Intranasal Vaccination offers protection against challenge with the pandemic 2009 H1N1 Influenza A Virus (IAV) in the porcine model

Dimitrios C. Sakkas[1], Diego E. Ventura Urbina[1], Peter J. Neasham[1], J. Fletcher North[1], Trinity L. Beell[1],Alexandra L. Awtrey[1],Camryn G. Mattox[1], Alyona Michael[1], Maria C. Naskou[1], David Burkhart[2], Shannon Miller[2], James D. Allen[3], Ted Ross[3] and Constantinos S. Kyriakis[1]
[1]Department of Pathobiology, College of Veterinary Medicine, Auburn University, AL
[2]Inimmune Corporation, Missoula, MT
[3]Cleveland Clinic Florida Research & Innovation Center, Port Saint Lucie, FL

Introduction. Current seasonal influenza vaccines have repeatedly demonstrated suboptimal efficacy, due to annual rapid antigenic evolution especially of influenza A viruses (IAVs). Development of "universal" influenza vaccines is crucial to induce broad, long-lasting protection against circulating and emerging strains. One approach towards universal vaccine uses the computationally optimized broadly reactive antigen (COBRA) methodology, based on designing recombinant antigens of the hemagglutinin (HA) surface glycoprotein. Additionally, intranasal delivery of antigens, can result in mucosal immune responses, further improving protection against challenge. In this study, the efficacy of this novel vaccine technology, combined with next-generation adjuvants, was tested in the porcine model.

Methods. A total of 24, six-weeks old piglets from an influenza negative herd were used in this study. They were randomly assigned to six experimental groups, each containing 4 animals. Pigs were vaccinated twice with a four-week interval, and four weeks after the second immunization they were challenged with A/CA/2009 (H1N1) virus. COBRA vaccines contained two hemagglutinin subtypes H1 and H3 and were formulated without an adjuvant or with one of three toll-like receptor agonist adjuvant. The aforementioned vaccine formations were compared to a whole inactivated virus (WIV) vaccine containing homologous to the challenge H1N1 strain formulated with a licensed squalene-based adjuvant. COBRA vaccines were delivered intranasally, while the WIV vaccine was delivered intramuscularly. Immunogenicity was assessed by hemagglutination inhibition (HAI) assay in serum and IgA ELISA in nasal swabs. Following virus challenge, the animals were monitored daily for clinical signs, and nasal swabs were taken to asses virus shedding. Pigs were euthanized at day 5 post infection and respiratory tissue samples were collected for virus titration and histopathological evaluation.

Results. Comparable with the homologous WIV vaccine, pigs that received the adjuvanted COBRA demonstrated high HAI titers against the challenge virus and low virus titers in nasal swabs. Vaccinated animals showed limited clinical disease and no remarkable gross pathology in lung and trachea.

Conclusions. The novelty of this study lies in the fact that the COBRA vaccine platform was administered intranasally in swine to enhance nasal mucosal immunity along with the systemic responses. HAI antibody titers against a wide panel of IAVs suggested broader protection compared to seasonal vaccines. Finally, IgA ELISA from nasal swabs indicated the production of nasal mucosal immunity, which is key in preventing infection and transmission.

Acknowledgments. This work has been funded by the National Institute of Health (NIH) Collaborative Influenza Vaccine Innovation Centers (CIVICs).

O21 Antibody Gene Therapy Protects Against Lethal Rabies Encephalitis

Jyoti Yadav[1,2], Nicholas J. Jones[4], Amanda L. Gross[1], Courtney J. Garrett[1,2], Giovanna Panepinto[1], S. Satheshkumar Panayampalli[5], L. Claire Godino[5], Ywh-Min Tzou[1], Kent R. Van Kampen[6], Miguel Sena-Esteves[7,8], Henry J. Baker[1,3], April D. Davis[4] and Douglas R. Martin[1,2]
[1]Scott-Ritchey Research Center, [2]Department of Anatomy, Physiology, & Pharmacology, [3]Department of Pathobiology, College of Veterinary Medicine, Auburn University, AL; [4]Wadsworth Center, New York State Department of Health, Slingerlands, NY; [5]Centers for Disease Control and Prevention, Atlanta, GA; [6]The Van Kampen Group, Payson, UT; [7]Horae Gene Therapy Center, [8]Department of Neurology, University of Massachusetts Medical School, Worcester, MA.

Introduction. Rabies virus neutralizing antibodies in serum do not cross the blood-brain barrier, making them ineffective in treating rabies encephalitis. We designed a recombinant adeno-associated virus vector (AAV-Rab) that crosses the blood-brain barrier and expresses broadly neutralizing antibodies to rabies virus, independent of the host immune system, in serum and brain to protect the nervous system from infection.

Methods. Mice were treated with a single intravenous injection of AAV-Rab as low ($1x10^{12}$) or high ($1x10^{13}$ vg/kg) doses, with or without a lethal challenge infection using an African canine rabies virus variant, administered intramuscularly. Rabies virus-neutralizing antibodies were analyzed in serum and brain. Additionally, cats were treated intravenously with low ($2x10^{12}$) or high ($1x10^{13}$ vg/kg) doses of AAV-Rab to monitor antibody production.

Results. In initial studies, AAV-Rab produced neutralizing titers in mouse serum up to 1,500 IU/ml and in cat serum up to 140 IU/ml. Titers remained high for 3 months in mice (maximum study duration) or 15 months in cats. Antibodies to rabies virus were detected by immunohistochemistry in neurons of numerous brain regions in mice and cats. When mice were treated by AAV-Rab 14 days prior to challenge with a lethal dose of rabies virus, all mice (16 of 16) treated with the high dose survived to the experimental endpoint ≥52 days post-challenge, while 14 of 16 mice in the low-dose cohort survived. When mice were challenged with rabies virus 3 days prior to high-dose AAV treatment, 11 of 12 animals survived. Neutralizing antibody titers in serum at the end of the study were >1,000 times higher than the minimum level recommended by the WHO (0.5 IU/ml). Untreated controls (11 of 12) died within 16 days of viral challenge.

Conclusions. A single intravenous dose of AAV-Rab expressed rabies virus neutralizing antibodies in serum and brain that protected mice from lethal infection and encephalitis. Cats treated with the same AAV-Rab produced neutralizing antibodies for over one year.

Acknowledgments. We gratefully acknowledge the expertise of Dr. Chengming Wang and Terri Wood for RFFIT data. Studies were supported by the Scott-Ritchey Research Center and the Intramural Grants Program of Auburn University. This work is a part of US Patent Office #PCT/US2020/051284, WO/2021/055614, filed March 25, 2021.

Faculty/Postdoctoral/Staff Platform Presentations

O22 Dual site administration of AAV gene therapy for the treatment of feline GM1 gangliosidosis

Courtney Garrett[1, 2], Juan Rodriguez[1], Douglas Martin[1, 2], Amanda Gross[1]
[1]Department of Anatomy, Physiology, and Pharmacology
[2]Scott-Ritchey Research Center, College of Veterinary Medicine, Auburn University, AL

Introduction GM1 gangliosidosis is a fatal neurodegenerative disease caused by a deficiency of lysosomal β-galactosidase (βgal). There are currently two ongoing clinical trials for the treatment of GM1 gangliosidosis using adeno-associated viral (AAV) gene therapy administered either intravenously (IV) or in the cerebrospinal fluid (CSF) via the cisterna magna (CM). Preclinical trials of either administration route show that single site treatment significantly increases lifespans in a feline model of GM1 gangliosidosis. We hypothesize that deficiency in efficacy caused by either single site injection will be addressed by the additive effect of combinatorial administration.

Methods. GM1 cats received $6.0x10^{13}$ vector genomes/kg of AAV9 split equally between IV and CM administration at approximately 2 months of age. Four animals will be followed to humane endpoint and four animals will be evaluated 16 weeks following treatment. Clinical assessments include neurological exams, CSF biomarkers, tissue elastography and 7T magnetic resonance imaging (MRI) and spectroscopy (MRS).

Results. Untreated GM1 animals survive approximately 8.0 ± 0.6 months while treated animals have a significant increase in average lifespan to 18.2 ± 6.1 months, with 2 animals in the long term cohort still alive at 25.3 and 23.0 months of age. Neurological abnormalities, which in untreated GM1 animals lead to an inability to stand and humane endpoint by 8 months of age, were attenuated in all treated animals. Aspartate aminotransferase (AST) and lactate dehydrogenase (LDH), biomarkers of central nervous system damage when measured in the CSF, were both reduced in in treated animals in comparison to untreated animals. Elastography, a non-invasive, ultrasound based method of determining tissues elasticity, showed a significant decrease in elasticity in untreated GM1 animals in the liver and spleen, indicating an increase in storage material. Following CM+IV AAV administration, elasticity values were normalized in both tissues at 7 months and 1 year. 7T magnetic resonance imaging (MRI) revealed a delay in progression of neurodegeneration and preservation of brain architecture.

Conclusions. While animals are ongoing we are unable to determine if CM+IV administration of AAV is better than either single administration route however, the combined treatment did significantly improve length and quality of life in GM1 cats. Additionally, based on neurological assessment, CSF biomarkers, elastography, MRI and MRS, it is clear that dual site administration also attenuates neurological disease progression and treats peripheral tissues.

Acknowledgments. This research was funded by the National Tay-Sachs and Allied Disease (NTSAD) Foundation.

O23 Autoclave sterilization of polymer-based canine training aids for biodetection requires contextual odor learning due to altered signal to noise ratio

Samantha Hagerty[1], Michelle Aono[1], Adam Rivers[3] and Melissa Singletary[1,2]
[1]Department of Anatomy, Physiology, and Pharmacology, Auburn University CVM, AL
[2]Canine Performance Sciences, Auburn University CVM, AL
[3]United States Department Agriculture, Agriculture Research Service

Introduction. Canines have demonstrated advanced biodetection capabilities across medical, agricultural, and public health sectors. However, training for biological targets presents unique challenges associated with pathogenicity which limits the availability of restricted and hazardous source material outside of controlled facilities. Previous work introduced the use of polymer-based odor capture and release (POCR)™ training aids to deliver true odor composites of viral-based biological materials without contamination risk. Field use of training aids carrying volatiles of emerging biological threats requires a rigorous 2-step autoclave method, and initial canine trials indicated a higher suggested configuration-dependent odor learning than non-sterilized POCRs™. In this work, we characterize the comparative odor profiles of live and POCR™-captured biological and non-biological materials before and after sterilization to better understand how this operationally relevant context effects target odors and informs development of optimized training protocols.

Methods. Headspace analysis was performed using proton transfer reaction-mass spectrometry (PTR-MS 1000 Ultra, IONICON Analytik), a method of direct and continuous volatile compound sampling without pre-concentration allowing for real-time measurement. Using ionized water as the primary reagent, sampled molecules interact based on proton-affinity and ionized products are directed toward a time-of-flight (TOF) mass analyzer followed by automated database matching to most likely identity candidates. The POCR™ training aid is composed of cured and heat-treated polydimethylsiloxane (PDMS) which has intrinsic chemical properties suitable for capturing volatile organic compounds (VOC). Detection targets included a non-biological odor, Amyl Acetate (AA) and a biological odor, Channel Catfish Virus (CCV)-infected oocytes in cell culture. Aqueous, or live, samples were analyzed prior to being charged onto the training aids, after charging, and following the autoclave cycle.

Results. While PDMS effectively captures and concentrates proximal volatiles, it intrinsically carries a high odor background associated with its crosslinking monomer fragments (dimethylsiloxane, trimethyl silyl, and methyl groups) and polymerization catalyst components (methylvinylcyclosiloxane, styrene, and methylbenzene) which are detectable in the headspace of uncharged and charged POCRs™. Autoclaving blank training aids resulted in a significant increase in signal intensity among the 12 signature polymer matrix peaks as well as water cluster compounds. Effective transfer of odor profiles from live material to the training aid was demonstrated for both non-biological (AA) and cell culture-based (CCV) training materials at comparable headspace concentrations. Autoclaving charged training aids resulted in decreased peak signals for respective odor profiles, though not statistically significant, and increased PDMS-specific peaks consistent with uncharged sterilized POCRs™. For both groups of training odors, the ratio of collective signal intensity among relevant peaks to polymer odor background intensity was quantified, demonstrating a shift in target-specific signal to spectral noise associated with autoclaving.

Conclusions. This work demonstrates altered headspace composition of PDMS-based training aids associated with the operationally necessary sterilization of materials carrying restricted biological materials, requiring configuration-dependent odor learning for canine biodetection. Although task-specific considerations are needed, we demonstrate consistent transfer of target odor profiles that persist after rigorous autoclaving protocols necessary for field use outside of controlled facilities. Further analyses are necessary to determine the extent of biomaterial changes induced by autoclave treatment and whether this impacts canine generalization performance in operational settings.

Acknowledgments. This research is funded by the United States Department of Agriculture G00016151.

O24 Associations between the fecal microbiome and gastrointestinal nematode parasites in North American Bison

Jeba R J Jesudoss Chelladurai[1], Theresa A. Quintana[1], Grace Schieferecke[2] and Guwin Weerawarna Nilaweera[2]
[1]Department of Pathobiology, College of Veterinary Medicine, Auburn University, AL
[2]Department of Biology, Kansas State University, Manhattan, KS

Introduction. The health of grazing ruminants is adversely affected by the effects of gastrointestinal nematodes (GIN), which chronically deplete nutrients and decrease production. Relatively little is known about the interactions of GIN and the microbiota of host animals. The overall goal of this work was to address this knowledge gap and increase our understanding of the complex interactions between trichostrongyle parasites and the microbiome in North American Bison. The specific objectives were to understand the diversity and composition of the core microbiome in bison with low and high strongyle parasite burdens using shotgun metagenomics conducted on the Illumina platform.

Methods. Utilizing a herd-stratified case-control experimental design, bison were categorized as either parasite infected or non-infected controls based on quantified parasite burdens. In this shotgun metagenomics pilot, a total of 18 samples from 9 North American bison herds in the midwestern USA were selected. Each sample represents a fecal pool from three individual bison, classified as either parasite infected or not infected. Following DNA extraction and QC, Illumina libraries were prepared and sequenced on a NextSeq 500 system at ~15 million read pairs each. Raw data in FASTQ format was analyzed using the DRAGEN metagenomics pipeline. Linear Discriminant Analysis was used to identify effect size.

Results. The metagenomic analysis identified a diverse range of microorganisms in the bison microbiome, including bacteria, archaea, fungi, and viruses. The most abundant bacterial phyla were Proteobacteria, Actinobacteria, Firmicutes, Euryarchaeota and Bacteroidetes. The diversity of the bison microbiome was assessed using alpha diversity and beta diversity metrics. The results showed that the bison microbiome was diverse, both within and between samples. Additionally, principal components analysis of the dataset revealed clustering, which was dependent on the taxa used in the analysis.

Conclusions. The results of this study provide new insights into the composition and function of the bison microbiome, as well as its interactions with parasites. Further research is needed to better understand the specific mechanisms by which the microbiome interacts with parasites and to develop new strategies for parasite control based on this understanding.

Acknowledgments. This project was funded by pilot project funds provided by Illumina to the PI through the Kansas State University Integrated Genomics Facility. Sample collection was funded by the Center for Excellence for Bison Studies at South Dakota State University.

O25 The Relationship Between Radiographic Spinal Score and FGF4L2 Genotype in Dachshunds

Stacey Sullivan[a], David Redden[b], Froydis Hardeng[c], Malin Sundqvist[d], Michelle Kutzler[e]
[a] College of Veterinary Medicine, Auburn University, [b] Edward Via College of Osteopathic Medicine, Auburn University, [N]orwegian Federation of Dachshund Clubs, [d] Swedish Dachshund Club, [e] College of Agricultural Sciences, Oregon State University

Introduction. The FGF4L2 retrogene insertion was recently found to be responsible for intervertebral disc disease (IVDD) in dogs. The insertion has been reported to be dominant for IVDD, suggesting that either 1 or 2 copies are equally detrimental to spinal health. Radiographic spinal score (K-n) is a measure of the degree of early disc degeneration that has been shown to be heritable and to correlate with the likelihood of symptomatic disc disease (SDD) as follows: K0 7% risk, K1/2 12% risk, K3/4 23% risk, and K5+ 69% risk. Reported breed average risk of SDD in dachshunds is 18-25%. Breeding schemes based on K-n have been adopted in Nordic and other countries. To date, the relationship between K-n and FGF4L2 genotype has been reported in only a small number of dachshunds. We hypothesize that a genotype comprised of either 1 or 2 FGF4L2 copies is associated with a worse K-n compared to a genotype with 0 copies.

Methods. This was a retrospective study (2012-2024) which included dachshunds registered with the Norwegian or Finnish Kennel Clubs for which both K-n and FGF4L2 genotype were known (n=407). The frequency and percentage of dachshunds within each K-n group (K0, K1/2, K3/4, K-5+) by FGF4L2 genotype (two copies, one copy, zero copies) were reported. K-n distribution differences among genotypes were analyzed using Cochran-Mantel-Haenszel test in SAS 9.4. Significance was defined as $p<0.05$.

Results. There is a significant difference in K-n distribution between dachshunds that have 0 or 2 FGF4L2 copies, with 0 FGF4L2 copies being associated with the best K-n. However, there is not a significant difference in the K-n distribution between dachshunds that have 0 or 1 FGF4L2 copies.

Conclusions. Despite the reported dominant behavior of the FGF4L2 retrogene insertion for IVDD in dogs, most dachshunds with 1 FGF4L2 copy have spinal scores similar to those with 0 copies, suggesting that there is a gene dose effect for SDD risk in dachshunds. We suggest that the FGF4L2 retrogene insertion is dominant for early disc degeneration, but that SDD risk is determined by FGF4L2 copy number. Given the high allele frequency of FGF4L2 in the dachshund, breeding to produce progeny with 1 FGF4L2 copy is expected to be a more achievable short-term goal for dachshund spinal health breeding programs than breeding for 0 copies. It is recommended that FGF4L2 genotype status be considered when choosing dachshund breeding stock.

Acknowledgments. The authors thank Ida Sorensen and Niklas Karlsson for compiling the data, and the Norwegian and Finnish Dachshund and Kennel Clubs for building and maintaining the public dachshund pedigree and health databases.

O26 Feline Infectious Peritonitis Risk Factors and Diagnostic Insights from PCR Analysis of Large-Scale Nationwide Submissions

Subarna Barua[1], Shila Sarkar[2], Kelly Chenoweth[3], Calvin Johnson[1], Diane Delmain[4], and Chengming Wang[1,3]

[1] Department of Pathobiology, College of Veterinary Medicine, Auburn University, AL
[2] World Health Organization, Country Office, Dhaka, Bangladesh
[3] Molecular Diagnostics Laboratory, Department of Pathobiology, College of Veterinary Medicine, Auburn University, Auburn, AL
[4] Department of Clinical Sciences, College of Veterinary Medicine, Auburn University, Auburn, AL

Introduction. Feline infectious peritonitis is a fatal and progressive illness caused by feline coronavirus (FCoV), affecting domestic and wild felids globally. This nationwide study aimed to investigate risk factors associated with FIP and determine optimal sample submission strategies for its diagnosis.

Methods. A total of 14,035 clinical samples from cats across the US were analyzed by means of reverse transcriptase quantitative PCR to detect replicating feline coronavirus (FCoV). $\chi 2$ and logistic regression analyses were conducted to assess the association between FCoV detection rates and risk factors such as age, gender, breed, and types of submitted samples.

Results. Higher FCoV detection rates were observed in younger cats, particularly those aged 0 to 1 year, and in male cats. Purebred cats, notably British Shorthairs [OR: 2.81; $P < .001$], showed a higher incidence of FCoV infection than other cats. Peritoneal fluid (OR, 7.51; $P < .001$) exhibited higher FCoV detection rates than other samples, while lower rates were seen in blood samples (OR, 0.08; $P < .001$) than in other samples. High FCoV detection rates were found in urine, kidney, and lymph node samples.

Conclusions. The study identified significant risk factors associated with FIP. Optimal sample submission strategies, particularly emphasizing the use of peritoneal fluid, kidney, and lymph node, were identified to improve FIP detection rates. Urine yielded a relatively high frequency of infection and viral loads compared with most other samples. Understanding the risk factors and optimizing sample selection for FIP diagnosis can aid in the early detection and management of the disease, ultimately improving outcomes for affected cats. These findings contribute valuable insights to FIP epidemiology and underscore the importance of continued research to enhance diagnostic strategies and disease management approaches.

Acknowledgments. The authors thank Dr. Laura Huber for her valuable advice on the statistical analysis in this work.

O27 Resident urinary microbiome in canine clinical samples: reference profiles, dysbiosis, and associations with disease states

Abbigail L. Lanier[1], Erin Kilbane[1], Thu Nguyen[1], Andrew Leisewitz[2], Emily C. Graff[1,3†], Xu Wang[1,3,4†]
[1]Pathobiology, Auburn University; [2]Clinical Sciences, Auburn University; [3]Scott-Ritchey Research Center, Auburn University; [4]HudsonAlpha Institute for Biotechnology

Introduction. The traditional belief that healthy urine is sterile has been significantly challenged in recent years, with mounting evidence arising from human medical research to overturn this universally accepted fact. Advances in next-generation sequencing technologies have allowed for the successful identification of diverse microbial taxa in urine samples, supporting the existence of a resident urinary microbiome (UMB) in companion animals. However, other studies failed to detect any 16S metagenomic reads that could be confidently classified as non-contaminants, leaving the presence of a distinct dog UMB an unresolved question. This discrepancy underscores the need for further rigorous investigations to elucidate whether a resident UMB exists in dogs and to understand its potential implications for canine health and disease.

Methods. To characterize the canine UMB, we applied a whole-genome shotgun (WGS) metagenomic sequencing approach to 12 de-identified dog urine samples collected at Auburn University Clinical Pathology Laboratory. These patients were from 9 different breeds with an age range of 4-14 years. Their diagnosis varies from urinary tract infection, acute pancreatitis, to different forms of cancer including lung cancer, bladder cancer, and lymphoma. After urinalysis, 2-6 mL of urine was transferred to a 15 mL conical-bottom tube and centrifuged for 5 minutes at 2000 rpm. The supernatants were removed, and urine pellet was resuspended in 0.5 mL urine to obtain concentrated sediment for subsequent metagenomic analyses.

Results. Given the varied genetic backgrounds, household environments, and clinical symptoms, we expect to observe variable microbial compositions in the UMB. Strikingly, our data demonstrated that the UMB compositions were highly similar across 6 of the 12 samples, thereby establishing a "reference" urinary microbiome. The microbial diversity is as high as that of the gut microbiome, with 14 highly abundance genera (>1%) accounting for 75% of the UMB. Our analysis revealed that the top 14 most abundant genera within the canine UMB maintained a consistent ranking order across all six reference urine samples. This uniformity strongly suggests the existence of a highly stable resident microbial community within the urinary tract. Importantly, among these six reference samples, three were obtained via free-catch methods and the remaining three through cystocentesis, indicating that the urine collection technique does not significantly influence the UMB composition. Furthermore, the remainder six urine samples exhibited a significant shift in UMB compared to the reference. These samples demonstrated a substantial increase in the abundance of a few bacterial species, with elevations ranging from 1 to 5 species and up to 1,000-fold, indicating potential UMB dysbiosis under disease conditions. When these species were excluded, the relative abundance of the remaining microbes was highly similar to that of the reference set.

Conclusions. Our findings support the presence of a stable resident UBM in dogs and indicate that alterations in microbial abundance may occur during specific disease states. These microbial changes hold potential as novel biomarkers for early detection of diseases in dogs.

Acknowledgements. This study is supported by the Frances Keith Endowment Fund and a Scott-Fund awarded to XW. Contribution from ECG is supported by the Focused Ultrasound Foundation.

Undergraduate Student Poster Presentations

P1 Temporal and temperature-dependent volatile odorant emissions of *Popillia japonica*

Christopher Consoli[1], Samantha Hagerty[1], Jason Oliver [2], Nadeer Youssef[2], David Held[3] and Melissa Singletary[1,4]
[1]Department of Anatomy, Physiology, and Pharmacology, Auburn University CVM, AL
[2]Tennessee State University McMinnville, TN
[3]Entomology and Plant Pathology, Auburn University CVM, AL
[4]Canine Performance Sciences, Auburn University CVM, AL

Introduction. Since being introduced to the United States, the invasive species known as *Popillia japonica* Newman (PJ), has cost millions in economic loss due to its harm to agriculture, prompting efforts to eradicate the pest in the most efficient way possible. The PJ in its larval stage, infests the root systems of turfgrass and agriculturally significant crops, only causing noticeable damage once they have taken over the surrounding area. The subterranean nature of the larvae poses another challenge for early identification of field infestations, and their damage effects may be unapparent until irreversible damage has occurred**.** One method that has shown promise is the utilization of detection canines to locate early signs of infestation. While alternative methods including trap-luring are also common for adult stages, these are limited in larval life stages. We aim to characterize the volatile organic compounds (VOC) unique to PJ and assess changes to this odor profile depending on time of day and temperature. Headspace analysis will also enhance the development of accurate and precise training protocols for detection canines.

Methods. Headspace analysis was performed using a real-time, direct sampling method called proton transfer-mass spectroscopy (PTR-MS 1000 Ultra, IONICON Analytik). Molecules sampled are ionized based on proton affinity and directed toward a time-of-flight (TOF) mass analyzer. A total of 4 larvae were collected from McMinnville, Tennessee and group-housed at 4°C. Data were collected from the larvae during various times of the day (AM and PM) and at various temperatures (25°C and 32°C) to observe how time and temperature affect the VOCs released. To collect data, larvae were placed in customized headspace sampling jars. After a short equilibration period, the headspace created by the larvae was analyzed by inserting the inlet sampling needle into the septa on the jars. Spectral data were then post-processed and analyzed for compound identification.

Results. Several peaks were unique to all PJ replicates at masses 47, 59, 61, 65, 69, 88, 95, 101, 102, and 107. Among these signature peaks, m47 and m59, demonstrated time and temperature-dependent changes. The instrument acquisition software contains an internal and National Institute of Standards and Technology (NIST)-linked database, which determines the most likely compound identity based on mass and fragmentation pattern similarity. These compounds were identified as ethanol (m47) and acetone (m59), with acetone showing significant differences based on time of day and temperature.

Conclusions. After collecting data on the headspace created by the larvae of *P. japonica*, ethanol and acetone were identified as significant VOCs released from the isolated larvae. However, further research regarding supplementary odors such as the habitat of these PJ larvae still needs to be conducted so that a more generalized canine training protocol may be created.

Acknowledgements. This research is funded by the United States Department of Agriculture G00016151.

P2 Elucidation of CAV2 Tropism

Devin Cooper[2] and Payal Agarwal[1,2]
[1]Scott Ritchey Research Center, College of Veterinary Medicine, Auburn University, AL
[2]Department of Pathobiology, College of Veterinary Medicine, Auburn University, AL

Introduction. Oncolytic virotherapy is a cancer treatment that uses oncolytic viruses to kill tumor cells. Conditionally replicative recombined CAV2 (Canine Adenovirus Type 2) is a potential oncolytic virus similar to human adenovirus 5 (Ad5. CAV2 genome is well mapped, but the receptors that it uses to infect a cell remain a mystery. It has been assumed that CAV2 uses the CAR receptor as Ad5. However, CAV2 can infect cells that do not express CAR receptor and are refractory to Ad5 infection. We have proposed that inhibition of CAR expression will result in low Ad5 infections but will not affect CAV2 infections. We also propose that DSG1 may be a potential receptor involved in CAV2 infections.

Methods. The techniques used for this project included polymerase chain reactions, agarose gel electrophoresis, siRNA inhibitions, and CAV2 and Ad5 infections of canine cell lines.

Results. We inhibited CAR mRNA translation using siRNA. The siRNA inhibition of CAR mRNA in canine cell lines MDCK and CML7 was successful. The cells transfected with anti-CAR siRNA showed low Ad5 infections, while CAV2 infection levels remained consistent. DSG1 mRNA levels were successfully analyzed in both cell lines using qPCR.

Conclusions. siRNA inhibition of CAR confirms that CAV2 can infect cells that no longer express the CAR receptor. Additionally, the qPCR results confirm the presence of DSG-1 mRNA in the cells. To further verify that CAV2 uses DSg-1 for infections, we are designing siRNA inhibition experiments targeting DSG-1.

Acknowledgments. I would like to acknowledge all members of the Agarwal lab, including Theresa Higgins, Sumbul Khan, Gracie Bunch, and Isabella Shimko-Lofano. Additionally, I would like to acknowledge, Dr. Bruce F. Smith and the other faculty of Scott-Ritchey Research Center. I would also like to thank Scott Ritchey Research Center, Dr. Cremaschi, and the Undergraduate Research Fellowship Program for funding.

P3 Sexual dimorphism of fetal and placental weights of rat fetuses exposed to prenatal cannabinoids

Paige M. Drotos[1], Cristine R. Camp[1], Adrian H. Courville[2], Miranda N. Reed[2], Rachel C. West[1]
[1]Department of Anatomy, Physiology, Pharmacology, College of Veterinary Medicine, Auburn University, AL
[2]Department of Drug Discovery & Development, Harrison School of Pharmacy, Auburn University, AL

Introduction. As general attitudes towards recreational cannabis use have become increasingly more accepting, the prevalence of cannabis use during pregnancy has sharply increased. Understanding the effects of cannabis consumption on fetal development is crucial, especially due to the understudied nature of the interaction. In this study, we assessed the effects of inhalation of delta-9 tetrahydrocannabinol (THC) throughout pregnancy using a rat model. As previous research has demonstrated a sexual dimorphism in the growth and development of fetuses and adolescents of mothers who consumed cannabis during pregnancy, our goal was to investigate the sexual dimorphism of placental and fetal development.

Methods. Pregnant rats were purchased and arrived at Auburn on gestational day (GD) 2. On GD5, dams were exposed to a vaporized dose of 100 mg/mL (THC) or vaporized polyethylene glycol (PEG). They received two 10-second puffs of THC or PEG over 15 minutes daily. There were 7 dams total, 3 were dosed with THC and 4 with PEG daily until GD18. On GD19, dams were sacrificed and fetuses and placentas collected and weighed. A total of 88 (49 PEG and 39 THC) rat fetuses and placentas were collected. Fetal tail snips were collected for genotyping to determine genetic sex. We isolated DNA from tail snips and used PCR to test for *Ddx3x* (found on the X chromosome) and *Ddx3y* (found on the Y chromosome). Fetal and placental weights were then organized by sex and assessed using a two-way ANOVA followed by a Tukey's multiple comparison test. P-values less than 0.05 were considered statistically significant.

Results. The average weight of the THC exposed fetuses was significantly heavier than the PEG fetuses ($p < 0.001$). The average weight of the THC exposed placentas was also higher than the PEG exposed placentas ($p < 0.05$). Interestingly, when biological sex was used as a variable, only the THC male fetuses were significantly larger than the controls (PEG male vs THC male $p < 0.05$, PEG female vs THC male $p < 0.01$). We saw the same phenomenon when accounting for sex in the analysis of the placental weights. The weight of the THC exposed female placentas was significantly ($p < 0.05$) higher than the placental weight of the PEG male pups and slightly higher, albeit not significantly ($p < 0.079$) compared to the PEG female placentas.

Conclusions. In this study we have demonstrated that sex influences the response to prenatal cannabinoid exposure in both the fetus and placenta. While both the fetuses and placentas of THC exposed pups were larger, the observed changes differ based on sex with male rats increasing in fetal weight and the placentas of female rats increasing in weight. Future studies will focus on if changes to fetal and placental metabolism caused the changes in weight.

Acknowledgments. We would like to recognize Victoria Caravaggio from the West Lab and additional members from the Reed lab for their support and assistance with this project. We also express our gratitude to Auburn University's startup funds for the financial support for Dr. West and NIH/NIDA R01 DA046723 for Dr. Reed.

P4 Assessing the Dosage Effect of AAV Gene Therapy in Intravenous Treatment of Feline GM1 Gangliosidosis

Ellie L. Hundley[1], Courtney J. Garrett[1], Amanda L. Gross[1], Douglas R. Martin[1, 2]
[1]Scott-Ritchey Research Center, [2]Department of Anatomy, Physiology, & Pharmacology, College of Veterinary Medicine, Auburn University, Auburn, Alabama, USA

Introduction. GM1 gangliosidosis is a neurodegenerative lysosomal storage disease a caused by the lack of β-galactosidase (β-gal). A lack of β-gal leads to the collection of GM1 ganglioside, which leads mainly to defects in the brain. However, both feline and human patients suffer from profound peripheral disease. Felines were used for this study because of their similarities to the human disease. Adeno-associated viral (AAV) gene therapy restores β-gal, which leads to the breakdown of GM1 ganglioside. Treatments were administered intravenously to GM1 cats to effectively treat the brain as well as the peripheral organs. The goal of this study is to compare the effectiveness of the treatment in peripheral organs, brain, and spinal cord.

Methods. There are four cohorts that were compared. The first cohort is a group of cats that are normal. The second cohort is affected with GM1, but no treatment was administered. The third and fourth cohorts also have GM1, and AAV treatment was given intravenously (IV) to the cats in two different doses. The low dose cohort received 1.5 X 10^{13} vg/kg and the high dose cohort received 6×10^{13} vg/kg. These felines were treated at12.8 ± 0.44 months of age. Clinical assessments were performed, which included neurological exams. Postmortem assessments included vector distribution via qPCR and enzyme distribution via specific activity assays.

Results. Untreated GM1 cats survive approximately 8.0 ± 0.6 months, while the treatment increased the lifespan to 28.3 ± 5.0 months in the high dosage cohort and 32.6 ± 13.5 in the low dose cohort. Additionally, all treated animals had an increase in quality of life, as determined by neurological assessment. The specific activity of β-gal increased in the treated cats, and in some cases, the increase in activity was greater in the cohort that received the high dosage treatment than the low dosage treatment. Surprisingly, there was more enzyme activity increase in some peripheral organs in the low dose cohort than in the high dose cohort. In the high-dose cohort, the average β-gal activity for liver, pancreas, heart, skeletal muscle, and spleen was 0.2 ± 0.1, 0.9 ± 1.0, 6.0 ± 0.9, 4.3 ± 0.7, and 0.05 ± 0.06 fold normal, respectively. In the low-dose cohort, the average β-gal activity for liver, pancreas, heart, skeletal muscle, and spleen is 0.9 ± 0.4, 0.5 ± 0.7, 13.7 ± 10.3, 3.3 ± 3.6, and 1.1 ± 0.3 fold normal, respectively. The vector distribution of the treatment was measured, and the vector was present in the treated cohort showing that the treatment reached the desired targets for treatment. The brain and spinal cord values are still being determined.

Conclusions. This study demonstrated the effectiveness of IV treatment of GM1 gangliosidosis with AAV gene therapy. AAV gene therapy was effective in restoring β-gal in both dosages, however, the two dosage cohorts improved enzyme activity levels differently in the peripheral organs. The high dose treatment would increase activity more than the low dose in some tissues, and in others, the low dose treatment was more beneficial to raising activity levels. Also surprising was that in general, the low dose cohort had the greatest increase in survival and quality of life. The vector distribution of the treatment was measured, and the treatment expression in the peripheral organs was successful.

Acknowledgements. This study was funded by Porter's Fund, private donations, and the Scott-Ritchey Research Center.

P5 Age-Related Changes in Tau and Beta-Amyloid in 3xTg Mice: Implications for Alzheimer's Disease Pathology

Henry Limbo[1], Vander LeKites[1], Natasha Grabau[1], Daniel Kroeger[1]
[1]Department of Anatomy, Physiology, and Pharmacology, College of Veterinary Medicine, Auburn University, AL

Introduction. Alzheimer's disease (AD) affects an estimated 6.7 million individuals aged 65 and older in the United States. At the molecular level, AD is marked by the accumulation of beta-amyloid (Aβ) and tau proteins, which contribute to neurodegeneration. While traditional research has focused on the role of insoluble Aβ and tau aggregates in AD pathology, recent studies suggest that their soluble forms which are present earlier in AD pathology may also play a critical role in disease progression. In this study, we will investigate the age-related changes in these biomarkers using the 3xTg mouse model of AD.

Methods. We will quantify changes in soluble and insoluble tau and Aβ levels in 3xTg and control mice at 3, 8, and 13 months using western blotting (WB) and immunohistochemistry (IHC). WB will provide semiquantitative data on overall levels and molecular weights of tau and Aβ species in the hippocampus. IHC will examine the localization of tau and Aβ in various brain regions across ages. Antibodies targeting soluble, insoluble, and total tau and Aβ will be used in both WB and IHC.

Results. We hypothesize that aging will be associated with an increase in soluble and insoluble tau and Aβ. We postulate that while both tau and Aβ species will increase with age, older brains will be more enriched in the insoluble aggregates. Finally, as well as aggregating in the hippocampus, we expect tau and Aβ to accumulate in regions of the brain vital in regulating sleep and wake based on previous studies showing disturbed sleep in individuals with AD.

Conclusions/Future Directions. Given the bidirectional relationship between sleep and Alzheimer's disease (AD), where AD-related protein aggregation impairs sleep, leading to further tau and Aβ accumulation, we plan to investigate whether enhancing sleep can reduce their buildup. First, we will enhance sleep in 3xTg mice by stimulating sleep-active GABAergic neurons in the ventral tegmental area (which we have already shown are implicated in promoting sleep). Following sleep interventions, we will assess cognitive function through behavioral tests and use WB and IHC to evaluate how sleep impacts AD progression. These results could inform potential AD treatments in humans.

P6 Generation and Characterization of Canine Tumor-Associated Macrophages

Nathan Newman[1], Alana Kramer[1], Jonathan Marable[1], Deepa Bedi[2], Maninder Sandey[1]
[1]Department of Pathobiology, College of Veterinary Medicine, Auburn University, AL
[2]Biomedical Sciences, College of Veterinary Medicine, Tuskegee University, AL

Introduction. Cancer immunotherapy is a promising new field of research that utilizes the patient's immune system to target cancer cells. However, cancer cells often induce an immunosuppressive tumor microenvironment (TME) that helps them avoid detection and subsequent targeting by the body's immune system. One such mechanism is recruiting M2 macrophages to the site of the malignancy. There are two broad types of macrophages, namely M1 (pro-inflammatory) and M2 (anti-inflammatory). M2 macrophages release cytokines and growth factors, such as interleukin-10 and transforming growth factor-β (TGF-β), to inhibit the function of cytotoxic T cells and natural killer (NK) cells, thereby dampening the antitumor immune response. In contrast, M1 macrophages, known for their pro-inflammatory and antitumor activities within the TME, play a crucial role in recognizing and eliminating cancer cells by presenting tumor antigens to T cells, generating an effective antitumor immune response. The main goal of this study is to generate tumor-associated macrophages (TAMs) from canine monocytes and to comprehensively characterize them both functionally and phenotypically.

Methods. Canine peripheral blood mononuclear cells (PBMCs) were isolated from healthy dogs, and monocytes were enriched using anti-CD14 magnetic beads via magnetic-activated cell sorting (MACS). The purified monocytes were cultured in RPMI medium supplemented with specific growth factors to differentiate into M1 (GM-CSF, IFN-γ & LPS) and M2 (M-CSF, IL-4 & IL-10) macrophages. For TAM differentiation, the monocytes were treated with tumor-conditioned media (TCM) derived from canine melanoma cells and supplemented with M-CSF, IL-4 & IL-10. The resulting M1, M2, and TAM populations were characterized based on expression of various immunomarkers, including CD14, CD206, MHCII, CD163, CD80, and CD86. Additionally, gene expression profiles of M1, M2 and TAMs were assessed through quantitative PCR.

Results. Monocytes with high purity were successfully purified from the peripheral blood using MACS. Murine and human M2 and TAMs are defined by their co-expression of CD163 and CD206. Canine M2 and TAMs showed a similar high level co-expression of CD163 and CD206, while no such co-expression was detected on M1 macrophages.

Conclusions. We have successfully developed an isolation and differentiation protocol to make canine M1, M2 and TAMs macrophages. In our future studies, we will evaluate the effect of CD206-targeted peptides on canine M2 and TAMs compared to M1 macrophages.

Acknowledgments. Rie Watanabe, Flow Cytometry and High-Speed Cell Sorting laboratory.

P7 Sexual Dimorphism of the Glucose Transporter GLUT1 in THC Exposed Rat Placentas

Carly Parker[1], Cristine Camp[1,] Adrian Courville[2], Miranda Reed[2], Rachel West[1]
[1] Anatomy, Physiology, Pharmacology Department, College of Veterinary Medicine, Auburn University, AL
[2] Department of Drug Discovery & Development, Harrison School of Pharmacy, Auburn University, AL

Introduction. The growing prevalence of recreational marijuana use has corresponded with an increase in prenatal exposure to cannabinoids through maternal consumption. Prenatal delta-9-tetrahydrocannabinol (THC) exposure has been linked to low birth weight; however, it is unclear if THC affects male and female fetuses and placentas differently. Previous research describes THC exposure affecting the expression of the glucose transporter protein 1, GLUT1, in the placenta. Insufficient levels of GLUT1 can reduce glucose availability, contributing to the pathogenesis of preeclampsia and intrauterine growth restriction. Our study aims to investigate the sexual dimorphism in GLUT1 production in male and female placentas exposed to THC.

Methods. Pregnant dams began exposure to vaporized doses of 100 mg/mL THC or polyethylene glycol (PEG), the vehicle control. Daily dosing began on gestational day (GD) 5 and continued until GD18. On GD19, dams were sacrificed and fetuses and placentas were collected. Placental tissue was homogenized in RIPA buffer then 20-30 µg of protein was used for gel electrophoresis and transferred onto a PVDF membrane. The membranes were blocked, followed by an overnight incubation in a primary antibody solution including antibodies for GLUT1 and Glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Membranes were washed then incubated for an hour in secondary antibodies. The membranes were imaged using a Licor Odyssey scanner and analyzed for relative protein concentration. The abundance of GLUT1 protein was normalized against the relative density of GAPDH housekeeping control.

Results. In both the vehicle and THC-exposed placentas, GLUT1 protein levels were significantly higher in female placentas compared to male placentas in ($p < 0.05$). However, there were no significant differences in GLUT1 levels between vehicle female placentas and THC-exposed female placentas. There was a modest yet not statistically significant increase in GLUT1 of male THC exposed placentas compared to vehicle control placentas ($p = 0.0647$).

Conclusions. The difference in GLUT1 expression in male and female placentas suggests inherent differences in glucose transport between male and female placentas. This difference remains present when pups are exposed to chemical stressors, such as marijuana. THC-exposed male placentas exhibited higher GLUT1 levels compared to the vehicle males, albeit not significantly. This suggests that THC potentially affects GLUT1 expression in male placentas. In contrast, there was no significant difference in GLUT1 levels between vehicle female placentas and those exposed to THC, suggesting that GLUT1 in female placentas is not affected by THC exposure. Future work will focus on better understanding if other metabolic pathways are influenced by sex or THC exposure in the placenta.

Acknowledgments. We would like to acknowledge Vicky Caravaggio in the West Lab and the lab members of the Reed lab for their support on this project. We also acknowledge our funding sources, Auburn startup funds to Dr. West and NIH/NIDA R01 DA046723 to Dr. Reed.

P8 Investigating the tumor promoting characteristics of the STK11 and MSK1 genes in canine mammary cancer

Caroline G. Parrish, Deepika Goyal, and R. Curtis Bird
Department of Pathobiology, College of Veterinary Medicine, Auburn University, AL

Introduction. MSK1 and STK11 are key regulatory genes in the signal transduction cascade controlling cell proliferation in cancer cells. The open reading frames of the genes encoding MSK1 and STK 11 expressed in mammary cancers of domestic dogs, *Canis lupus familiaris*, were analyzed to find highly conserved sequences for designing an rtPCR reaction that can specifically amplify the targeted region of each of the coding sequences, as well as using gel electrophoresis and to access expression levels in each cell line. Then, Sanger sequencing was employed to verify the results and gene identity.

Methods. To optimize the PCR protocol for amplifying the targeted sequences, rtPCR primers were created by using the genomic sequences of the MSK1 and STK11 genes in *Canis lupus familiaris*. The canine genome was then analyzed to identify primer pairs also conserved in *Homo sapiens* that were within the protein coding sequence and spanned an intron-exon junction. Once the successful primers were synthesized, they were utilized in an rtPCR reaction to find the targeted sequences. Canine mammary tumor cell lines CMT12, 27, 28, and 47 were cultured and the RNA extracted to use with each of the primers to access expression levels among different cell lines. A fibroblast cell line was also used for comparison against the canine mammary cell lines. The different cell lines had varying amounts of RNA added to show levels of expression of the genes. The rtPCR protocol was optimized by analyzing the amount of RNA and $MgSO_4$ added to each reaction, and then changing these amounts based on the results obtained when the bands were observed through non-denaturing agarose gel electrophoresis. Additionally, DMSO was added to the reaction to improve the specificity of primer annealing. The annealing temperatures of the primers were also adjusted accordingly by increasing incrementally by 0.5° C along with cycle number to optimize amplification. Gel extraction along with DNA precipitation was performed to prepare samples for Sanger sequencing. The results were then further analyzed by comparing the sequences observed to target sequences expected.

Results. The successful cell culturing of the canine mammary tumor cell lines that would be used to extract RNA, was done through careful passage and allowing the cells to go through a few passages before extracting the RNA at 80% confluency. This allowed us to obtain a relatively high concentration and purity of RNA to be used in rtPCR reactions. Reaction parameters were optimized for the rtPCR reaction. When the $MgSO_4$ concentration was changed from 1 mM to 1.5, this resulted in a brighter and sharper band when analyzed. The addition of DMSO, at just 1 µL/reaction allowed the primers to be more specific in their binding. The theoretical length determined from the sequence of the MSK1 gene primers was 400 bp and 298 bp, and for STK11 genes was 398 bp, 297 bp, and 297 bp, respectively, in length. The gel electrophoresis results showed this length of nucleotides for each gene sequence, and further Sanger sequencing results verified gene identity.

Conclusions. The primers chosen for each of the respective genes were analyzed and sequenced showing the expression of each gene in the different canine mammary cell lines.

Acknowledgment. We thank AURIC, Auburn University's Department of Undergraduate Research, the College of Sciences and Mathematics, and the College of Veterinary Medicine for funding and support.

P9 Pig-specific Contraceptives

Nathaniel Pike[1], and James W. Gillespie[1]
[1]Department of Pathobiology, College of Veterinary Medicine, Auburn University, AL

Introduction. Bacteriophages or "phages" have been engineered to display species-specific peptides that can stimulate production of anti-sperm antibodies. A barrier for bacteriophagemediated immunocontraceptive technologies is effective delivery of vaccine products to wild pigs in a field environment. Preliminary work has been done indicating that this can be achieved in wild pigs with the ultimate goal of contraception and population control. As this technology is planned to be delivered in an environment with many variables, the delivery system must be able to withstand various rapidly changing variables in the field. This study attempts to address that challenge by developing chitosan encapsulated bacteriophage particles for oral delivery. Here we sought to determine the size and shape of these particles and the effectiveness of encapsulation.

Methods. Chitosan nanoparticles were created using an ionic gelation method using sodium tripolyphosphate (STPP) as a crosslinker. Chitosan was dissolved at 5 mg/mL in 1% acetic acid. Nanoparticles were formed by addition of sodium tripolyphosphate with stirring at 150 rpm. Particles containing phages were prepared using the same conditions and further diluting a phage stock into the chitosan mixture. Following particle formation, aggregates were removed by centrifugation at 12,000 ´ g and supernatants were collected. Nanoparticles were characterized for their size and encapsulation efficiency. Size was determined by dynamic light scattering (DLS) on a Malvern ZetaSizer Nano ZS90. Samples were diluted 100-fold in ddH_2O, pH 7.0 and analyzed in triplicate to calculate a mean size. A biological titer of phages from the input sample and those remaining unencapsulated in the supernatant of the nanoparticle mixture were determined by infection into *E. coli* cells. Encapsulation efficiency was calculated as the recovery of phages titered after nanoparticle formation compared to the total input of phages.

Results. Chitosan particles were successfully prepared using the ionic gelation method using common reaction conditions as determined by an increase in sample turbidity with added crosslinker. Analysis of the nanoparticle solution without added phages, revealed that the size of the particles was approximately one micron in diameter and precipitated in solution. Addition of bacteriophages significantly decreased the particle diameter to ~150 nm. Titering of the supernatant collected from these nanoparticles produced no colonies and showed that all phages had been encapsulated successfully.

Conclusions. We demonstrate that nanoparticles encapsulating bacteriophages could be created using an ionic gelation method. These particles encapsulated phage at a high rate and met ideal size standards for uptake by immune cells. Further studies are planned to further test parameters for both nanoparticle preparation and encapsulation of phage.

Acknowledgments. Research was supported by a Research Support Program (RSP) award from the Office of the Vice President of Research & Economic Development (VPRED) to JWG.

P10 Construction of Recombinant Hyr1 Vaccine Preparations for Induction of Hyr1-specific Antibodies

Nicholas D. Renfree[1], Laura Huber[1], and James W. Gillespie[1]
[1]Department of Pathobiology, College of Veterinary Medicine, Auburn University, AL

Introduction. Antimicrobial resistance (AMR) is one of the greatest threats to human health and one of the top health challenges facing the 21st century. Discovery and development of new antimicrobial drugs have failed to deal with the significant threat posed by AMR. Therefore, new alternatives to treat patients who are infected with multiple MDR pathogens are greatly needed. In this study, we focus on a specific epitope of the *Candida albicans* Hyr1 protein that has structural homology to other bacterial proteins and want to induce expression of specific antibodies for that epitope to provide an alternative treatment option for infections with *Candida albicans*. To create the epitope-specific antibodies, we must immunize an animal model, such as mice, with the specific protein or epitope we plan to generate polyclonal antibodies towards. Here, we produced a recombinant Hyr1 protein in yeast cells that could subsequently be used for immunization of mice to stimulate production of polyclonal Hyr1-specific antibodies.

Methods. Genomic DNA was isolated from *Candida albicans* SC5314 cultures using the Zymo Research Quick-DNA Fungal/Bacterial Miniprep Kit. We performed a polymerase chain reaction with Hyr1-specific primers to amplify the Hyr1 gene from the *C. albicans* template gDNA. Gel electrophoresis was used to confirm the Hyr1 gene of interest was successfully amplified and purified from genomic DNA. The purified DNA fragment was digested with NotI/XbaI restriction endonucleases and inserted into a pPICZalpha yeast expression plasmid from the EasySelect Pichia Expression Kit. Purified plasmids carrying the Hyr1 gene were transfected into *P. pichia* yeast cells and transformants selected where the gene was inserted into the yeast genome. Transformed cells were cultured and protein expression induced with methanol to produce Hyr1 protein. The recombinant Hyr1 protein was purified by affinity chromatography. A nickel resin was used to bind a histidine tag that was fused to the rHyr1 protein. Bound proteins were eluted and collected for analysis by SDS-PAGE.

Results. Genomic DNA was isolated from *C. albicans* cells, the Hyr1 gene cassette amplified by PCR using Hyr1-specific primers and purified from contaminating sequences by Qiagen PCR Purification Kit. The Hyr1 gene cassette was inserted into pPICZ plasmids as above and 10 *E. coli* colonies were screened for correct sequence and orientation. Plasmids were linearized and transfected into *P. pichia* yeast cells before plating on selective agar plates. Eight colonies were screened by SDS-PAGE for stable incorporation of the Hyr1 gene into the yeast genome and expression of recombinant Hyr1 protein. Only one of the eight screened colonies produced a protein band with the expected molecular weight product and was selected for larger scale culture and purification by affinity chromatography.

Conclusions. We successfully prepared recombinant Hyr1 protein from *P. pichia* cells that can be administered to mice and stimulate an Hyr1-specific antibody response. After isolation of Hyr1 proteins, we plan to immunize mice with recombinant Hyr1 proteins to stimulate production of Hyr1-specific antibodies, followed by a challenge with a lethal dose of *Candida albicans* to test if the Hyr1-specific antibodies can fight off the *Candida albicans*.

Acknowledgments. We thank the Department of Pathobiology and the Office of Research and Graduate Studies (ORGS) for seed funding.

P11 Novel Anti-Luteinizing Hormone Antibodies and Their Biological Effects in Mice

Gabrielle C. Schultz[1], Arthur D. Zimmerman[1], Malia A. Walton[1], Johanna Ehrhardt[1], Anniston E. Dodson[1], Emma E. Hruska[1], Aime K. Johnson[1,2] and Douglas R. Martin[1,3]
[1]Scott-Ritchey Research Center, Auburn, AL
[2]Department of Clinical Sciences, Auburn University College of Veterinary Medicine, Auburn University, AL
[3]Department of Anatomy, Physiology and Pharmacology, Auburn University College of Veterinary Medicine, Auburn University, AL

Introduction. Luteinizing hormone (LH) is a glycoprotein hormone secreted from the anterior pituitary by gonadotrophin cells, and is an important part of the hypothalamic-pituitary-gonadal (HPG) axis. LH plays a significant role in maturation of primordial germ cells, and in men, activates Leydig cells of the testes to produce testosterone. In women, LH stimulates steroid release from the ovaries which helps to regulate the menstrual cycle, ovulation, and possible implantation of an egg. Dysregulation of LH levels can lead to a variety of complications including low testosterone/estrogen, irregular menstrual cycles, infertility, and early or delayed puberty. Additionally, elevated LH levels in both men and women can increase the likelihood of developing dementia, specifically Alzheimer's disease (AD). In the current study, we examine the use of an adeno-associated virus (AAV)-mediated anti-LH antibody treatment for reducing LH levels in normal (CD1) mice. Treatment with anti-LH antibodies significantly disrupts estrous cyclicity, alters levels of LH in serum, and affects LH receptor (LHR) immunoreactivity within different tissues. Overall, AAV-mediated anti-LH antibody treatment displays potential as a novel therapeutic for a wide variety of applications.

Methods. 3-month old CD1 mice were treated intravenously with 6.0×10^{13} vector genomes (vg)/kg of one of four AAV-mediated anti-LH antibodies. Mice estrous cyclicity, serum LH levels and LHR immunoreactivity within the brain and uterus were assessed. Mice estrous cyclicity was determined by daily vaginal cytology, serum LH levels were measured using a chemiluminescent immunoassay (CLIA), and LHR immunoreactivity was measured using immunohistochemistry (IHC).

Results. Results show disrupted estrous cyclicity in several treatment groups. In general, mice treated with AAV-anti-LH antibodies spend increased time in estrus as compared to control-treated mice. In most treatment groups, mice have lower serum LH levels by 4 months post-treatment as compared to control-treated mice. Lastly, mice treated with either 6C10D8 or 1B7H11 anti-LH antibodies have decreased LHR immunoreactivity within the hypothalamus and within the uterus.

Conclusions. In conclusion, treatment with anti-LH antibodies leads to biologically relevant alterations in serum LH levels, estrous cyclicity, and LHR immunoreactivity. Future studies are necessary to validate and characterize these antibodies further and to determine which antibody would be most effective therapeutically and in larger animal species. Both 6C10D8 and 1B7H11 are strong candidates as effective anti-LH antibodies as they are both highly distributed and have long-lasting effects when delivered using an AAV9 vector.

Acknowledgments. We acknowledge financial support from the Scott-Ritchey Research Center.

Veterinary Student Poster Presentations

P12 Demonstrating effective odor capture of invasive species volatiles using polymer-based training aids for canine pest detection

Sydney De Lorenzo[1], Samantha Hagerty[1], Melissa Singletary [1,2]
[1]Department of Anatomy, Physiology, and Pharmacology, Auburn University CVM, AL
[2]Canine Performance Sciences, Auburn University CVM, AL

Introduction. The Asian Long horned Beetle (ALB) is a highly invasive and damaging pest as it is estimated to cost $70 billion dollars in damage every year worldwide. The dispersal habits of the ALB are invasive, as one study found that 98% of ALB were recaptured 560m away from original site. The Cooperative ALB Eradication program administered by state, federal, and local governments estimated that trees at least 1,500km from known infestations need to be surveyed. The USDA has determined that detection dogs are vital to pest eradication efforts as they improve efficiency and efficacy of pest detection compared to human visual identification. However, there are limitations in training due to access to materials, specifically ALB frass, or the excrement from the beetle produced at multiple life stages. However, different life stages of the ALB have different odor profiles, which can impact detection performance. The goal of the current study is to determine the comparative headspace composition of target (ALB) and distractor materials in their raw form and after being charged onto Polymer odor capture and release training aids (POCR™).

Methods. Samples collected and used for the study included live ALB frass (target odor) and distractor odors. Distractors included frass from softwood maple trees, novel frass from hardwood maple trees, and bark collected from softwood maple trees. POCR™ were prepared using each sample. The headspace of each sample was analyzed using Proton Transfer Reaction-Mass Spectrometry. PTR-MS is a method of direct, real-time headspace sampling based on proton affinity of reacting volatiles. Principal Component Analysis of the measured spectra was conducted to determine sample relatedness and a common odor fingerprint for further quantification of signal intensity and variance between groups.

Results. Spectral data demonstrated consistent odor profiles measured for each sample group. While these representative spectra included distinguishable peaks, there was also a heavy overlap between groups corresponding to host species (maple tree). Factor analysis demonstrated that, among all groups, the frass collected from a different variety of maple tree (hardwood) was most distinct. There is also peak variation seen between ALB frass collected from the same location at different times of the year (March and June), indicating possible temperate and life cycle influence on frass emissions from the ALB. Intragroup comparison between live and POCR™-captured headspace indicated effective transfer of odor signature to the training aids with signal intensities that were, on average, 1.77-fold higher than live material.

Conclusions. Volatile fingerprints were found to be associated with each training material. ALB-specific volatiles were identified and further analyses of frass collection from different seasons or life cycles may reveal additional information. The peaks found in live material for each source were also found in charged POCRs™, demonstrating effective transfer of the odor profile. The alignment between the distractor odor profile and the target confirmed our choice in distractors, as it challenges the dogs to distinguish between very similar volatile odor compounds, making the more successful in field operations.

Acknowledgments. Student Support: Boehringer Ingelheim and Auburn University Canine Performance Sciences Project Funding: United States Department of Agriculture Animal and Plant Health Inspection Service Grant G00016067

P13 The Impact of the Non-Nutritive Sweetener Allulose on the Gut Microbiome and Metabolic Syndrome

Courtney Dunning[1], Katti Crakes[1], Jotham Suez[1]
[1]W. Harry Feinstone Department of Molecular Microbiology & Immunology, Bloomberg School of Public Health Johns Hopkins University, Baltimore, MD, U.S.A.

Introduction. In recent years, artificial sweeteners have become ubiquitous in the modern diet, often marketed as a healthier alternative to sugar for weight management and diabetes control. Emerging research suggests that these sweeteners, such as sucralose and saccharin, may have unintended effects on the gut microbiome, potentially leading to metabolic disruptions, including insulin resistance, glucose intolerance, and increased risk of obesity. A new sweetener, Allulose, has been approved by the FDA since 2015 and comes from a natural source unlike other sweeteners. Because of Allulose being a natural non-nutritive sweetener (NNS), will its impacts on the gut microbiome differ from those like saccharin and sucralose or will it lead to the same defects regarding metabolic health?

Methods. For this project, both in vivo and in vitro methods were used to investigate the impact of non-nutritive sweeteners (NNS) on the gut microbiome. For in vivo experiments, C57BL/6J mice received water (n=10) or water supplemented with sucralose (n=10), saccharin (n=10), or allulose (n=10) and a concurrent high-fat diet over 12 weeks. Stool samples were collected weekly for microbiome analysis and body weights, food intake, and glucose tolerance was monitored periodically. In vitro experiments included the effects of NNS in cultured ex vivo murine-derived mixed microbiome community grown in Bacteroides medium in aerobic conditions. In addition, an aerobic sole carbon source screen was performed on several bacterial strains that were inoculated into a minimal medium (M9) with supplemental glucose, sucralose, saccharin, or allulose as a sole carbon and substrate for bacterial growth. Growth of bacteria (OD 600nm) was measured over 10 days.

Results. Saccharin and allulose were found to modify the microbiome of C57BL/6J mice five weeks after supplementation with non-nutritive sweeteners, compared to a water control. Allulose, similar to saccharin, also demonstrated the ability to inhibit the growth of a murine-derived mixed microbiome community in vitro under aerobic conditions. While common gut bacteria did not proliferate in the presence of sucralose or saccharin in minimal medium, *Lactobacillus rhamnosus, Lactobacillus reuteri and Enterococcus faecalis* exhibited increased growth when allulose was present, possibly utilizing it as a sole carbon source.

Conclusions. Although some strains of bacteria were able to utilize allulose as a sole carbon source, other experiments, such as the murine-derived mixed microbiome community screen, showed great inhibition of growth when exposed to allulose in vitro. These contrasting results show that more studies are needed to evaluate the effects of allulose on the microbiome. Also, given that the microbiome findings are preliminary, additional research and replicative studies are necessary to further elucidate the impact of non-nutritive sweeteners on gut microbiome composition and metabolic health.

Acknowledgments. NIH Training Veterinary Students for Career in Biomedical Research - 5T35OD024982

P14 Retrospective Analysis of Hypertrophic Pododermatitis Treated Across Multiple Referral Institutes

Micah Goode[1], Lidnesey Boone[2], Erik Hofmeister[2]
[1] DVM Canadite 2027 Auburn University College of Veterinary Medicine
[2] Department of Equine Surgery, Auburn University College of Veterinary Medicine
[3] Department of Anesthesia, Auburn University College of Veterinary Medicine

Introduction. Hypertrophic Pododermatitis is a disease that causes hypertrophy of the horn-producing tissue mostly in the frog region. There is no known etiological agent for Hypertrophic Pododermatitis and predisposing factors are highly debated. There is also no definitive treatment for the disease. Our goal with this study is to look into how different institutes' cases present, are treated, and diagnosed.

Methods. All cases within the last 10 years were pulled from LSU and Auburn Veterinary Teaching Hospitals. Signalment, all diagnostic methods, length of stay, treatments, success rates, and number of relapses were all pulled from the record. Descriptive statistics will be used to evaluate the effectiveness of each method of treatment and diagnosis and help declare predisposing factors.

Results. Draft horses made up 50% of cases a much higher rate than they present for other diseases. 78% presented with complaints about the canker. 28% had a definitive diagnosis with histopath. Forelimb and hindlimb had equal occurrence and multiple limbs were often affected. Surgical debridement with antibiotics is the only treatment used with surgical methods and antibiotic types showing large variations in success rates. 43% of horses had a relapse with 35% of them having more than one. Age played a large role in predisposition as well with a mean age of 13.

Conclusions. Our study agrees with a draft predisposition but not a limb disposition pointed out in other studies. An antibiotic combination of Metronidazole, copper sulfate, and oxytetracycline has proven to be the best combination to decrease recurrence. Narrow methods of treatment when compared to other studies done. The largest role in decreasing relapse rate has proven to be early treatment and daily bandage changes. The large rate of success with antibiotics provides some evidence for the disease being bacterial and not viral.

Acknowledgment. Drs. Hofmeister, Boone, and Creamer were all helpful in getting and interpreting the data.

P15 Bilateral Pyelonephritis and Suspected Uremic Encephalopathy in a New-World Camelid

Kristen Hoehler[1], Rachel Neto[1], Jessica Lambert[1], Katelyn Waters[2], Jenna Bayne[2]
[1]Department of Pathobiology, College of Veterinary Medicine, Auburn University, AL
[2]Department of Clinical Sciences, College of Veterinary Medicine, Auburn University, AL

Introduction. Pyelonephritis may manifest as acute or chronic renal failure and is typically a consequence of lower urinary tract infections. Cases of pyelonephritis in camelids are underreported, with only rare, isolated publications afflicting camels, with *Escherichia coli* and *Staphylococcus lugdunensis* as underlying etiologies. Clinical signs vary with chronicity, but commonly include lumbar pain, poly/oliguria, urinary incontinence, renal azotemia and, potentially, uremia. Uremic encephalopathy is poorly documented in veterinary species; characterized by varying neurologic signs including lethargy, recumbency, ataxia, tremors, and blindness; and histologically supported by Alzheimer type II astrocytes and white matter spongiform change.

Methods. An 18-year-old intact female llama was presented to the AULATH for urinary incontinence, ataxia, progressive hindlimb paraparesis, muscle atrophy, and weight loss. CBC and chemistry profiles showed a non-regenerative anemia, neutrophilia, and elevated creatinine. Euthanasia was elected after a 6-day hospitalization.

Results. Postmortem examination revealed severe bilateral renomegaly with multifocal pockets of suppurative and hemorrhagic exudate within the medulla and cortex. Histologically, the inflammation was centered on ducts and tubules and accompanied by myriad intralesional bacteria. Bacterial culture of the kidneys returned heavy growth of *E.coli*. Evaluation of the nervous system revealed significant astrocytic swelling throughout, comparable to Alzheimer type II astrocytes.

Conclusions. Necropsy findings, clinical presentation, and serum chemistry profile support a diagnosis of ascending bilateral pyelonephritis, likely due to chronic cystitis, with suspected uremic encephalopathy. This case exemplifies a common renal disease affecting a species that has been largely unrepresented, as well as a rare and severe sequela of chronic renal dysfunction.

P16 p-Cresol inhibits hepatocellular proliferation *in vitro*

Jessica Meier, Farnaz Yousefi, and Shaan Mooyottu[1]
[1]Department of Pathobiology, College of Veterinary Medicine, Auburn University, AL

Introduction. Epidemiologic data and our recent results indicate that *Clostridioides difficile* infection (CDI)—a serious gastrointestinal illness in patients—triggers gene expression and metabolic shifts that promote inflammation, lipid accumulation, and oxidative damage in the liver, which are critical in the development and progression of Metabolic dysfunction-Associated Steatotic Liver Disease (MASLD), although the mechanisms are unknown. Previous research and our preliminary data indicate that *C. difficile* produces a large amount of p-cresol—a toxic clostridial metabolite in the gut—which potentially mediates the hepatotoxic effect of *C. difficile*. To this end, we investigated the effect of p-cresol on the murine hepatocyte cell line AML-12. We also investigated the effect of piperazine, an FDA-approved compound that forms a p-cresol-piperazine complex, on the hepatotoxicity of p-cresol *in vitro*.

Methods. AML-12 cells were cultured in DMEM medium supplemented with 10% fetal bovine serum, 10 µg/ml insulin, 5.5 µg/ml transferrin, 5 ng/ml selenium, penicillin (100 units/ml), and streptomycin (100 µg/ml), maintained at 37°C with 5% CO2. The cells were seeded at a density of 0.3 million cells per well in six-well plates, each well containing 2 ml of the complete growth medium. Treatments included p-Cresol at concentrations of 300 µM and 600 µM (comparable to the serum p-cresol levels in mouse models of CDI), piperazine at 150 µM, and an equimolar combination of p-Cresol (300 µM) and piperazine (150 µM), with a control group receiving no treatment. Following a 48-hour incubation, live and dead cells were counted using the trypan blue exclusion method, with counts performed using a Corning Cyto cell counter and analyzed with Cytosmart software to ensure accurate quantification of cell viability and proliferation. The statistical analysis was performed using one-way ANOVA to identify overall differences among treatment groups, followed by independent two-sample t-tests to compare each treatment group against the control for live cell count, dead cell count, total cell count, and cell viability.

Results. AML-12 cells treated with 600 µM p-Cresol exhibited a significant reduction of both live and total cell counts compared to the control group, suggesting a strong inhibitory effect on cell proliferation ($p < 0.05$). The PC 300 treatment showed a trend towards reduced cell counts, but the differences were not statistically significant. The 150 µM piperazine treatment alone did not significantly affect cell counts or viability. However, the combination of 300 µM p-cresol and 150 µM piperazine significantly reduced live and total cell counts compared to the control ($p<0.05$), but not to PC 300 alone. All treatment groups maintained high cell viability percentages, above 96%, indicating that most cells remained viable despite the treatments.

Conclusions. p-Cresol at 600 µM significantly inhibits cell proliferation and reduces both live and total cell counts, while 300 µM shows a trend toward inhibition. Piperazine (PIP 150) alone does not significantly impact cell counts or viability, and the combination of PC 300 and PIP 150 significantly reduces cell proliferation compared to control but not compared to PC 300 alone. These findings suggest a concentration-dependent inhibitory effect on AML-12 cell proliferation, and the addition of piperazine binding does not significantly alter this effect.

Acknowledgments. Boehringer Ingelheim Veterinary Summer Scholars Program.

P17 Developmental toxicity of S-(1,2-dichlorovinyl)glutathione (DCVG) in zebrafish (*Danio rerio*)

Zachary R. Oestmann, Maryam Hariri, & Katharine A. Horzmann
Department of Pathobiology, College of Veterinary Medicine, Auburn University, AL

Introduction. Trichloroethylene (TCE) is a chlorinated solvent and metal degreaser that was commonly used in the mid-twentieth century. Due to inappropriate disposal, it is commonly found in groundwater, especially around National Priorities List sites. Ingestion of TCE contaminated water is known to cause developmental toxicity. TCE is rapidly metabolized in the body and TCE metabolites are linked to some of the adverse health effects. S-(1,2-dichlorovinyl) glutathione (DCVG), a phase II metabolite of TCE, is linked to kidney cancers; however, the contribution of DCVG in developmental toxicity is unknown. The goal of this study was to test the hypothesis that DCVG contributes to developmental toxicity seen in zebrafish (*Danio rerio*) exposed to TCE.

Methods. Zebrafish embryos were collected and exposed to 0, 5, 50, or 500 parts per billion DCVG (ppb; µg/L) for 120 hours post fertilization (hpf). At 120 hpf, a visual motor response test, heart rate analysis, and morphology measurements were performed to evaluate for developmental toxicity and neurotoxicity.

Results. Behavior data showed a significant increase in velocity and distance traveled for larvae exposed to 500 ppb DCVG compared to the 0 ppb controls. Heartrates were significantly increased for larvae exposed to 5, 50, and 500 ppb DCVG compared to the 0 ppb controls. Head width was also significantly decreased at 50 ppb DCVG compared to 0 ppb controls.

Conclusion. The results of this study indicate that DCVG does contribute to TCE-related developmental toxicity, contribute to the One Health initiative, and highlight the need to investigate the toxicity of metabolites for contributions to toxicity at specific target organs.

Acknowledgements. I want to thank my mentor, Dr. Katharine Horzmann, her graduate students, Auburn University CVM, Dr. Nancy Merner, and the BIVSP for this opportunity. I also want to thank the National Institute of Health for providing funding, specifically the grant: NIEHS R15 ES033361-01.

P18 Can SR9238 block hepatotoxicity in cases of chronic phenobarbital exposure: A One Health perspective

Hannah Vaughn, Sangeet Makhija, Ryan Gibson, and Kristine Griffett
Griffett Lab, Department of Anatomy, Physiology, Pharmacology, College of Veterinary Medicine , Auburn University, USA

Introduction. Phenobarbital is one of the most prescribed anti-epileptic drugs in veterinary medicine. However, during prolonged use, it can lead to liver damage as it causes a xenobiotic response. There have been no identified studies evaluating if phenobarbital-induced liver damage can be blocked. Therefore, we used a LXR (Liver X Receptor) compound, SR9238, to investigate if it can be used to block hepatoxicity. LXRα and LXRβ are nuclear receptors involved in cholesterol efflux and transport, as well as regulating lipogenesis and glucose metabolism. SR9238 is a liver specific LXR inverse agonist that works to repress certain lipogenic and inflammatory pathways. It has shown to repress hepatocellular damage and inflammation in previous ethanol and alcohol-related steatohepatitis (ASH) studies. Therefore, we hypothesized that when giving SR9238 with phenobarbital, liver damage could be reduced.

Methods. To test this hypothesis, we evaluated gene expression in mice after administering SR9238 and phenobarbital. Two groups of mice were evaluated. The first group was administered SR9238 without phenobarbital to evaluate sole function on liver tissue. The second group was administered SR9238 and phenobarbital. Liver tissues were collected, and RNA extracted. Finally, a qPCR was ran on a BioRad metabolic plate. This allowed us to compare certain genes involved in the metabolism of phenobarbital and compare expression to control groups, with the goal to find a way to block toxic metabolites.

Results. Aldh1a1 and Cyp2e1 are the genes responsible for alcohol metabolism. In both male and female mice, we found that SR9238 increased these genes in the liver, suggesting that alcohol and related derivatives would be metabolized quicker with the LXR compound.

Cyp3a4, Cyp1a1, and Cyp3a11 are also involved in metabolism of phenobarbital and other drugs. We found significant differences in basal expression in male and female mice, as well as with those treated with the LXR compound. This suggests that dosing of phenobarbital may need to be adjusted depending on sex.

Conclusions. SR9238 increased many inducible enzymes involved in drug metabolism, which will hopefully lead to increased clearance of the drug. Decreased enzymes involved in lipid biosynthesis could decrease fatty liver and progression into liver disease. There also is a difference between male and female gene expression that needs to be investigated further. Since SR9238 is liver specific, we do not believe it will interfere with phenobarbital therapy in the brain. The data generated from this phenobarbital study can also provide proof-of-concept for the coadministration of LXR compounds to combat opioid-induced liver disease due to metabolic pathway similarities.

Acknowledgments. The authors would like to thank the remainder of the Griffett lab and the Anatomy, Physiology, and Pharmacology department at Auburn University College of Veterinary Medicine. NIH: R01NS126204.

P19 Antimicrobial activity of canine platelet lysate against *Staphylococcus in vitro*

Meghan Yaffa[1], MelikaSadat Mollabashi[2,3], Scarlett Sumner[1], Maria Naskou[2,3]
[1]Department of Clinical Sciences, College of Veterinary Medicine, Auburn University, AL
[2]Department of Pathobiology, College of Veterinary Medicine, Auburn University, AL
[3]Scott Richey Research Center, Auburn University, AL

Introduction. Antimicrobial resistance is a pressing issue in both veterinary and human medicine today. The rise of resistant organisms has led to significant economic consequences as well as increased morbidity and mortality. Platelets and their derivatives offer a potential additional wound treatment with antimicrobial properties. In additional to their role in hemostasis, platelets, provide benefits for wound healing and treatment by promoting cell migration, keratinocyte epithelization, and regulating fibroblast matrix deposition. Platelet lysate (PL), an acellular product manufactured from platelet conentrates such as platelet rich plasma, contains high concentrations of growth factors and cytokines. Moreover, PL and other platelet derived products from various species have shown antimicrobial properties *in vitro and in vivo.* In this study, we hypothesized that canine PL would have a dose-dependent effect against *Staphylococcus (S.) aureus* and *S. pseudintermedius*, as well as a synergistic effect with oxacillin and amikacin.

Methods. Canine PL was manufactured from whole blood using a manual centrifuge and freeze-thaw technique. Bacterial growth curve assays were performed using Biotech Gen5 Software for each bacterial strain. The treatment groups applied to each bacterial strain included saline, PL, and platelet-poor plasma (PPP), each tested at 20% and 80% concentrations. Oxacillin and amikacin were tested with and without PL and PPP.

Results. PL and PPP suppressed the growth of *S. aureus* at 20% and less so at 80%. The converse was true against *S. pseudintermedius*; PL and PPP suppressed the growth at 80% and less so at 20%. A greater bacterial suppression was noted with PL compared to PPP for both bacteria at 80%. PL and PPP appeared synergistic with amikacin against *S. aureus*. PL and PPP decreased the effectiveness of oxacillin at both 20% and 80% against *S. aureus* and at 80% against S. pseudintermedius.

Conclusions. PL shows antimicrobial effects in a dose-dependent manner, which may differ based on the bacteria being treated. PL showed synergism with amikacin and does not appear synergistic with oxacillin against susceptive *Staphylococcus.* Further research is warranted to evaluate the optimal use of PL as an antimicrobial.

Acknowledgments. This research was funded by The Scott Fund and The Swaim Fund for Excellence in Wound Healing, Scott-Richey Research Center, College of Veterinary Medicine, Auburn University. Student support was funded by Boehringer Ingelheim Veterinary Scholars Program and Auburn College of Veterinary Medicine.

Graduate Student Poster Presentations

P20 Environmental antimicrobial resistance threats to pollinators

Kiranmayee Bhimavarapu[1], Pankaj Gaonkar[1], Alinne L. R. Santana-Pereira[1], Geoffrey R. Williams[2], Camila Rodrigues[2], Andre L.B.R. da Silva[2], Laura Huber[1]
[1]Department of Pathobiology, College of Veterinary Medicine, Auburn University, AL
[2]Department of Clinical Sciences, Auburn University, AL

Introduction. Antimicrobial use in food animals contributed to the emergence of Antimicrobial Resistance (AMR) in the environment. Honey and pollen have been shown to be reliable bioindicators of environmental AMR. However, the interconnectedness of antimicrobial use in animals, AMR environmental contamination and the impact on pollinator health is unknown. This study aims to infer the mechanisms of AMR spread from the environment to its pollinator, and the risk of pollinator disease due to exposure to AMR following the use of composted or fresh poultry litter as a fertilizer.

Methods. In this controlled semi-field experiment, two high tunnel greenhouses were divided into 3 subsets. Yellow squash was planted and fertilized with either fresh or composted poultry litter. *Apis mellifera* honeybee colonies were introduced when squash plants flowered. Soil, pollen and honeybee samples were collected 5 times within a period of 56 days. The relative concentration of antimicrobial genes (ARGs), and mobile genetic elements (MGEs) in all samples were compared between groups using qPCR. Bees were screened for the presence of pathogenic and opportunistic organisms. The metagenome of soil and bee samples from 3 time points were analyzed.

Results. Preliminary analysis revealed higher levels of ARGs and MGEs in fresh litter when compared with compost. Metagenomic analysis showed a significant difference in the microbiome composition in the foraging bees exposed to fresh litter vs compost. *Bartonella*, a microorganism commonly found in bees but not part of the core bee microbiome, is more prevalent in bees exposed to fresh litter than in bees that were exposed to compost. Dysbiosis was observed following exposure of bees to the environment fertilized with poultry waste which has contributed to the increase of the opportunistic pathogens. ARGs richness also increased in soil and bees exposed to litter than those exposed to compost. Tetracycline, polymyxin and beta-lactam were the most abundant genes observed in soil and bees.

Conclusions. Fertilizing crops with either fresh poultry litter or compost contaminates the environment with AMR. Foraging bees in contact with these fertilized crops experience impact in their overall microbiome, and consequently, an increase in opportunistic organisms that could cause disease and mortality. The overlap between soil and bee ARGs indicates horizontal gene transfer across environments. Our future studies will investigate the direct transmission of ARGs from fertilized soil to pollen and honeybees through whole genome sequencing of shared organisms. We will also explore the direct effect of antibiotic residues on AMR emergence and spread.

Acknowledgments. Huber lab, AU Bee lab, E.V. Smith experimental farm. This research is funded by Animal Health & Disease Research Funds, Research and Graduate studies and the Agricultural Experiment Station, Auburn University, Alabama.

P21 Interferon beta stimulation of 3D trophoblast spheroids

Victoria T. Caravaggio[1], Cristine R. Camp[1], and Rachel C. West[1]
[1]Department of Anatomy, Physiology, and Pharmacology, College of Veterinary Medicine, Auburn University, AL

Introduction. During pregnancy, the placenta has a uniquely critical role in protecting the fetus from infection and inflammation. The syncytiotrophoblast (STB) layer of the placenta is the first line of defense with powerful antimicrobial and antiviral activity. The placenta can mount a robust type-I interferon response, providing a rapid antiviral response by activating interferon-induced transmembrane proteins (IFITMs), and interferon stimulated genes (ISGs). In this study, we aimed to use a physiologically relevant 3-dimensional (3D) trophoblast stem cell (TSC) spheroid model of the placental innate immune response. Our spheroid model allows the cells to recapitulate their microenvironment and proliferate and organize as would be seen in natural growth conditions. The aim of this study was to evaluate the ability of the syncytiotrophoblast cells to mount an immune response in response to interferon beta stimulation in a 3D spheroid model.

Methods. TSCs were used to generate undifferentiated 3D TSC or differentiated STB spheroids. Spheroids were grown in either TSC stem or STB differentiation culture media conditions. TSC spheroids were kept in 3D conditions for 24 hours while STB spheroids were kept in differentiation conditions for 72 hours. Spheroids were then treated with 250 ug/mL of interferon beta (IFNB) for 8 hours. After 8 hours RNA was isolated, and cDNA synthesized. RT-qPCR was used to quantify relative levels of several interferon stimulated genes. A one-way ANOVA followed by a Tukey's multiple comparison test was used to determine statistical significance ($p < 0.05$).

Results. We evaluated mRNA levels of the interferon-induced transmembrane proteins *IFITM1*, *IFITM2*, *IFITM3*, the interferon stimulated genes *ISG15* and *ISG20*, and interferon beta *INFb1*. There were significantly higher mRNA levels of *ISG15* and *ISG20* in spheroids stimulated by INFB (*ISG15,* $p<0.01$), with the highest expression levels in the differentiated STB spheroids compared to unstimulated controls (*ISG15,* $p<0.0001$) (*ISG20,* $p<0.01$). A similar expression pattern was observed for *IFITM2* ($p<0.01$) and *IFITM3* ($p<0.01$), with the highest expression in the stimulated STB spheroids ($p<0.01$). The one exception to this pattern was in *IFITM1* with the highest expression in the INFB stimulated TSC spheroids ($p<0.001$), followed closely by the stimulated STB spheroids ($p<0.05$).

Conclusions. The significant difference in gene expression between the undifferentiated TSC spheroids and differentiated STB spheroids is evidence of the important immunological protection that the STB cells provide in the placenta. The robust increase in mRNA levels of interferon stimulated genes after immune stimulation in the STB spheroids indicate that the STB layer readily responds to IFNB, whereas the TSC spheroids lack the same ability to mount a strong immune response. These experiments reveal that the development of the TSCs into the STB layer confers the initiation of the type-I interferon response when stimulated. These findings provide evidence that our 3D spheroid model is a suitable model to mimic and study the placental immune response.

Acknowledgements. This project was funded by the American Society for Reproductive Medicine Ky Cha Award in Stem Cell Technology and Auburn University startup funds.

P22 Comparative Analysis of Expression of NECTIN1 in Canine Mammary Tumors

Deepika Goyal, Caroline G. Parrish, and R. Curtis Bird
Department of Pathobiology, College of Veterinary Medicine, Auburn University

Background. Nectin1 is a member of the Nectin family of $Ca2^{+}$ independent immunoglobulin-like cell adhesion molecules. The members of the Nectin family participate in various cellular activities such as cell differentiation, migration, proliferation, and survival. In general, members of the Nectin family have three domains: extracellular containing three Ig-like loops with one V-like and two C-like domains, one transmembrane region, and one cytoplasmic tail. These Nectin molecules help to organize the adherens and cadherin junctions. A limited number of studies have been conducted to find the role of Nectin1 in tumor development and progression. Nectin 1 expression was found to be decreased in gastric cancer and melanoma cells. The lower expression of Nectin1 is correlated with the spread of melanoma cells in both Zebra fish and humans.

Objective. To analyze the comparative expression of Nectin1 in Canine Mammary Tumor cell lines and normal canine fibroblast cells.

Methods. rtPCR with RNA extracted from canine mammary tumor cell lines CMT28,27,12 47 and normal fibroblast cells was conducted using the primers designed for the canine Nectin1 gene. Subsequently, agarose gel electrophoresis is performed to evaluate the outcome, size, and purity of the PCR product. Sequencing of the amplified products was employed to verify through Sanger sequencing (MGH DNA core facility). Semiquantitative PCR was done to compare the expression of Nectin1 in each cell line and normal dog fibroblast cells.

Results. The expression of Nectin1 was observed to be reduced in canine mammary tumor cell lines compared to normal dog fibroblast cells.

Conclusions. Our research reveals that Nectin1 expression is uniformly decreased in canine mammary tumor cell lines compared to normal dog fibroblast cells. This decrease may be important in the advancement of mammary tumor progression in dogs, indicating that Nectin1 has the potential to act as a biomarker for tumor development or progression. Nectin1 could also function in tumor suppression or as a marker of cell differentiation which is lost typically upon neoplastic transformation. Additional studies are needed to investigate the functional consequences of Nectin1 downregulation and its viability as a target for therapeutic strategies in canine mammary tumors.

Acknowledgment. We thank AURIC, Auburn University's Department of Undergraduate Research, the College of Sciences and Mathematics, and the College of Veterinary Medicine for funding and support.

P23 Investigating the Impact of Lid Sealing Methods on Volatile Organic Compound Exposure Accuracy: Are the Results of Toxicity Studies Underestimated?

Maryam Hariri[1], Katharine A. Horzmann[1], and Ann S. Ojeda[2]
[1]Department of Pathobiology, Auburn University, AL
[2]Department of Geosciences, Auburn University, AL

Introduction. Volatile Organic Compounds (VOCs) are significant environmental toxicants with widespread public concern due to their vast application and potential harmful impacts on human health. The industrial solvents, trichloroethylene (TCE) and perchloroethylene (PERC) are VOCs of public health concern; however, the volatile nature of the chemicals poses a challenge to researchers working to identify adverse health effects. Variations in toxicity assay results often are attributed to differences in dosing conditions, exposure methods, and experimental design. Given their volatility, even small variations in handling these compounds during toxicity studies can result in substantial differences in the reported outcomes. The zebrafish (*Danio rerio*) serves as a valuable vertebrate model in toxicology research, particularly for screening the toxicity of various compounds. However, exposing zebrafish embryos, where VOCs are mixed in water, may underestimate toxicity because the compound volatilizes rapidly, leading to effectively reduced exposure. Lethal concentration 50 (LC50) values are commonly used in regulatory safety evaluations to compare toxic effects across different species and chemicals.

Methods. In this study, we hypothesized that exposure conditions do influence toxicity and examined how different common lid sealing methods affect the measured toxicity of TCE and PERC in zebrafish embryos through comparison of the LC50s. We evaluated TCE and PERC LC50 values through 120 hours post fertilization (hpf) in zebrafish embryos continuously exposed in crimp cap vials, upright screw cap vials, or inverted (upside-down) screw cap vials. We measured the actual concentrations of TCE in the inverted screw cap vials through 120 hpf using Gas Chromatography-Mass Spectrometry (GC-MS) to evaluate how effectively this lid sealing technique maintains exposure levels over time.

Results. The results revealed significant differences between lid sealing methods in the LC50 values for TCE and PERC. The 120 hpf LC50 for TCE in both crimp cap vials and inverted screw cap vials was approximately 17 parts per million (ppm; mg/L), whereas in upright screw cap vials the LC50 was around 69 ppm. Similarly, the 120 hpf LC50 for PERC followed the same trend: both crimp cap vials and inverted screw cap vials had LC50 values around 7 ppm, while the upright screw cap vials had an LC50 around 35 ppm. These findings suggest that crimp caps or inverted screw caps provide a significantly better seal for dosing volatile compounds compared to upright screw cap vials. Additionally, the GC-MS results showed that the upside-down screw cap vials were efficient in providing concentrations close to the initial dosing levels, however, VOC concentration still decreased throughout the 120 hpf period.

Conclusion. Overall, this study confirms that differences in exposure methods can affect the actual VOC concentration in toxicity assays. VOC toxicity may be underestimated in some research experiments due to unrecognized VOC volatilization, but more accurate results can be obtained by simply inverting the exposure vial.

Acknowledgments. This work was supported by NIEHS R15 ES033361, the Boehringer Ingelheim Vetmedica, Inc. Veterinary Scholars Program, and the Auburn University College of Veterinary Medicine.

P24 An Enhanced cDNA Synthesis Method for Improved an Influenza Virus Sequencing: Overcoming Limitations of Traditional Techniques

Shakiba Kazemian, Stephen Pedroza, Peter J. Neasham, James F. North, Miria F. Criado and Constantinos S. Kyriakis
Department of Pathobiology, College of Veterinary Medicine, Auburn University, Auburn, AL, United States.

Introduction. Influenza A virus (IAV) continues to be a significant global health threat, affecting both human and animal populations. Whole viral genome sequencing, essential to monitor virus evolution and interspecies transmission, relies on cDNA synthesis followed by PCR amplification. However, traditional cDNA synthesis methods, particularly in clinical samples, often result in suboptimal gene coverage and reduced sequencing accuracy. This work aimed to modify and optimize existing cDNA synthesis methods to improve gene coverage and sequencing outcomes using the Oxford Nanopore MinION platform.

Methods. We developed a novel, compact, and efficient protocol for IAV cDNA synthesis by utilizing a modified 1-step reverse transcription polymerase chain reaction (RT-PCR) approach. This method incorporated multisegment primers designed to target all eight gene segments of the IAV genome. RNA was extracted from virus-infected samples, and cDNA synthesis was performed using SuperScript IV Uniprime, followed by purification to ensure a high-quality template for amplification. A multisegment primer scheme was employed to streamline amplification, with primers targeting gene segments either individually or in shared combinations. MinION sequencing libraries were prepared using the Oxford Nanopore Native Barcoding Kit, and the final library was sequenced on an R10.4.1 flow cell. Data were analyzed using IRMA to generate coverage graphs and assess sequencing performance.

Results. The optimized cDNA synthesis method significantly improved PCR amplification efficiency, producing high-quality sequencing data with increased read depth and uniform coverage across gene segments. These results were validated through the successful sequencing of different H1N1 isolates, including clinical and stock virus samples.

Conclusions. This method not only reduced labor and processing time but also enhanced sequencing accuracy, offering a reliable solution for influenza virus genome analysis. The optimized approach can be broadly applied to various influenza strains, providing a valuable tool for both clinical diagnostics and influenza research. Its enhanced performance using the MinION platform supports the ongoing need for improved methods in viral genomics, facilitating better insights into virus evolution, transmission dynamics, and immune responses.

Acknowledgements. This work has been funded by the National Institute of Health (NIH) Centers of Excellence for Influenza Research and Response (CEIRR), Agriculture Research Service, US Department of Agriculture (ARS-USDA) and College of Veterinary Medicine, Auburn University.

P25 Exploring Oncolytic viral therapy to target Osteosarcoma

Sumbul Khan[1,2], Therasa A Higgins[1,2], Isabella Shimko-Lofano[1], and Payal Agarwal[1,2]
[1]Scott Ritchey Research Center, College of Veterinary Medicine, Auburn University, AL
[2]Department of Pathobiology, College of Veterinary Medicine, Auburn University, AL

Introduction. Osteosarcoma (OS) is a mesenchymal neoplasm. OS makes up approximately 3% of all malignancies in children. The mortality rate has not changed in the last few decades; therefore, the development of advanced cancer therapies is essential. We hypothesize that next-generation conditionally replicative oncolytic virus with an armed anti-PD1 HcAb will lyse the OS cells and initiate the immune response against tumor cells in the tumor microenvironment.

Methods. We have infected canine OS cell lines with CAV2 AU M3 and evaluated its cytotoxicity by performing cell viability (Luciferase) and cytotoxicity assay (LDH). To assess whether the virus can stimulate an immune response or not, we performed an immunogenicity assay (ATP assay). To evaluate the secretion and expression of anti-PD1 HcAb using western blot. Flow cytometry was done to confirm the binding of anti-PD1 HcAb to PD1 receptors.

Results. We have found that CAV2 AU M3 is lytic in OS cell lines (D17, CF-11, and MCKOS) at 1000, 100, and 10 MOI in both 2D (Monolayers) and 3D (Tumor spheroids) cell cultures and it also produced and secreted anti-PD1 HcAb, which binds to PD1 receptor efficiently.

Conclusions. CAV2 AU M3 is a potent armed oncolytic virus that can lyse and kill OS cells and produce anti-PD1 HcAb.

Acknowledgments. I would like to thank my lab mates, Gracie Bunch, and Devin Cooper, for their support. I would also like to thank our collaborators Dr. Rimas Orentas, Dr. Maninder Sandey, Dr. Roberto Molinari, Dr. Yev Brundo, and Dr. Bruce Smith, as well as Dr. Rie Watanabe, Dr. James Gillespie, and Dr. Emily Graff for research support. Additionally, I would like to thank our funders, NIH NCI R15, Scott-Ritchey Research Center, and CVM orgs.

P26 Endoscopic Approach to the Canine Olfactory Area

Pia K. LaPorte[1], Andrew L. Leisewitz[2], and Melissa A. Singletary[1, 3]
[1]Department of Anatomy, Physiology, and Pharmacology, College of Veterinary Medicine, Auburn University, AL
[2]Department of Clinical Sciences, College of Veterinary Medicine, Auburn University, AL
[3]Canine Performance Sciences, College of Veterinary Medicine, Auburn University, AL

Introduction. It is unknown how differences in the respiratory and olfactory nasal cavity microenvironments influence function within and across species. The microenvironment of other mucosal sites demonstrate an integral relationship regarding the function of epithelial development, immunological regulation, and overall homeostasis. This limited understanding of the olfactory and respiratory nasal cavity microenvironments is being addressed through anatomical, histological, immunohistochemical, and microbiotal comparisons in the dog. Development of an endoscopic approach allows for excellent visualization of and non-invasive access to the caudal portions of the canine nasal cavity. This can increase sample size, provide repeated access and open up avenues for longitudinal data collection *in vivo*.

Methods. A flexible endoscopic approach was used for mucosal swab collection on four canine cadavers. The endoscope approach was from the external nares and was advanced caudally until reaching the main olfactory region of the nasal cavity. At this location a cytology brush was advanced through the endoscope to swab the olfactory mucosa, and then it was returned to the endoscope before removal to avoid contamination by the respiratory region. The brush head was collected and placed in an eNAT molecular preservation fluid and stored in -80°C freezer. The nasal cavities of each dog were later dissected for Respiratory Epithelium (RE) and Olfactory Epithelium (OE) from the nasal septum and ethmoidal turbinates. Tissue and swab samples were sequenced by SeqCenter with the Illumina Sequel platform for identification of the microbiota.

Results. Preliminary analyses of the tissue and cytology brush swabs indicate similar microbial taxonomy and read numbers between endoscopic swab sampling and direct sampling of the tissues.

Conclusions. Endoscopic brush sampling of the olfactory region of the canine nasal cavity collects representative microbiotal samples of the area, allowing for analysis of the microbial community. This can be useful in the treatment of nasal diseases and the continued discoveries of the implications of the olfactory microbiome on overall olfactory function.

Acknowledgments. We would like to acknowledge the contributions of the Singletary Lab and the staff of the Small Animal Service of the Bailey Teaching Hospital. Financial support was provided through laboratory start-up funding through Auburn University.

P27 Comparison of MOPP versus LOPP for first-line treatment of canine multicentric T-cell or hypercalcemic lymphoma

Cecelia Lounsberry[1], Stephanie Lindley[1], Noelle Bergman[1], Michelle LaRue[2], Samantha Haas-Linden[3], Deborah Keys[4], Ashley A. Smith[1,3]
[1] Department of Clinical Sciences, Auburn University College of Veterinary Medicine, Auburn, AL, USA
[2] Veterinary Specialists of North Texas, Fort Worth, TX, USA
[3] Southeast Veterinary Oncology & Internal Medicine, Jacksonville, FL, USA
[4] Kaleidoscope Statistics, Veterinary Medical Research Consulting, Athens, GA

Introduction. Alkylator-based protocols such as LOPP (lomustine, vincristine, procarbazine, prednisone) and MOPP (mechlorethamine, vincristine, procarbazine, prednisone) are used as frontline chemotherapy for canine T-cell and/or hypercalcemic lymphoma. This study compared response rate, progression-free survival (PFS), and median survival time (MST) for dogs with T-cell and/or hypercalcemic lymphoma treated with first-line LOPP or MOPP. Prognostic factors influencing outcome data were also evaluated.

Methods. This multi-institutional, retrospective study included dogs with multicentric, intermediate to large cell lymphoma that were T-cell and/or hypercalcemic. Dogs with gastrointestinal or cutaneous involvement were excluded. Statistical analyses using SAS 9.4 included construction of Kaplan-Meier curves, comparison between treatment groups with log-rank tests, and the use of univariable and multivariable Cox proportional hazards regression for prognostic variables.

Results. Ninety-nine dogs were included. Complete response rate was significantly higher in dogs treated with LOPP compared to MOPP (87% v. 65%, p=0.011). The median PFS was significantly longer in dogs treated with LOPP versus MOPP (186 days vs. 95 days, p=0.024). The MST was not significantly different between protocols. The frequency of adverse events was similar between groups. Remission was more likely in dogs with a cranial mediastinal mass or $CD4^+CD8^-$ immunophenotype. Dogs with a cranial mediastinal mass had a longer PFS. MST was only influenced by the use of rescue chemotherapy.

Conclusions. This study supports the use of alkylator-based protocols for the treatment of T-cell lymphoma. Dogs treated with LOPP versus MOPP had a higher complete response rate and longer PFS. Similar MST between groups could be related to the preservation of lomustine as a rescue agent in MOPP treated dogs. Lomustine may be a preferred alkylating agent for T-cell lymphoma patients. Presence of a cranial mediastinal mass and $CD4^+CD8^-$ immunophenotype were associated with an improved CR/PFS and CR, respectively. There were no prognostic variables shown to improve MST in this study. Prospective studies are needed to confirm these findings.

Acknowledgments. The author would like to thank the veterinarians, technicians, and staff of the Auburn University Veterinary Teaching Hospital's Oncology Service, Veterinary Specialists of North Texas, and Southeast Veterinary Oncology and Internal Medicine for the excellent care of these patients. There was no financial support for the research, authorship, and/or publication of this article.

P28 Assessing How Blood Serum Biomarkers Predict Biological Age in Chimpanzees

Emily J Marshall[1], Alexandra de Sousa[1], Brier Rigby Dames[2], Ryan Gibson[1], Melissa Singletary[1], Christine J Charvet[1]
[1]Department of Anatomy, Physiology, and Pharmacology, College of Veterinary Medicine, Auburn University, AL
[2] Department of Computer Science, University of Bath, UK

Establishing the age of an animal is important for medical care and general animal welfare as well as in wildlife conservation. Machine Learning (ML) is a computer algorithm that is capable of prediction and classification tasks. Utilizing ML to address biomedical research needs is a more streamlined process to solve complex problems such as predicting age in an individual utilizing data collected within a healthcare setting. Blood serum biomarkers, such as alkaline phosphatase, globulin, and total protein, are found circulating in the blood and can be measured at routine blood draws. These biomarkers are useful and can be utilized to predict age in many species due to temporal patterns as an organism ages. The aim of this project is to utilize blood samples collected from chimpanzees to predict biological age. Data were collected from Alamogordo Primate Facility, University of Texas M.D. Anderson Cancer Center, Yerkes National Primate Research Center (NPRC), Oregon NPRC, Wisconsin NPRC, California NPRC, National Institute of Aging Animal Center at Poolesville, and University of Illinois Chicago. The collected data were then processed for albumin, alkaline phosphatase, creatinine, chloride, globulin, total protein, sodium, potassium before being analyzed by ML modeling. Random Forest models were assessed and a final model was selected by RMSE (Root Mean Square Error value. Preliminary results show that we are able to predict the age of chimpanzees with a (R^2–which is calculated by subtracting Sum of Squares Residual divided by Sum of Squares Total from one)77.7% accuracy. The model shows a low AIC and BIC score of -198.2 and -191.9 respectively. Therefore, these ML models can rely on blood-based biomarkers to predict ages.

Acknowledgments. This work was funded by the Auburn College of Veterinary Medicine, including the Scott Fund and the Animal Health and Research Disease Fund.

P29 Safety of Multi-site Ultrasound Neuromodulation to Improve Metabolic Function in Domestic Cats

Shelby McAlister[1,2], Jordan Doss[2], Stephanie Maples[2], Robert Cole[3], Megan Grobman[3], Christopher Puleo[4], Emily Graff[1,2]
[1]Department of Pathobiology,[2]Scott Ritchey Research Center, College of Veterinary Medicine, [3]Department of Clinical Sciences, College of Veterinary Medicine, [4]General Electric (GE) Research

Obesity affects millions of domestic cats, resulting in impaired glucose regulation which often leads to metabolic disease including diabetes mellitus, and shortened lifespan. Current therapies for diabetic cats can require daily injections that negatively impact the owner-animal bond. Thus, there is a critical need for therapies to improve insulin regulation and obesity in cats. Novel therapies using peripheral focused ultrasound to target the sympathetic nervous system prevent or reverse the development of diabetes in obese and insulin resistant rodent models. Multi-site stimulation at the liver and the gastrointestinal tract results in diabetic remission in multiple models of obesity. We intend to develop this innovative therapy for client owned cats to improve therapeutic options. For this specific study, our overall objective is to demonstrate safety of neuromodulation in healthy domestic cats to improve insulin sensitivity and induce weight-loss. We installed a GE LOGIQ E10 ultrasound system configured for targeted neuromodulation. Five healthy adult cats were enrolled in the study. Health status was determined based on physical exam, a complete blood count (CBC), serum biochemistry, urinalysis, and thyroid panel. A continuous glucose monitoring (CGM) system was placed and interstitial blood glucose values were monitored for 48 hours. After 48 hours, the cats were sedated and a single dose of a peripheral focused ultrasound treatment was delivered to the porta hepatis for 30 minutes followed by 30 minutes to the superior mesenteric plexus. The stimulation site and physical activity of the cats were monitored daily for two weeks following treatment. Interstitial glucose was monitored for an additional 12 days. At 30 days, post stimulation serum biochemistry, urinalysis and thyroid panel were repeated. There was no heat, redness, swelling, or pain at the site of stimulation and no loss of appetite or change in behavior at any point during the study or 60 days following stimulation. There was no evidence of liver, renal, or thyroid disease based on CBC, and biochemistry and endocrine profiles. The daily average interstitial glucose concentration was 100 $\pm$ 26 mg/dL pre-stimulation with a trend towards decreased interstitial glucose 48 hours following stimulation (78 $\pm$ 17 mg/dL; $p=0.2$). This trend was also observed in serum glucose and 30 days post stimulation ($p=0.2$). Ultrasound stimulation appears to decrease mean daily interstitial glucose concentrations with no change in glucose variability in apparently healthy domestic cats. Similar to what is reported in people, none of the cats experienced hypoglycemic or adverse events associated with peripheral ultrasound stimulation. Preliminary results suggest that single-dose multi-site ultrasound neuromodulation is safe in healthy domestic cats and may provide a novel and more accessible therapy for obese and diabetic cats. Future studies will investigate a large cohort of obese and overweight cats to determine a more extensive safety profile and begin to explore metabolic effects.

P30 The Interrelationship between anti-Müllerian hormone, antral follicle count, and hormone treatment in mares

Hayley Moore[1], Candace Lyman[1], Peyton Draheim[2], Jessica Klabnik[1]
[1]College of Veterinary Medicine, Auburn University, Auburn, AL
[2]Hagyard Equine Medical Institute, Lexington, KY

Introduction. Anti-Müllerian hormone (AMH) concentration is correlated with antral follicle count (AFC) in the mare. Because of this relationship, potential lies in the ability of AMH to act as a prognostic tool for assessing a mare's overall fertility or, more specifically, predicting a mare's potential as an oocyte donor to produce in vitro-derived embryos. It is unknown if AMH concentrations remain correlated when mares are undergoing hormone therapy protocols aimed at increasing AFC to promote oocyte aspiration efficiency. The aim of this project is to determine if AFC is correlated to AMH values in mares undergoing cycle manipulation with only follicle ablation (controls) or undergoing hormone therapy targeted to increase AFC with either progesterone and estradiol (P&E) or with a proprietary drug that supports follicular growth. We hypothesize that AMH concentration will be positively correlated with AFC in mares undergoing cycle manipulation.

Methods. Data was analyzed from two previous experiments. In the first experiment, 10 mares underwent a crossover design in which mares did (P&E group) or did not (Control group) receive a dose of a slow-release formulation of progesterone and estradiol 17β (P&E, a total dose of 1.5 g and 50 mg, respectively) on Day 0. On Day 0, all mares underwent transvaginal follicle ablation. On Days 0, 3, 6, and 10, AFC was determined via transrectal ultrasound and serum was stored for AMH enzyme-linked immunosorbent assay (ELISA). In the second experiment, a group of 6 mares (Proprietary Drug group) underwent transvaginal follicle ablation (Day -10) followed by prostaglandin (5 mg dinoprost tromethamine IM, Day -4) to lyse existing luteal tissue and act as a means of cycle synchronization. The proprietary drug was administered on Days 0 through 4. On Days 0 through 4 and Day 7, AFC was determined via transrectal ultrasound and serum samples were stored for AMH ELISA. Data were analyzed for normality via Shapiro-Wilk and correlation was determined by Kendall rank correlation coefficient. Day 0 AMH and AFC data was removed prior to analysis, as this was the first day in which cycle manipulation (i.e., drug administration or ablation) occurred in most mares. Significance was set at $P<0.05$ for all analyses and performed in R version 4.3.1.

Results. There was no significant correlation ($R = 0.17$; $p = 0.19$) between AMH and AFC in the Control group (n=10), a moderately positive correlation ($R=0.51$; $p = 0.00011$) in the P&E group (n=10), and a weak positive correlation ($R = 0.36$; $p = 0.0062$) in the Proprietary Drug group (n=6).

Conclusions. This study demonstrates that there is a statistically significant positive correlation between AMH concentration and AFC in mares undergoing two treatments that support follicular growth. Future studies are needed to determine if AMH concentration can be a tool used to help assess oocyte aspiration potential and fertility in the mare.

Acknowledgements. Funding for the original experiments was provided by the Theriogenology Foundation, Oklahoma State University, and Auburn University.

P31 Method for single nuclei isolation from equine sarcoid tissue

Kira J Noordwijk[1], Xu Wang[2], R. Reid Hanson[1], Lindsey H. Boone[1], and Bruce Smith[2,3]
[1]Department of Clinical Sciences, College of Veterinary Medicine, Auburn University, AL
[2]Department of Pathobiology, College of Veterinary Medicine, Auburn University, AL
[3]Scott-Ritchey Research Center, College of Veterinary Medicine, Auburn University, AL

Introduction. Equine sarcoid is a common skin tumor found in horses of all breeds, colors and ages worldwide. There are six main types of sarcoids including occult, verrucous, nodular, fibroblastic, malevolent, and mixed. Quarter Horses, Arabians, and Appaloosas have been reported to have an increased risk of developing sarcoids. Horses affected by sarcoids may need to be removed from work due to issues like tack irritating the tumors or risk of disfigurement. Ulcerated sarcoids are often a significant welfare concern for owners. Although several treatment options are available, more aggressive sarcoids are difficult to treat and often are non-responsive to therapies. These therapies can be very costly, sometimes cost-prohibitive, to the owners. Functional therapies, based in the molecular characteristics of these tumors would allow horses to remain in training and use.

Methods. Sarcoid tissue samples were collected from horses which presented to the Auburn University, J.T. Vaughan Large Animal Teaching Hospital (AULATH). Samples were flash frozen and stored in liquid nitrogen. 150g of tissue was crushed in a liquid nitrogen cooled mortar and pestle. The crushed tissue was dissociated in lysis buffer consisting of 1x PBS, 10 mM Tris-HCL, 0.0125% Triton X-100, 1 mM DTT, 0.2 U/µL RNase inhibitor, and 2 U/µL DNase inhibitor. The supernatant was collected through a three-stage incubation protocol. The supernatant was centrifuged, washed, and resuspended in a buffer solution consisting of 1x PBS, 2% Bovine Serum Albumin (BSA), 0.2 U/µL RNase inhibitor, and 2 U/µL DNase inhibitor. After isolation, suspensions of nuclei will be visualized to determine nuclei concentration, suspension quality, and nuclei sizes prior to continuing to single-nuclei RNA sequencing. To count nuclei, 10 µL aliquots were combined with Acridine Orange/Propidium Iodide (AO/PI) and assayed with a Countess II FL (Invitrogen; ThermoFisher Scientific©). A second sample was stained with Trypan Blue and nuclei were counted using the BioRad TC20 Automated Cell Counter (Bio-Rad Laboratories, Inc.©) as well as counted manually to establish an accurate nucleus count. The sample was stained with AOPI, placed on a microscope slide and assayed on a Keyence microscope under 60x magnification for quality assessment of nuclei.

Results. Two sarcoid tissue samples, one fibronodular and one malevolent sarcoid, were processed with described protocol. Samples were confirmed as sarcoid based on histopathology. Both samples produced 4-5.0x10^6 nuclei/mL of excellent quality. Mild debris was noted on the first attempt which improved on the second sample with removal of the 20µm cell strainer step. Under 60x magnification, multiple nuclei were identified on both samples with completely intact cell membranes and minimal to no signs of disruption to the cell membrane of the nucleus.

Conclusions. The study aim of this pilot project for processing of sarcoid tissue were to obtain the optimal concentration (700- 1200 nuclei/ µl of intact nuclei without disruption of the cellular membrane for sequencing and library preparation. and for nuclei to be intact. An optimal concentration of intact nuclei was obtained using this protocol. However, this protocol did result in a mild amount of debris after processing that required an additional filtration step to remove the debris by running the sample through a 20µm cell strainer. This debris is speculated to come from excessive shear during frozen processing. Both samples produced nuclei sufficient to proceed with single-nuclei RNA sequencing.

Acknowledgments. This research was funded by the American College of Veterinary Surgeons Surgery Resident Research Grant.

P32 Effect of administration of intranasal minerals on immune and clinical outcomes of dairy calves experimentally challenged with bovine herpesvirus 1 (BHV-1)

Jessica G. Prim[1], Manuel F. Chamorro[1*], Florencia Meyer[3], Thomas Passler[1], Shollie Falkenberg[2], Jenna Stockler[1], Jessica Rush[1], Roberto A. Palomares[4]
[1]Departments of Clinical Sciences and [2]Pathobiology, College of Veterinary Medicine, Auburn University, AL
[3]Department of Biochemistry, Molecular Biology, Entomology & Plant Pathology, Mississippi State University, MS
[4]Department of Population Health, College of Veterinary Medicine, University of Georgia, GA

Introduction. Bovine Respiratory Disease Complex (BRDC) remains a significant cause of economic loss and animal welfare concerns in the cattle industry. Minerals such as zinc, copper, and magnesium play crucial roles in immune function, potentially reducing the incidence and severity of BRDC. Results from previous studies suggest that injectable trace minerals (ITM) enhance immune responses following modified-live virus (MLV) vaccination in calves. Additionally, reduction of clinical scores following experimental infection with respiratory viruses has been reported in calves previously treated with ITM; however, the effect of intranasal mineral administration on immune responses and clinical outcomes following experimental infection with respiratory viruses in calves is unknown. This study explores the effect of a mineral formulation administered intranasally on immune and clinical outcomes in dairy calves following challenge with Bovine Herpesvirus 1 (BHV-1).

Methods. Fourteen 3-month-old dairy calves were randomly assigned to one of two treatment groups. The INM group (n=7) received 4.4 mL of a mineral solution containing 14 mM Zn, 0.25 mM Cu, and 1.5 mM Mg intranasally (IN). The Control group (n=7) received 4.4 mL of phosphate-buffered saline (PBS) IN. Treatments were administered to calves from both groups on days -2, 2, and 6 relative to BHV-1 challenge (day 0 of study). On day 0, all calves were challenged with 2 mL of a BHV-1 inoculum ($3x10^7$ $CCID_{50}$/mL) intranasally. Clinical signs were assessed on days -2, 0, 2, 6, 10, 14, 21, and 28 using a respiratory score sheet. Nasal swabs (NS) were collected for virus isolation and serum samples were collected to evaluate BHV-1 neutralizing antibody titers. Data were analyzed by ANOVA for repeated measures, with statistical significance considered when $p < 0.05$.

Results. Clinical parameters before and after virus challenge were similar between groups. All calves were negative to BHV-1 in NS and seronegative to BHV-1 before challenge. Detection of BHV-1 in NS after challenge was similar between groups. A numerically greater mean ± SEM Log2 serum neutralizing antibody titer was observed in the Control group compared with the INM group on day 28 (32.9 ± 47.9 vs. 10 ± 11, respectively); however, this was not significantly different (P=0.1).

Conclusions. Based on results from this study, treatment with INM did not have a significant effect on health outcomes, antibody responses, or virus isolation in dairy calves experimentally challenged with BHV-1.

P33 The Development of Virus-Like Particles Targeting Avian Origin Hemagglutinin 5 for Production of Hyperimmune Antiserum

Philip G. Strate, J. Fletcher North, Peter J. Neasham, and Constantinos S. Kyriakis
[1]Department of Pathobiology, College of Veterinary Medicine, Auburn University, AL

Introduction. Highly pathogenic avian influenza (HPAI) viruses, pose an ever growing concern for animal and public health and safety. The ongoing outbreak of the H5N1 HPAI virus in dairy cattle, with several confirmed human cases, highlights the capacity of the virus to cross species and potentially infect humans. A highly effective therapeutic hyperimmune antiserum against HPAI viruses could serve as a first line of defense in case of a human outbreak or, worse, a new pandemic. To address the threat of emerging HPAI H5 strains, our goal is to develop separate virus-like particles which comprise antigenic motifs characteristic of three diverse H5 hemagglutinin (HA) glycoprotein lineages. The HA is the main surface glycoprotein of the virus and antibodies directed against the HA can be neutralizing. Our expectation is that immunization of animals with these diverse H5 motifs will result in hyperimmune serum with cross-reactive antibodies against common epitopes of novel H5 subtypes which may emerge. Here, we present the virus-like particle (VLP) development process, which underpins future work to characterize the efficacy of the vaccine to produce hyperimmune product against novel H5 HPAI influenza.

Methods. The VLPs were developed utilizing the Bac-to-Bac Baculovirus Expression System (Invitrogen Life Technologies, Carlsbad, California). A total of three VLPs were produced. To replicate the structural aspects of IAVs, recombinant matrix protein M1 and ion channel M2 of A/Viet Nam/1203/2004, were co-expressed alongside each of the three recombinant H5 HAs: A/Colorado/18/2022, A/Viet Nam/1203/2004, and A/Cambodia/NPH230032/2023. Each HA representing antigenically distinct lineages of H5 HPAI viruses. All recombinant genes were synthesized and ligated into four separate pFastBac1 vectors (Thermo Fisher Scientific, Waltham Massachusetts). Each plasmid was transformed into DH10Bac *Escherichia coli* (*E. coli)* bacterial cells. Recombinant clones were selected and expanded in broth culture. DH10Bac *E. coli* were lysed and plasmids collected. The bacmid DNA was amplified by polymerase chain reaction (PCR) and recombinant gene size confirmed by gel electrophoresis. Each recombinant bacmid plasmid was transfected into *Spodoptera frugiperda* 9 easy-titer (SF9-ET) cells, to produce the baculovirus expressing each protein. High Five cells were then used for VLP production, following infection with baculoviruses expressing the matrix and each of the selected HAs. VLPs were purified via ultracentrifugation and their structure confirmed with electron microscopy, while the integrity of the HAs was confirmed by Western blot.

Results. Following the successful generation of four different baculoviruses, we were able to begin VLP production and characterization. Detailed characterization of each VLP is currently ongoing.

Conclusions. Using the Bac-to-Bac Baculovirus Expression System, we were able to successfully transduce DH10Bac *E. coli,* allowing for transposition of each desired recombinant gene into the bacmid plasmid. Plasmid isolation and DNA composition analysis were also successful, indicating that the genes of interest were properly incorporated into the bacmid plasmid, allowing for recombinant baculovirus replication upon transfection in insect cells. The process of virus quantitation and amplification indicated successful passage in both SF9-ET and High Five cells, paving the way for protein and VLP characterization. VLPs obtained will be used in upcoming work investigating the efficacy of each H5 expressing VLP for inducing an immune response for hyperimmune serum production, characterization, and use in upcoming challenge studies.

P34 Exploring 1-Trichloromethyl-1,2,3,4-tetrahydro-beta-carboline (TaClo)-induced neurotoxicity following the early-life exposure in the zebrafish model (*Danio rerio*)

Ji-Hang Yin[1], Katharine Horzmann[1]
[1]Department of Pathobiology, College of Veterinary Medicine, Auburn University, AL

Introduction. 1-Trichloromethyl-1,2,3,4-tetrahydro-beta-carboline (TaClo) is an endogenous neurotoxicant formed in the brain after exposure to trichloroethylene (TCE) or tetrachloroethylene (PERC). TCE and PERC are industrial solvents and have the potential to induce developmental toxicity, neurotoxicity, and the development of Parkinson's disease; however, it is unknown if their metabolite, TaClo, is responsible for these effects. This study tested the hypotheses that (1) embryonic exposure to environmentally relevant concentrations of TaClo induces developmental toxicity and neurotoxicity in zebrafish, (2) embryonic TaClo exposure leads to long-term neurotoxic effects in adult zebrafish, and (3) the mechanisms of TaClo-induced neurotoxicity parallel those proposed in Parkinson's disease.

Methods. Fertilized zebrafish (*Danio rerio*) embryos were dosed with embryo water (control), 0.001% DMSO (carrier control), TaClo at 5, 50, and 500 ppb, and 1.75 μM MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) (positive control) for 24 or 120 hours post fertilization (hpf). Endpoints for developmental toxicity evaluation at larval stages include survival and hatching percentage, body measurement, and heart rate analysis. Behavior was evaluated through a 24 hpf photomotor response test (PMR) and 120 hpf visual motor response test (VMR). Dopaminergic neuronal expression in the ventral diencephalon (vDC) was evaluated using whole mount in-situ hybridization and immunofluorescence assay (WIFA) to assess neurotoxicity in 120 hpf zebrafish. Additional treated larval zebrafish were rinsed and raised under normal conditions until 6 months post fertilization (mpf) for neurobehavioral tests, including novel tank test (NTT) and open field test (OFT). Lastly, reactive oxygen species, antioxidant levels, apoptosis, mitochondrial activity, and microglial and astrocytic function were evaluated using WIFA or flow cytometry to investigate the underlying mechanisms of neurotoxicity.

Results. Our results demonstrated a bimodal dose-response with TaClo at 5 ppb, triggering significant behavioral alternations in the PMR and VMR. At 5 ppb TaClo and 1.75 μM MPTP, treated larvae at 120 hpf had a significant decrease in dopaminergic neuronal expression, and a significant increase in apoptosis and microglial activation in vDC regions. The MPTP-treated larval group showed a significantly lower glutathione peroxidase activity (Gpx), whereas the highest activity was in the TaClo 500 ppb group. In all the TaClo-treated groups, larval zebrafish had significantly lower astrocytic expression than the carrier control. Although no neurobehavioral changes were noticed in the TaClo-treated adult zebrafish, MPTP-treated zebrafish showed a long-term behavioral impairment in both sexes and an increased anxiety status in females in NTT.

Conclusions. Early-life exposure to environmentally relevant concentrations of TaClo triggered Parkinson's disease-like neurotoxicity in larval zebrafish, with similar apoptosis, neuroinflammation, and astrocytes pathways altered.

Acknowledgments. This research project is funded by NIH-NIEHS (R15 ES033361). We thank Dr. Jennifer Panizzi and Amanda Strozier at the Department of APP for kindly providing us with Tg(mpeg1.1:mCherry) zebrafish embryos. We also would like to thank Dr. Rie Watanabe for her guidance and support on flow cytometry.

P35 *C. difficile* Infection Induces a Pro-Steatotic and Pro-Inflammatory Metabolic State in Liver

Farnaz Yousefi and Shaan Mooyottu
Department of Pathobiology, Auburn University, Auburn, AL, 36849

Introduction. Recent studies suggest links between *Clostridioides difficile* infection (CDI) and liver disorders, with Metabolic dysfunction-associated steatotic liver disease (MASLD) significantly increasing CDI risk and severity. Moreover, gut dysbiosis, often leading to *C. difficile* overgrowth, is implicated in chronic liver conditions. In this study, we explore this potential association from a reverse angle, hypothesizing that CDI directly induces a pro-steatotic and pro-inflammatory metabolic state in the liver, the hallmark of metabolic steatotic liver diseases.

Methods. Fifty-four adult C57BL/6 mice were divided into Control, Antibiotic control (Abx), and *C. difficile* infection (C. diff) groups. The Abx and C. diff groups received an antibiotic mixture to induce gut dysbiosis and facilitate *C. difficile* colonization. The C. diff group was then inoculated orally with 1.4×10^4 *C. difficile* spores. Mice were euthanized 48 h post-inoculation to collect liver and cecum for metabolomic, transcriptomic, metagenomic, and multi-omics correlation analyses.

Results. Gut dysbiosis and *C. difficile* colitis were confirmed via metagenomic analysis and histopathology. Liver metabolomics, transcriptomics, and joint pathway analyses revealed significant alterations in lipid metabolism, highlighting dysregulation in glycerolipid metabolism, steroidogenesis, energy metabolism, and mitochondrial functions in the C. diff group compared to controls ($p < 0.05$). Metabolites and pathways associated with oxidative stress and inflammation were significantly enriched ($p < 0.05$). Steatosis-associated metabolites, such as palmitic acid and glycerol 3-phosphate, and inflammation-associated metabolites, such as leukotriene B4 and prostaglandin G2, were significantly increased, while free and oxidized glutathione levels significantly decreased ($p < 0.05$). Gene ontology and KEGG pathway analyses predicted changes mimicking alcoholic liver disease. Gut metagenome-liver metabolome correlation analysis identified specific bacterial species in the dysbiotic gut microbiota of the C. diff group correlating with differentially enriched liver metabolites involved in oxidative stress, amino acid, and uric acid metabolisms.

Conclusions. CDI triggers gene expression and metabolic shifts that could facilitate lipid accumulation, oxidative damage, and inflammation, suggesting that CDI could be a risk factor for the onset and progression of metabolic liver diseases, emphasizing the role of the gut-liver axis. Further studies are warranted to confirm this risk.

P36 Independent origin of genomic imprinting in viviparous fish: parent-of-origin effects in placenta gene expression in matrotrophy Poeciliid

Ying Zhang[1], Yuridia Reynoso[2], Yuan Kang[3], Jeff Huang[3], David Reznick[2†], Xu Wang[1,4,5†]
[1]Department of Pathobiology, Auburn University; [2]Department of Evolution, Ecology and Organismal Biology, University of California, Riverside, [3]Department of Anatomy, Physiology & Pharmacology, Auburn University; [5]HudsonAlpha Institute for Biotechnology

Introduction. Genomic imprinting is an epigenetic phenomenon in which allelic expression depends on the parent of origin. To date, imprinted genes have been identified only in therian mammals and flowering plants, with the mammalian placenta and plant endosperm serving as hotspots for imprinting. The placenta is a major evolutionary innovation in mammals, functioning as an essential organ that connects the developing fetus to the mother's uterus and facilitates the exchange of nutrients, gases, and waste products. Similarly, the endosperm is a plant structure that nourishes developing embryos within seeds. The occurrence of imprinting coincides with the evolution of the placenta and endosperm, suggesting that genomic imprinting may be a byproduct of placental reproduction. We hypothesize that imprinting should evolve in other organisms with placental reproduction. Interestingly, such examples exist in nature to test this hypothesis: placentation has independently evolved at least 9 times within the fish family Poeciliidae. Notably, *Poeciliopsis prolifica* is a matrotrophic species exhibiting extensive maternal provisioning during embryo development.

Methods. Two semi-inbred strains of *P. prolifica,* line A and line B, were crossed to produce embryos from reciprocal directions. Embryonic tissue, pericardial sac (placenta), and maternal follicle (maternal origin) were collected for RNA-seq transcriptome analysis. The quality of raw reads was examined with fastQC and low-quality reads and adapters were trimmed by Trimmomatic. High-quality filtered reads were mapped to the *P. prolifica* genome. The alignment files were merged for *de novo* SNP calling using GATK. Informative SNPs were identified as heterozygous SNPs with a sequencing depth of 6 or higher. Allelic expression ratios were quantified by calculating the number of reads containing line B allele divided by the total read depth at each SNP position. Candidate imprinted genes were identified using cut-off of allelic expression ratio < 0.35 and > 0.65 in each of the two reciprocal crosses. Selected candidate imprinted genes were validated using allele-specific pyrosequencing.

Results and Conclusions. A total of 351,361 exonic SNPs were called from RNA-seq data of from 17 fish, of which 315,085 are within 24,222 protein-coding genes, providing a truly transcriptome-wide coverage with an average density of 13 SNPs per gene. In F1 maternal follicle samples, the distribution of allelic ratios in the transcriptome mirrors that of the mother, confirming their entirely maternal origin. In contrast, in F1 placental tissue, the allelic expression distribution is centered around 50%, resembling the distribution observed in embryonic tissue. These results reveal that the pericardial sac constitutes the embryonic contribution to the placenta. In the F1 placental tissue, we identified 556 maternally expressed candidate imprinted genes enriched in ion transport, signaling, and cardiovascular development pathways. Additionally, we identified 84 paternally expressed candidates. Interestingly, functional enrichment analyses revealed that these paternal genes are highly enriched in skeletal muscle development pathways. Two maternally expressed candidate imprinted genes, *Clic2* and *Smg9*, as well as two maternally expressed candidates, *Dpp4* and *Ankrd13d*, were selected for allele-specific pyrosequencing validation. They were all confirmed to have significant parent-of-origin effects in gene expression. Strikingly, *Dpp4* is a known marker for human noninvasive extravillous trophoblasts and is among the top three most highly expressed genes in human placental tissue. This indicates convergent evolution between placental teleosts and mammals, suggesting a potentially important function of *Dpp4* in the Poeciliid placenta.

P37 *Corynebacterium* enrichment is a hallmark of the vaginal microbiome in cows carrying persistently infected Bovine Viral Diarrhea Virus (BVDV) fetuses

Yue Zhang[1], Rachel Phillip[1,2], Paul H. Walz[1], Shollie M. Falkenberg[1,2†], Xu Wang[1,3†]
[1]Pathobiology, Auburn University; [2]Sugg Laboratory, Auburn University; [3]HudsonAlpha Institute for Biotechnology ([†] co-corresponding authors)

Introduction. Bovine viral diarrhea virus (BVDV), a member of the genus *Pestivirus*, is a significant pathogen responsible for substantial economic losses in the beef and dairy cattle industries in the United States, with annual costs potentially exceeding $1 billion. Persistently infected (PI) cows are animals that have been infected with BVDV *in utero* and carry the virus for their entire lives. As a primary reservoir for BVDV, these cows continuously shed the virus into the environment and posing a significant risk to the health of other cattle in the herd. Furthermore, there is new evidence that BVDV may potentially disrupt the maternal recognition of pregnancy or the immune protection of the conceptus, thereby causing cattle infertility and further economic loss. The microbiome of the reproductive system plays a crucial role in maintaining reproductive health, which is highly relevant to the *in utero* BVDV infection. Imbalance of the vaginal microbiome, a condition known as bacterial vaginosis, may increase susceptibility to fetal infections. However, a concrete link between bacterial vaginosis and BVDV PI has not been established. This research aims to characterize the vaginal microbiome of cows carrying BVDV-PI and noninfected fetuses and identify the vaginal microbiome correlates in BVDV persistent infection.

Methods. Vaginal swab samples were collected from cows carrying healthy (N=6) and BVDV-PI (N=6) fetuses using the ESwab kits (Cat No. 480C, Copan Diagnostics). DNA samples were extracted from the swabs and medium using Qiagen QIAamp DNA microbiome Kit and Zymo HostZERO microbial DNA kit. Whole-genome shotgun (WGS) metagenomic libraries were prepared using the NEBNext Ultra II FS DNA Library Prep Kit. After quality control, the libraries were sequenced on an Illumina NovaSeq X Plus sequencer to achieve at least 50 million reads per sample. We applied a bioinformatic analysis pipeline described in our previous studies, including raw sequencing data quality control, adapter dimer and low-quality base trimming, *de novo* metagenomic contig assembly, taxonomy annotation, and relative abundance quantification at different taxonomic levels. The vaginal microbiome profiles from PI animals and control animals were compared.

Results and Conclusions. Our study is the first to characterize the virginal microbiome at WGS metagenomic level in beef cattle. In cows carrying healthy fetuses, the vaginal microbiome is predominantly composed of the phylum Bacillota (formerly known as Firmicutes), accounting for approximately 53.7% of the microbial community. The second most abundant phylum is Pseudomonadota (Proteobacteria), representing about 20.2% on average. Actinomycetota (Actinobacteria) is the third most abundant phylum, comprising 14.3% of the microbiome. Collectively, these three phyla constitute nearly 90% of the vaginal microbiome in cattle. In PI fetus carrying animals, Firmicutes abundance remains the same (54.8%, $P > 0.05$). However, we observed a two-fold increase in Actinobacteria abundance ($P < 0.05$), while Proteobacteria abundance decreased by approximately 60%. Further investigation at the genus level revealed that the increase in Actinobacteria was solely driven by *Corynebacterium* (5.2% in controls vs. 16.3% in PI animals), a genus known to be opportunistic pathogens associated bovine urinary tract infections. In contrast, reduction in Proteobacteria was due to decreases in *Escherichia coli* and *Vibrio parahaemolyticus* (14.9% in controls vs. 1.9% in PI animals). The observed alterations in the vaginal microbiome of cows carrying PI fetuses have significant implications for the dynamics of the microbial community and the reproductive health of the animals, which will inform the early detection and management of BVDV infections.

Acknowledgements. This study is supported by an AUCVM start-up fund to X.W.

Faculty/Staff Poster Presentations

P38 Alcohol Administration Disrupts Intestinal Autophagy to Exacerbate Liver Injury

Murugadas Anbazhagan[1], Ahmed Bakheet[1], John D. Dickinson[2] and Paul G. Thomes[1]
[1]Department of Anatomy Physiology and Pharmacology, College of Veterinary Medicine, Auburn University, Auburn, AL, [2]Department of Internal Medicine, University of Nebraska, Omaha, NE.

Background. Autophagy is crucial for intestinal barrier integrity as it maintains key functions of intestinal epithelial cells, such as secreting antimicrobial peptides and mucins, and removing harmful microorganisms. This process helps maintain gut homeostasis and prevents harmful gut products from reaching the liver and causing injury. Our study examined whether alcohol disrupts intestinal autophagy to compromise the gut barrier and whether such autophagy dysfunction promotes alcohol-induced liver injury.

Methods. Male wildtype and global autophagy-deficient ATG16L1 hypomorphic (ATG16L1 hm/hm) mice were subjected to chronic-binge ethanol administration (NIAAA feeding model) or to a control diet. We measured autophagy markers and intestinal permeability in the ilea of these animals, as well as serum endotoxin and markers of liver injury. Mouse and human ileal organoids were treated as indicated in the results.

Results. Compared with controls, Ileal segments from wild-type mice fed chronic-binge ethanol (EtOH) showed lower mRNA levels for the autophagy-regulating transcription factor EB (TFEB) and its downstream targets, PGC1α and LAMP1, along with other autophagy and lysosomal markers including ATG4b, ATG14, and ATP6V0d2. The levels of the stem cell marker LGR5 and epithelial cell markers LYZ1 and IGFBP4 were also reduced in these animals. Human ileal organoids exposed to 0.5% EtOH for 24 hr exhibited lower levels of LC3B, LAMP1, LAMP2, Cathepsin B, PGC1α, and several other autophagy and lysosomal markers, including their regulator, TFEB. Compared with wild-type mice, ileal organoids from ATG16L1 hm/hm mice showed impaired autophagy flux. Compared with EtOH-fed wild type mice, EtOH-fed ATG16L1 hm/hm mice exhibited 1.6- and 2-fold increases in FITC-dextran leakage (ex vivo) and serum endotoxin, respectively, along with a 1.6-fold rise in serum ALT and liver triglycerides. Proinflammatory markers CXCL1 and CXCL2 were also elevated in the livers of EtOH-fed ATG16L1 hm/hm mice.

Conclusion. we conclude that alcohol exposure disrupts autophagy in intestinal epithelial cells, thereby compromising their functions. Such disruption of autophagy leads to increased intestinal permeability and leakage of bacterial products into the circulation, causing liver injury and inflammation. Our findings also suggest that autophagy deficiency exacerbates alcohol-associated liver injury.

P39 Dysregulated Lysosome Biogenesis and Abnormal Lysosomal Activity Potentially Drive Human Alcohol-Associated Liver Disease

Ahmed Bakheet[1], Murugadas Anbazhagan[1] and Paul G. Thomes[1]
[1]Anatomy, Physiology and Pharmacology, College of Veterinary Medicine, Auburn University, Auburn, Alabama, USA.

Background. Lysosomes are critical for degrading cellular macromolecules, thereby maintaining cellular homeostasis. In rodents fed ethanol, lysosomes are downregulated, leading to accumulation of undegraded polymeric substrates. However, the connection between these changes in human alcohol-associated liver disease (ALD) is not fully understood. Here, we investigated lysosomal alterations in livers of human subjects with alcohol-induced hepatitis (AH) and cirrhosis (AC) to assess the role of lysosomes in ALD pathology.

Methods. Livers of normal human subjects or those with AH or AC were subjected to immunostaining, RT-PCR, and Western blot (WB) analyses.

Results. Compared with normal livers, mRNA levels encoding Transcription Factor EB (TFEB), which regulates autophagy and lysosome biogenesis, were elevated 4.5-fold in livers with AH and by 2.5-fold in those with AC. The upregulation of TFEB mRNA was associated with increased expression of two of its targets, *PGC1a* and *VAT6V1H* (acidifies lysosomes). In AH livers, mRNAs encoding the lysosomal-associated membrane proteins *LAMP1* and *LAMP2* were unchanged. However, in AC livers, both *LAMP1* and *LAMP2A* mRNA levels were elevated, compared with normal livers. WB analyses of AH and AC liver homogenates revealed significantly higher levels of TFEB, the lysosomal protease cathepsin B (Cat B), and the lysosome damage-sensing protein Galectin-3 (Gal-3). Compared with normal livers, LAMP1 levels were lower in AH livers but were higher in AC livers. Immunostaining showed enhanced TFEB expression in both the cytoplasm and nuclei of hepatocytes of AH livers. Additionally, Cat B, Gal-3, and the autophagosome marker LC3B, showed increased staining in hepatocytes of AH livers compared with normal livers. In normal livers, LAMP1/2 and Cat B appeared as small, punctate structures, whereas in AH livers, they were larger and aggregated. In AH livers, Cat B was widely distributed throughout the entire hepatocyte, and it exhibited enhanced interaction with lipid droplet (LD) membranes. Gal-3, LC3B, and P62 were also associated with LD membranes. High colocalization of cat B and LAMP2 with Gal-3 was observed in AH livers.

Conclusion. Our findings suggest that in human ALD, there is enhanced lysosome biogenesis, and this could be a response to increased processing of substrates like lipids. This may lead to lysosomal substrate accumulation, damage, and leakage, leading to hepatocyte injury, thereby contributing to ALD pathogenesis.

P40 A Survey of the Clinical Usage of Non-Steroidal Intra-Articular Therapeutics in Dogs by Veterinary Practitioners

Camila Sepulveda[1], Lindsey Boone[1], Erik Hofmeister[1], Margaret Kane[1], Kara Lascola[1], Anne Wooldridge[1], and Kayla Corriveau[1]
[1]Department of Clinical Sciences, Auburn University, AL.

Introduction. Osteoarthritis (OA) is a degenerative condition of the entire joint, whose progressive characteristics can lead to significant functional impairment and pain in the canine patient. Since the last decade, interest has increased regarding the use of non-steroidal intra-articular therapeutics (NSIATs) as part of the multimodal management of canine OA. This study aims to know the clinical experience of practitioners using these therapeutics to better understand their clinical usage, perceived outcomes, and clinical reasoning for product usage.

Methods. An electronic questionnaire inquiring about intra-articular (IA) injections and the use of NSIATs including platelet-rich plasma (PRP), autologous conditioned serum (ACS), autologous protein solution (APS), cellular therapy, viscosupplements, and radionuclides was distributed to canine practitioners. The surveyed population included surgical and sports medicine rehabilitation diplomats and/or veterinarians with a certification in animal rehabilitation. The survey collected information about practitioners' experience and the use of NSIATs: clinical usage, justification of product preference, treatment protocol used, subjective assessment of clinical efficacy, and frequency of an observed inflammatory response after IA administration.

Results. A total of 174 surveys were included in the results. Intra-articular injections were performed by 164 participants. The most common joint injected with steroidal and/or NSIATs was the elbow, followed by the stifle, shoulder, hip, and tarsus. NSIATs were used by 144 participants. The top reason for participants' decision as to which NSIATs they preferentially used was scientific data and articles published regarding the product's safety and efficacy. Platelet-rich plasma and viscosupplements were the most commonly used NSIATs by practitioners, followed by cellular therapy, radionuclides, ACS, and APS. Practitioners reported that the most common reason to use PRP and viscosupplements was chronic articular pathology needing 'maintenance' or routine injections. Most participants did not combine PRP with other IA therapeutics. In the case of viscosupplements, it was usually combined with corticosteroids or used as a sole therapy. According to the participant's subjective assessment, most of the positive responders presented some or substantial clinical improvement after PRP or viscosupplements administration. In most cases, the incidence of acute joint flares after PRP or viscosupplements IA injection was between 0% to 2%. Canine orthopedic percentile, major disciplinary focus, workplace setting, and year of graduation did not influence the use of NSIATs.

Conclusions. This survey provides information about IA injections and the clinical usage of NSIATs. Most canine practitioners who performed IA injections used NSIATs among which PRP and viscosupplements were the most commonly employed.

Acknowledgments. The authors would like to thank all the canine practitioners who took the time to participate in the survey and the different veterinary associations and veterinary colleagues for their help in distributing the electronic link to the survey.

P41 Hematology and serum chemistry in the first inbred strain of *Monodelphis domestica*, a marsupial laboratory model for biomedical research

Darielys M. Diaz[1], Abbigail L. Lanier[1], Wenqi Cao[1], Bettina Schemera[2], Luan Henker[1], Rachel Neto[1], Emily C. Graff[1], Peter W. Christopherson[1], John L. VandeBerg[3], Xu Wang[1,2,4,5*]
[1]Department of Pathobiology, Auburn University; [2]Division of Laboratory Animal Health, Auburn University; [3]Institute and Division of Human Genetics, School of Medicine, The University of Texas Rio Grande Valley; [4]Scott-Ritchey Research Center, Auburn University; [5]HudsonAlpha Institute for Biotechnology

Introduction. The gray short-tailed opossum (*Monodelphis domestica*), also known as the laboratory opossum. is a solitary marsupial native to regions of Brazil and Bolivia. Due to its small size, ease of maintenance in laboratory settings, and high reproductive success, this species has increasingly been utilized in various fields of basic and biomedical research, including cardiovascular disease, lipid metabolism, skin cancers, genomic imprinting, X chromosome inactivation, immune system evolution and function. As their contributions to scientific research expand, the availability of comprehensive resources detailing their physiological background becomes crucial for facilitating comparative analyses and retrospective studies. Although previous studies have reported hematological parameters in laboratory opossums, the animals used were from diverse genetic backgrounds, potentially introducing variability into the results. We have established the first inbred line of *Monodelphis domestica*, known as the LSD strain, which has an inbreeding coefficient greater than 0.99. This nearly homozygous line serves as an ideal model for controlling genetic variability, much like the inbred mouse strains used extensively in biomedical research.

Methods. A total of 48 laboratory opossums from the LSD strain were enrolled in this study (N = 24 males and N = 24 females). The age of the animals ranged from 5 to 14 months, all of which are considered adults that have reached sexual maturity. On the day of sampling, each opossum was individually euthanized using CO_2 inhalation followed by cervical dislocation, in accordance with approved IACUC protocol. Immediately following euthanasia, intracardiac blood was collected using 1 mL syringes with 22-gauge needles and placed into EDTA tubes for analysis. Hematologic and serum chemistry analyses were performed at the Auburn University College of Veterinary Medicine Clinical Pathology Laboratory.

Results and Conclusions. Mean values and reference intervals were calculated for the hematology and serum biochemistry data of both male and female opossums. In hematologic parameters, no significant differences were detected between the sexes (all $P > 0.05$, t-test). Two parameters showed marginal significance: males exhibited 6% higher hemoglobin levels ($P < 0.10$), and females had higher red blood cell distribution width (RDW) values ($P < 0.10$). Regarding serum chemistry results, females displayed significantly greater levels of blood urea nitrogen (BUN) and cholesterol (both $P < 0.01$, t-test), while none of the remaining parameters showed significant differences between the sexes. Our study established the baseline reference values in an inbred strain of opossums, which is crucial for detecting deviations from normal healthy conditions to define disease state, providing a controlled framework for biomedical research using this important animal model.

Acknowledgements. This study is supported by a laboratory start-up fund from Auburn University College of Veterinary Medicine.

P42 Characterization of the Transient Perinatal Rise in Luteinizing Hormone in Cats and Dogs

Johanna Ehrhardt[1], Arthur D. Zimmerman[1] and Douglas R. Martin[1,2]
[1]Scott-Ritchey Research Center, Auburn, AL
[2]Department of Anatomy, Physiology and Pharmacology, Auburn University College of Veterinary Medicine, Auburn University, AL

Introduction. Puberty is a developmental period of progression toward sexual maturity, typically resulting in fertility. Importantly, pubertal onset is directly linked to and dependent upon appropriate function of the hypothalamic-pituitary-gonadal (HPG) axis. Specifically, gonadotropin releasing hormone (GnRH) from the hypothalamus is secreted in a pulsatile manner resulting in production and release of the pituitary gonadotropins, luteinizing hormone (LH) and follicle-stimulating hormone (FSH). These gonadotropins support gametogenesis and regulate sex steroid hormone secretion, leading to sex-specific, physical changes. Puberty onset typically begins between 8-13 years of age in females and 9-14 years of age in males, and these variations are due to a multitude of complex factors including proper activation of the HPG axis during development. Activation of the HPG axis occurs both in fetal life during the second trimester and postnatally, beginning at 2 weeks in humans, during an event termed minipuberty, referred to here as transient perinatal HPG activation. This hormonal event is both important and necessary for priming of the HPG axis to attain proper pubertal onset and reproductive viability, and may play a role in brain development, formation and maintenance of sensory systems, and normal cognitive function later in life. However, this period has only been partially characterized in a few mammalian species. Therefore, given the broad importance and impact of minipuberty, we characterize hormonal changes during minipuberty in two important companion species, cats and dogs.

Methods. To characterize this developmental period, biweekly blood draws were taken from kittens beginning at postnatal day 0 to 3 months of age and from puppies beginning at postnatal day 0 until 4 months of age. Serum LH levels were measured using a chemiluminescence immunoassay (CLIA) and serum GnRH titers were determined via a standard enzyme-linked immunosorbent assay (ELISA).

Results. Results show multiple transient elevations in serum LH levels during the first 3 months in kittens and 4 months in dogs. On average, kittens had earlier and higher peaks of LH as compared to puppies. In addition, there is a similar transient increase in serum GnRH titers in both cats and dogs although these titers were also higher in cats.

Conclusions. To our knowledge, this is the first characterization of this hormonal event in these important translational research models. Additionally, regardless of species, this is one of few studies measuring changes in hormone levels within the same animals longitudinally. These findings can help determine how hormonal alterations during this period impact developmental outcomes.

Acknowledgments. We acknowledge financial support from the Scott-Ritchey Research Center.

P43 Characterization of H9Nx avian influenza viruses in chicken embryo-derived primary cell cultures

Rocio Gerez[1], Diego E. Ventura[1], Mariana A. Pinheiro[1], James T Krehling[1], Miria F. Criado[1]
[1]Department of Pathobiology, College of Veterinary Medicine, Auburn University, AL

Introduction. The H9 subtype of Avian Influenza Virus (AIV) is the most widespread low pathogenic avian influenza virus (LPAIV) globally, posing significant threats to both the poultry industry and public health. While wild waterfowl serve as natural reservoir, H9 AIV has been shown to infect a wide range of species, including mammals and humans. The emergence of poultry-adapted strains elevates the need for effective control measures due to their zoonotic potential and rapid evolution, which may alter key biological properties such as tissue tropism, virulence, and immune evasion. In this study, we examined the effects of H9Nx avian influenza virus (AIV) strains isolated from various avian species on chicken embryo-derived primary cell cultures. The objective was to investigate tissue susceptibility and resistance, and host-pathogen interactions. The findings from these experiments will help identify viral traits that enhance transmission, reveal strain-specific patterns, and highlight cellular processes or components modulated during infection.

Methods. AIV H9Nx stocks isolated from chicken (H9CK), turkey (H9TK), ruddy turnstone (H9RT), and wood duck (H9WD), were propagated and titrated using standard protocols. The H9 AIV strains were used to infect primary cell cultures derived from chicken embryo, including fibroblast (CEF), kidney (CEK), lung (CELu), liver (CELi), trachea (CET), and duodenum (CED) with a multiplicity of infection (MOI) of 0.1. The experimental endpoint was set at 7 days post-inoculation, with samples collected daily in triplicate. Susceptibility or resistance to infection was assessed through the observation of cytopathic effect (CPE), along with immunofluorescence and Real-time RT-PCR. Statistical analyses were performed in GraphPad Prism using two-way ANOVA, followed by Tukey's multiple comparison test.

Results. A distinct pattern of infection was observed among the different H9 AIV strains across the primary cell cultures. CPE was observed in all cultures for all strains, except for H9TK, H9RT, and H9WD in CEF, and H9TK and H9WD in CELi. Immunofluorescence confirmed the presence of specific tissue markers that co-labeled with AIV proteins. Differences in viral replication kinetics were evident, as shown by distinct viral growth curves in the real time RT-PCR analysis. H9CK, as anticipated, demonstrated the highest level of adaptation, achieving viral titers above 5 Log_{10}/ml for both cells and supernatants across all cultures, except for CEF, CELi, and CED where positive results were found with lower titers. H9TK reached titers exceeding 4 Log_{10}/ml in CET, CEK, CELu and CELi, but viral titers were undetectable at most time points in CEF and CED, with all supernatant samples in CED testing negative. H9RT exhibited positive results at all time points in CEF, CET, CEK, CELi cell samples, but was only detectable CET and CEK supernatant samples. Lastly, H9WD produced detectable viral titers in both cells and supernatants in CET, CEK, and CELu, with detectable viral titers below 4 Log_{10}/ml in at least one time point in CELi, CEF, and CED cell samples; however, for these latter cultures, all supernatant results were negative.

Conclusions. H9Nx AIV strains exhibited different tropism for the primary cell cultures. Overall, CEK, CET, and CELu were the most susceptible tissues, whereas CED and CEF exhibited the highest resistance for infection. These findings emphasize the tissue-specific replication capabilities of H9 AIV strains and provide insight into their differential pathogenicity, which can contribute to future research on host susceptibility and viral transmission.

Acknowledgments. Laci Mackay for technical support with immunofluorescence images. Financial support: Agriculture Research Service, US Department of Agriculture (ARS-USDA) and Department of Pathobiology, College of Veterinary Medicine, Auburn University.

P44 Enhancing the Musculoskeletal System Course (VMED 9590) Assessments with AI and Respondus 4.0

R. Reid Hanson[1], Lindsey Adams[2]
[1]Department of Clinical Sciences, College of Veterinary Medicine, Auburn University, AL
[2] Biggio Center for the Enhancement of Teaching and Learning, Auburn University, AL

Introduction. This project showcases the transformative impact of Artificial Intelligence (AI) and Respondus 4.0 on assessment development in the VMED-9590 Musculoskeletal course in Canvas. By streamlining question bank creation and preserving lecture-specific content, we have enhanced exam integrity and reduced instructor workload. These advancements boost student engagement through tailored practice materials, resulting in a richer learning experience. Our journey illustrates how AI can elevate content quality and improve the educational experience for students.

Methodology. Step 1: Lecture Content– Collect lecture slides, videos, textbooks, and other course materials to generate comprehensive and diverse questions.
Step 2: ChatGPT (AI)– Use AI to generate a variety of question types—multiple choice, short answer, matching, and true/false—at varying levels of difficulty to ensure a balanced assessment across the course content.
Step 3: Transfer to Word Document– Transfer the AI-generated questions into a Word document for easy refinement and formatting. Ensure it's properly structured for Respondus 4.0 before proceeding with the upload.
Step 4: Respondus 4.0 and Canvas– Use Respondus 4.0 to upload your formatted Word document directly into Canvas. This step streamlines the integration of the question banks into the LMS.
Step 5: Question Banks– Your newly created question banks are ready! You can now use them to create multiple versions of exams, practice tests, or pop quizzes to enhance the learning experience.

Results. Saving time on creating question banks allowed us to enhance the course for an improved student experience. With the time saved, we were able to make the following changes:

Question Banks:
- Two additional Lecture Question Banks (8-16 questions each)
- Total currently 43 Lecture Question Banks for Exams

Lecture Materials:
- 45 Equine Case Studies: Developed detailed case studies to deepen students' understanding of equine care and treatment.
- 16 Equine Practice Quizzes: Created practice quizzes for continuous assessment and self-paced learning.
- 5 Equine Lecture Quizzes (+5 Makeup Versions): Generated quizzes for each lecture with corresponding makeup exams.
- 4 Lecture Exams (+4 Makeup Versions): Developed comprehensive exams for lectures and alternate versions for makeup exams.

Course Aesthetics:
- Welcome Page: Designed a more engaging and informative course welcome page.
- Equine Lecture Pages: Created 16 introductory content pieces and learning objectives for each lecture.
- Syllabus: Updated and refined the syllabus for clearer navigation and improved student understanding.

Conclusions. AI's capabilities extend beyond exams—we generated comprehensive lecture content by analyzing textbook materials. The AI summarized key concepts, created questions, and helped structure the lecture based on the textbook, turning static reading material into engaging interactive lessons.

Acknowledgements. We would like to acknowledge the invaluable support and resources provided by the Biggio Center, as well as the guidance of Dr. Melinda Camus, Associate Dean for Academic Affairs.

P45 Genomic Analysis of a Bacteriophage-Resistant *Salmonella* Newport and its Attenuation in Calves

Steven R. Kitchens, James C. Wright, Paul H. Walz, Xu Wang, and Stuart B. Price
Department of Pathobiology, College of Veterinary Medicine, Auburn University, AL

Introduction. Bacteriophage (phage) treatment for the reduction of multiple drug-resistant *Salmonella* Newport in dairy calves is being examined in our lab from a clinical disease and food safety perspective. An unintended consequence of phage treatment could be the emergence of fully virulent but phage-resistant *Salmonella*. Working from the hypothesis that resistance would attenuate virulence in *S*. Newport, we generated a spontaneous mutant resistant to 4 out of 5 lytic phages used in our treatment regimen.

Methods. Two pairs of 8–10-week-old calves were challenged orally with the phage-resistant *S.* Newport mutant, with one pair administered a dose of 7.45×10^9 colony forming units (CFUs) and the second pair 1.47×10^{10} CFUs. A third pair of calves was challenged with a total dose of 1.96×10^{10} CFUs composed of a 1:1.3 ratio of parent:mutant in a competition assay designed to determine how well the mutant competed against the parent strain *in vivo*. Whole genome sequencing of the parent and mutant *Salmonella* was performed with the Illumina Miseq (short-read sequencing) and with Oxford Nanopore Technologies (ONT) (long-read sequencing). Variant detection (GATK Good Practices Pipeline) solely with short-reads for the parent and mutant strains resulted in inconclusive results. Utilizing ONT long-reads and Illumina short-reads, polished hybrid assemblies of the parent and mutant genomes were aligned (Mummer4). S. Newport parent and phage-resistant mutant strains were additionally sequenced PacBio Revio platform. Independently assembled HiFi reads were aligned, and variants detected using Mummer4.

Results. The four calves inoculated with the phage-resistant S. Newport mutant strain alone showed much reduced Salmonella fecal shedding, and mild clinical disease was only observed with the higher dose. The calves in the competition assay showed severe diarrhea, high fecal shedding of both parent and mutant Salmonella strains, and signs of fever and lethargy. Two nonsynonymous SNPs were detected between the hybrid assembly parent and mutant genome, one being in a predicted phage tail fiber protein gene, and the second SNP was found in *rfbM* (O-antigen biosynthesis gene). The SNP in *rfbM* was confirmed after alignment between the PacBio HiFi read assemblies.

Conclusions. The decreased shedding and clinical signs in the calves that received the phage-resistant strain alone indicate the mutation responsible for phage-resistance in *S*. Newport had an attenuating effect on virulence, perhaps by decreasing the mutant's fitness and/or its ability to colonize its host. The results of the competition experiment suggest the parent assisted the mutant strain to amplify in the calves' gut. Future experiments are planned to complement the mutation in *rfbM* to determine if phage-sensitivity can be restored in the phage-resistant *S*. Newport mutant.

Acknowledgments. We thank Forrest Shirley, Karen Wolfe, and Joanna Hyland for their assistance with the calves and the bacteriophage characterizations. This work was supported by the Animal Health and Disease Research Program of the College of Veterinary Medicine and the U.S. Department of Agriculture, Agricultural Research Service.

P46 A decade of insights: long-term clinical environmental microbiome changes in Bailey Small Animal Teaching Hospital from veterinary medicine and One Health perspectives

Nguyen T[1], Diaz D[1], Graff EC[1,2], Leisewitz AL[3], Smith BF[1,2], Sullivan SA[3], Kuo KW[3], Kennis RA[3], Lea CR[3], Delmain D[3], Chamorro ED[3], Oster SC[3], Moore PA[3], Lindley SE[3], Corriveau K[3], Hodson L[3], Etheredge T[3], Wilborn RR[3], Bergquist B[3], Moon RS[3], Castro L[3], Clark-Price S[3], Sumner SM[3], Winter RL[3], Perkins A[3], Kaur R[1], Dalton K[1], Bartol FF[4], Baird AN[3], Johnson CM[1], Wang X[1,2,5]
[1]Pathobiology, Auburn University; [2]Scott-Ritchey Research Center, Auburn University; [3]Clinical Sciences, Auburn University; [4]Anatomy, Physiology and Pharmacology, Auburn University; [5]HudsonAlpha Institute for Biotechnology

Introduction. Bailey Hospital opened its door in 2014. We are celebrating a decade of unwavering commitment to veterinary excellence, groundbreaking research, highly successful DVM education, and holistic One Health approaches. Environmental samples were collected throughout the hospital prior to operation, providing a unique opportunity to compare the pre-use environmental microbiome with the current one after ten years of veterinary practice across all specialties. This comparison will serve as a valuable resource for studying how humans, animals, and hospital environments interact and influence one another. The microbiome will provide insights into baseline data of pathogenic and non-pathogenic microbes residing in the hospital, environmental impact of veterinary practice, sanitation effectiveness, nosocomial infection control, as well as antimicrobial resistance profiles.

Methods. Swab samples were collected across multiple surfaces and specialties within Bailey Small Animal Teaching Hospital using ESwab kit (Copan Diagnostics, Italy). A total of 40 samples were collected in 2014, and N=587 samples were collected from Clinical Pathology, Neurology, Internal Medicine, and Cardiology in 2024. Sample collections of the remaining 16 specialties will be completed within this year. To investigate how environmental microbiome has changed after ten years of veterinary medicine operation and explore the bacterial communities among different specialties, DNA from 2014 and 2024 environmental samples were extracted using QIAamp PowerFecal Pro DNA kit (Qiagen, Germantown, MD, USA). Whole-genome shotgun (WGS) metagenomic libraries were constructed using NEBNext Ultra II FS DNA Library Prep Kit for Illumina (New England Biolabs, Ipswich, MA, USA). Pooled libraries were sequenced on an Illumina NovaSeq6000 or NovaSeq Xplus machine to achieve 50 million reads per sample (Novogene Corporation Inc., Sacramento, CA, USA).

Results. In the initial pilot study of 40 WGS libraries, approximately 40 million reads per sample were generated. Negative control samples exhibited a significantly higher proportion of adapter dimer content and a lower yield, as expected. After quality control and filtering of low-quality reads, the WGS reads were mapped to the human, canine, and feline genomes to determine the proportion of non-microbial reads. The levels of human and animal reads generally corresponded to the levels of human and animal activities in the sampled rooms and areas. On average, 82% of the reads originated from diverse microbes in the clinical environment, which will be subjected for further analysis.

Conclusions. Our analyses of clinical environment microbiome data have significant potential to advance the understanding of hospital microbial ecology. They are expected to substantially impact infection control, zoonotic disease prevention, evaluations of sanitation effectiveness, and antimicrobial resistance surveillance, thereby ensuring the safety of both patients and staff. Additionally, these findings will provide insights into integrating One Health principles into the design and zoning of new veterinary hospitals.

Acknowledgements. This study is supported by the Frances Keith Endowment Fund and a laboratory start-up fund from Auburn University College of Veterinary Medicine award to X.W.

P47 Susceptibility of different avian cell lines to H4Nx avian influenza viruses

Mariana A. Pinheiro[1], Thais M. Lima[2], Rocio Gerez[1], Diego E. Ventura[1], James T. Krehling[1], Miria F. Criado[1]
[1]Department of Pathobiology, College of Veterinary Medicine, Auburn University, AL
[2]Department of Cellular and Molecular Biology, University of Sao Paulo, Ribeirao Preto, Brazil

Introduction. Avian influenza virus (AIV) poses a significant threat to wildlife and poultry, affecting global food security and public health. The H4Nx strains have been found in various birds worldwide, raising concerns due to their adaptability and potential for cross-species transmission. This study assesses the susceptibility and permissiveness of different H4Nx AIV strains in continuous avian cell lines to better understand avian host-virus interactions.

Methods. Three strains of H4Nx avian influenza viruses isolated from blue-winged teal (H4BWT), turkey (H4TK), and mallard (H4ML) were propagated and titrated in embryonated chicken eggs following standard protocols. Viral kinetics were analyzed in three continuous avian cell lines from chicken (UMNSAH/DF-1, CRL-3586), quail (QM5, CVCL M211), and duck (CCL-141). The Madin-darby canine kidney (MDCK, CCL-34) cells were used as a control. Each cell type was infected at a multiplicity of infection (MOI) of 1, with samples collected at 12-, 24-, 48-, 72-, 96-, and 120-hours post-inoculation (hpi) and daily observations of cytopathic effects (CPE). Infectious virus titers were determined by plaque assay and viral loads were quantified using real-time PCR. Statistical analyses were conducted with GraphPad Prism software.

Results. All cell lines used were permissive to H4Nx infection, exhibiting cytopathic effects. In the duck cell line, the highest viral titers were obtained by H4BWT and H4ML, although the peak of viral replication in cells occurred at 12 hpi for H4TK (4.3 $\log_{10}$ plaque forming units (PFU)/ml), followed by H4BWT (6.2 $\log_{10}$ PFU/ml) and H4ML (4.5 $\log_{10}$ PFU/ml) at 24 hpi. The peak of viral budding in the supernatant was observed at 24 hpi for H4TK (4.3 $\log_{10}$ PFU/ml) and 48 hpi for H4BWT (6.2 $\log_{10}$ PFU/ml) and H4ML (4.5 $\log_{10}$ PFU/ml). In quail cells, H4BWT had the highest titers in the cells (5.1 $\log_{10}$ PFU/ml) and supernatant (6.3 $\log_{10}$ PFU/ml), with peak at 12 and 24 hpi, respectively. Quail cells were more susceptible to initial replication of H4TK than H4ML, with high viral titers detected 12 hours earlier in cells and supernatant; however, both strains reached similar titers at the peak of infection (4.3-4.9 Log_{10} PFU/ml). Finally, the chicken cell line showed the lowest susceptibility for replication with low viral titers in the cells and a minimum amount of viable virus released to the supernatant for all analyzed strains. As expected, the MDCK cells had the highest viral titers in supernatant observed for H4ML (8.3 $\log_{10}$ PFU/mL) at 96 hpi, and for H4BWT (8.6 $\log_{10}$/ml) and H4TK (7.3 $\log_{10}$ PFU/mL), both at 120 hpi. Real-time RT-PCR results showed similar patterns observed in the plaque assays.

Conclusions. Selected H4Nx AIV replicated in all tested continuous avian cell lines showing susceptibility to infection, with the lowest infection efficiency in chicken cells. The current data provide insights into the H4 replication dynamics, which will be explored in detail in future experiments to understand the mechanisms of susceptibility and resistance to infection.

Acknowledgments. This work was supported by the US Department of Agriculture (ARS-USDA) and Department of Pathobiology, College of Veterinary Medicine, Auburn University.

P48 Investigation of food marking and contamination behaviors informs feeding practice in research colonies of laboratory opossums

Abbigail L. Lanier[1], Bettina Schemera[2], Patricia E. Rynders[2], Trisha Kendall[2], Myra Petrofski[2], Jennifer Spooner[2], Paul B. Samollow[3], John L. VandeBerg[4], and Xu Wang[1,5,6*]
[1]Department of Pathobiology, Auburn University; [2]Division of Laboratory Animal Health, Auburn University; [3]Department of Veterinary Integrative Biosciences, Texas A&M University; [4]Division of Human Genetics, School of Medicine, The University of Texas Rio Grande Valley; [5]Scott-Ritchey Research Center, Auburn University; [6]HudsonAlpha Institute for Biotechnology

Introduction. The laboratory opossum, *Monodelphis domestica*, was established as a research model in 1979 and has been successfully maintained in laboratory setting since then.
The opossum is an important laboratory model for various research disciplines in biomedical sciences, Due to their divergence from eutherian mammals ~160 million years ago, metatherian mammals, or marsupials, possess unique characteristics that allow them to be considered as "alternative mammals." Despite the characteristics that differentiate marsupials (metatherians) from eutherian mammals, most of the physiological functions of these two infraclasses remain conserved. The differences as well as the similarities between the infraclasses enable the laboratory opossum to serve as a useful and, in some cases, unique model for various genetic, physiological and disease processes. Proper feeding approaches are essential for promoting animal growth and well-being.

Methods. In this study, we systematically evaluated food scattering and potential food contamination from feces across four feeding methods: direct placement of food pellets on bedding and using three different types of containers. We conducted timed daily observations of food scattering and marking behaviors in 22 animals, capturing photographs at specific intervals over the course of a week. Body weight was measured before and after the trial.

Results. Our findings revealed that the containers did not prevent food scattering behaviors, as evidenced by comparable survival curves for food scattering across all methods ($P > 0.05$, log-rank test). While the paper tray and ceramic dish delayed the occurrence of food marking by feces, indicated by a significant extension in the time to marking events ($P = 0.009$ and $P < 0.001$, respectively), these containers introduced new animal welfare concerns. The paper tray increased bleeding incidents in digits and paw pads eightfold ($P = 0.0002$), presumably due to sharp edges. The ceramic dish was associated with urine marking, and small but statistically significant weight loss (0.7%, $P < 0.05$). By 144 hours, all cages showed food contamination regardless of the feeding method.

Conclusions. The results suggest that containers provide minimal benefit in preventing food contamination, and some types of containers may pose health risks. Therefore, we propose that placing food pellets directly on the bedding, a practice used for 45 years of laboratory opossum maintenance, is acceptable for promoting optimal health and operational efficiency for this species. Our results fill a significant gap in care practices and offers insights into optimal colony management for this important research model.

Acknowledgments. This study was supported by Auburn University College of Veterinary Medicine Animal Health and Disease Research Program, USDA National Institute of Food and Agriculture (NIFA) Hatch project ALA05-2-18041, and Alabama Agriculture Experiment Station (AAES) Agriculture Research Enhancement, Exploration, and Development (AgR-SEED) award to XW. ALL is partially supported by the Frances Keith Endowment Fund.

P49 Showcase! The Advanced Capabilities of Auburn University Flow Cytometry and High-Speed Cell Sorting Laboratory (RRID:SCR_025507)

Rie Watanabe[1], James Gillespie[1], and Richard Curtis Bird[1]
[1]Department of Pathobiology, College of Veterinary Medicine, Auburn University, AL

Introduction. Explore the cutting-edge applications of flow cytometry and high-speed cell sorting at Auburn University's Flow Cytometry Lab. Our team will highlight recent research breakthroughs enabled by our state-of-the-art instruments and offer guidance for incorporating flow cytometry into your research.

About the laboratory. Our lab features advanced instruments, including the CytoFLEX LX flow cytometer and CytoFLEX SRT cell sorter, capable of supporting diverse research needs from immune cell phenotyping to small particle analysis.

Brief specifications and benefits of instruments:

CytoFLEX LX cell analyzer:
6-lasers (NUV, Violet, Blue, Yellow, Red, IR)
21 unique fluorescent detection channels
Capability of sample loading from tubes or 96-well plates

CytoFLEX SRT cell sorter:
4-lasers (Violet, Blue, Yellow, Red)
15 unique fluorescent detection channels
4-way sorting, single cell sorting (purity, enrichment mode)

Application to Research. Flow Cytometry and Cell Sorting can be applied in a variety of research fields, from standard immune cell phenotyping, apoptosis and cell cycle analysis, to small particle analysis such as exosomes. We will share in-house data to demonstrate our capabilities that can be applied to your next project. Examples of established research applications.

1) Immune cell phenotyping
2) Cell cycle or apoptotic analysis for cell populations
3) Identification of the percentage of cells with an introduced gene of interest by viral vector or transfected and selected plasmid
4) Single cell sorting to obtain single cell clones
5) 4-way sorting to separate different cell types
6) Small particle detection: demonstration data using 130nm polystyrene particles

Support and Education. In addition to offering cutting-edge tools and anslysis, we provide free consultations to help optimize experimental design and data analysis. Our educational offerings, including seminars and customized graduate courses to empower researchers at all experience levels. Join us on Phi Zeta Day to learn more and discover how our flow cytometry lab can accelerate your research!

Acknowledgments. Auburn University Flow Cytometry and High-Speed Cell Sorting Laboratory is supported by the Department of Pathobiology, College of Veterinary Medicine, and Mission Enhancement Fund from Auburn University Provost's Office.

P50 Alzheimer's Disease Histopathological Phenotype in Feline GM1 and GM2 Gangliosidosis

Arthur D. Zimmerman[1], Ashli Q. Evans[1], Malia A. Walton[1] and Douglas R. Martin[1,2]
[1]Scott-Ritchey Research Center, Auburn, AL
[2]Department of Anatomy, Physiology and Pharmacology, Auburn University College of Veterinary Medicine, Auburn University, AL

Introduction. Alzheimer's Disease (AD) is a degenerative brain disorder and the most common form of dementia. The presence of amyloid-beta (Aβ) plaques, neurofibrillary tangles (NFTs), and loss of neuronal connections are hallmarks of this disease. While current therapies may reduce symptoms of AD, they do not slow AD progression, and the overwhelming majority of new, potential therapies fail in clinical trials. Major roadblocks for development of new, effective therapeutics for the treatment of AD have been the lack of translatable animal models and our incomplete understanding of the pathological onset and progression of the disease. In recent years, much evidence has shown that aged cats may represent a natural, sporadic model for the study of AD. Additionally, there is a growing body of literature showing a link between gangliosides and the development of Aβ plaques and NFTs in certain cases of AD. We hypothesize that gangliosidosis-affected cats are a novel model for studying progression of AD pathogenesis and for testing current and new AD therapies. Therefore, in the present study, we examine feline models of both GM1 and GM2 gangliosidosis for the presence of Aβ plaques and NFTs.

Methods. We examined four different brain regions (corona radiata, parietal cortex, temporal cortex and hypothalamus) using immunohistochemistry (IHC) in gangliosidosis-affected cats as well as age-matched controls for the presence and subtypes of Aβ plaques and NFTs. Brain tissue from cats aged 2-8 months for GM1 cats, 0.5-4 months for GM2 cats and aged-matched controls was examined in this study. Whole brain images were scanned at 20X using the Olympus Evident VS200 slide scanner.

Results. Results show both age-related and region-specific changes in the presence, number and subtype of Aβ plaques as well as changes in the expression pattern of phosphorylated tau (pTau-Ser422). Results also suggest that changes in Aβ accumulation and pTau expression in gangliosidosis cats correlate spatially and temporally with AD progression in human patients. Changes appear earlier in regions affected during early stages of AD, and increase in frequency during the later stages of gangliosidosis in a manner comparable to later stages of AD. In addition, the progression of Aβ plaque subtypes throughout the brain of gangliosidosis-affected cats may model the same pattern of progression in AD.

Conclusions. Overall, we show that gangliosidosis-affected cats could be a novel model for the study of AD pathogenesis as they present with the hallmarks of AD, Aβ plaques and NFTs. We assert that GM1 and GM2 cats could represent a significant advancement for studying disease progression of an AD-like phenotype in a naturally-occurring, sporadic animal model. In future studies, we will compare Aβ and pTau expression patterns in gangliosidosis-affected cats to that of aged-cats, a documented model of human AD.

Acknowledgments. We acknowledge financial support from the Scott-Ritchey Research Center.